Systematics of *Thomandersia* Baill.

Alexandra H. Wortley

Wadham College

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Abstract

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This thesis addresses three aspects of the systematics of *Thomandersia*. Firstly, a taxonomic account of *Thomandersia* is provided. Secondly, the anatomy of *Thomandersia* is described. Finally, the phylogenetic position of *Thomandersia* in Lamiales is investigated and the potential of morphological and molecular datasets to resolve relationships in Lamiales is assessed.

Six species of *Thomandersia* are recognised, based upon previous taxonomic treatments, an assessment of variability within the genus, and a rationale for describing infrageneric taxa. An improved key to species, species descriptions, distribution maps and illustrations are presented.

A preliminary analysis of molecular sequence data suggests that previous, morphology-based classifications of *Thomandersia* in Acanthaceae do not reflect phylogenetic relationships. The floral and wood anatomy of *Thomandersia* are re-examined in this light and discussed in the context of Lamiales. Unique characters of *Thomandersia* include a particularly large calycine nectary, expanded placentas, irregular corolla aestivation, and spherical, scaly seeds. *Thomandersia* is found to share anatomical features with all other Lamiales investigated. Detailed anatomical studies of retinacula, a character shared with Acanthaceae, and extrafloral nectaries, which may be homologous to nectaries in other Lamiales, are presented. The majority of anatomical characters are homoplastically-distributed in Lamiales and therefore of little use for diagnosing monophyletic groups.

Morphological and molecular phylogenetic investigations support the resurrection of Thomandersiaceae, a family of isolated phylogenetic position with a large number of molecular and morphological autapomorphies. At the same time, the investigations highlight problems in resolving interfamilial relationships in Lamiales. The ultimate aim of phylogenetic analysis in Lamiales is an accurately-resolved tree with strongly-supported relationships between all taxa. Morphological characters are of limited use in achieving this goal: when a large morphological matrix is combined with molecular data only small, poorly-supported increases in resolution are achieved. The increasing availability of molecular sequence data is expected to make it possible, in the near future, to resolve an accurate, supported phylogeny of Lamiales and elucidate the sister group relationships of isolated taxa such as Thomandersiaceae.

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Chapter 1. Systematics of *Thomandersia* Baill.

1.1 Introduction

Systematics is a vital biological discipline (Hawksworth & Bisby, 1988; Gaston & Mound, 1993; Prance, 1995; Wheeler, 1995a; Wilson, 2000; House of Lords, 2002). It includes two major research agendas, completing the species inventory and inferring phylogenetic relationships (Minelli, 1993; Lammers, 1999; Donoghue & Alverson, 2000; Prance, 2001; Wortley et al., 2002; Wilson, 2003). This thesis contributes to both aspects of the systematics of *Thomandersia*, through a taxonomic account, anatomical study and molecular phylogenetic analysis.

Thomandersia Baill. comprises six species of small trees and shrubs from western and central Africa (Baillon, 1891; De Wildemann & Durand, 1898-1902; De Wildemann, 1905; 1906; Heine, 1966a). It was first described under the name *Scytanthus* (Bentham, 1876), and renamed *Thomandersia* in 1891 (Baillon, 1891). It can be identified by its strongly anisophyllous leaves, conspicuous, swollen calycine nectaries, and fruits in which the seeds are subtended by a hardened, hook-like retinaculum (Heine, 1963; 1966a; Bouquet, 1969; Corner, 1976). *Thomandersia* is an abundant component of wet tropical forest ecosystems and has widespread enthnobotanical applications. It contains a number of medicinally-active secondary chemicals (Szafranski et al., 1991; Ngadjui et al., 1994; Ngadjui, 1995) and is often planted in villages in the belief that it can drive away demons and bring good fortune in hunting (Bouquet, 1969; Burkill, 1985).

The phylogenetic relationships of *Thomandersia* have proven controversial. It has traditionally been classified in Acanthaceae on the basis of morphological characters, most notably the retinacula, which are considered unique to Acanthaceae (e.g. Lindau, 1894; Van Tieghem, 1908; Leonard, 1951; Bremekamp, 1965). Other sources of evidence, particularly pollen morphology (Bremekamp, 1942; Raj, 1961) and, more recently, phylogenetic analyses of molecular sequence data, suggest *Thomandersia* should be excluded from Acanthaceae. However it remains unclear in which family it should be placed. Both molecular and morphological data indicate that *Thomandersia* is a member of Lamiales, a large order in which classification is under ongoing revision by Richard Olmstead (Olmstead, in progress). Investigating the phylogenetic relationships of *Thomandersia* within Lamiales forms a major part of this thesis.

This chapter describes the current state of knowledge regarding the systematics of *Thomandersia*, including taxonomy, morphology, anatomy, and a preliminary molecular analysis of its phylogenetic relationships. The questions that will be addressed in the thesis are outlined, together with their implications for Lamiales and angiosperm systematics in general.

1.2 Starting points

The species-level taxonomy of *Thomandersia* was last revised in the 1960s (Heine, 1966a). Six species were recognised in Heine's revision, which provides a foundation for further investigations of species circumscription and delimitation.

The classification of *Thomandersia* to family has so far been based on comparative anatomy and morphology (Bremekamp, 1942; Heine, 1966a; Sreemadhaven, 1976).

As in many pre-cladistic classifications, attention has focused on a few, selected characters, in particular the retinacula. *Thomandersia* is most frequently classified in Acanthoideae (Acanthaceae), all members of which have retinacula (e.g. Bentham, 1876; Baillon, 1891; Lindau, 1897; Burkill & Clarke, 1899; Heine, 1963; Long, 1970; Hedren et al., 1995; Scotland et al., 1995; Scotland & Vollesen, 2000), with the exception of *Avicennia*, recently assigned to Acanthoideae on the basis of molecular data (Schwarzbach & McDade, 2002). Within Acanthoideae, *Thomandersia* has usually been assigned to Ruellieae (e.g. Bentham, 1876; Baillon, 1891; Lindau, 1894; Lindau, 1897; Burkill & Clarke, 1899), even though it lacks cystoliths and colporate pollen, which characterise the tribe (Scotland & Vollesen, 2000).

Systematics aims to produce classifications based on monophyly, through phylogenetic analysis of large numbers of characters, and is increasingly reliant on molecular sequence data (Cracraft, 1973; Nelson, 1974; Janowitz, 1979; Nyffeler, 1990; Welzen, 1997; APG, 1998; Backlund & Bremer, 1998; Hibbett & Donoghue, 1998; Judd et al., 1999; Chase et al., 2000; Mishler, 2000; Soltis & Soltis, 2000; Soltis et al., 2000; Bremer et al., 2002; APGII, 2003). The debate surrounding the relative utility of molecular and morphological datasets is described in Chapter 4. In practice, molecular datasets are becoming rapidly larger and more prevalent in phylogenetic analysis (Givnish & Sytsma, 1997; Slowinski & Page, 1999; Hillis & Wiens, 2000; Soltis & Soltis, 2000; Daly et al., 2001; Baker & Gatesy, 2002; Endress, 2003; Scotland et al., 2003).

Within angiosperms, molecular sequence-based phylogenetic analyses have identified many well-supported, monophyletic clades (APG, 1998; Parkinson et al., 1999;

Graham & Olmstead, 2000; Kuzoff & Gasser, 2000; Mishler, 2000; Savolainen et al., 2000; Soltis et al., 2000; Bremer et al., 2001; APGII, 2003; Davies et al., 2004). However, work is still needed to increase the number of taxa for which molecular data are available and thereby "flesh out" the details of angiosperm phylogeny, including more precise family circumscriptions and the relationships of problematic taxa (APG, 1998; Soltis & Soltis, 2000; Bremer et al., 2002; APGII, 2003). *Thomandersia* is one taxon whose relationships are yet to be established using molecular data. A key starting point for this investigation is therefore to generate a preliminary molecular phylogeny for Lamiales including *Thomandersia*; this is described below.

1.3 Preliminary molecular phylogenetic analysis of Lamiales

1.3.1 Introduction. The circumscription of Lamiales has changed dramatically over the past 20 years. For example, Cronquist's (1981) Lamiales comprised four families: Boraginaceae, Lamiaceae, Lennoaceae and Verbenaceae; Cantino (1982) included only Lamiaceae and Verbenaceae; and Takhtajan (1987) included Lamiaceae, Verbenaceae and *Callitriche*. In Takhtajan's system, Lamiales plus Boraginales, Gentianales, Scrophulariales and Solanales made up Lamiidae, which was approximately equivalent to Dahlgren's (1983) Lamiiflorae. Initial molecular investigations supported the monophyly of Lamiidae *sensu* Takhtajan (Downie & Palmer, 1992; Olmstead et al., 1992), but Lamiales *sensu stricto* was often non-monophyletic, so Lamiaceae and Verbenaceae were classified as part of a larger Scrophulariales (e.g. Downie & Palmer, 1992). Subsequently, Lamiales was expanded to accommodate the uncertain relationships between Lamiaceae, Verbenaceae and related taxa (Olmstead et al., 1993; Olmstead & Reeves, 1995; Wagstaff & Olmstead, 1997; Spangler & Olmstead, 1999). Lamiales presently comprises approximately

22,000 species in 20 families (APG, 1998; Albach et al., 2001a; Bremer et al., 2001; Olmstead et al., 2001; Bremer et al., 2002; APGII, 2003; Olmstead, in progress).

Most of the previous attempts at phylogeny reconstruction in Lamiales have focused on particular families, such as Acanthaceae, Bignoniaceae or Gesneriaceae (Scotland et al., 1995; Smith et al., 1997b; Spangler & Olmstead, 1999). The first higher-level phylogenetic analysis including the order employed 29 morphological characters across 23 taxa, and was poorly-resolved (Lu, 1990). More recently, broadening taxonomic coverage and increasing amounts of molecular data have begun to improve resolution within Lamiales (Wagstaff et al., 1998; Spangler & Olmstead, 1999; Olmstead et al., 2001; Beardsley & Olmstead, 2002; Schwarzbach & McDade, 2002). For example, a three-gene analysis of 1,129 informative characters and 65 taxa, although concentrating on Scrophulariaceae, identified many well-supported families and resolved a number of interfamilial branches (Olmstead et al., 2001).

In the present study, Lamiales phylogeny is estimated using two molecular markers – the chloroplast genes *rbcL* and *ndhF* – to provide a starting point for investigations into the relationships of *Thomandersia* within the order. Firstly the dataset is used to test the hypothesis that *Thomandersia* is a member of Acanthaceae, and secondly to uncover alternative sister group relationships within Lamiales.

1.3.2 Methods. Taxon sampling. A two-gene molecular dataset was compiled, to test the hypothesis that *Thomandersia* is a member of Acanthaceae. An exemplar taxon sampling strategy was employed (Yeates, 1995; Wiens, 1998a; Prendini, 2001). Exemplar-based sampling avoids the assumption that families are monophyletic, and

has been found in simulation to be an accurate method (Wiens, 1998a). Forty-one exemplar species were sampled, spanning the root node of all Lamiales families and including seven members of Acanthaceae (Appendix 1). All subfamilies of Acanthaceae and all proposed positions of *Thomandersia* were represented. Two species of *Thomandersia* – *T. hensii* and *T. laurifolia* – were sampled. Similar sampling strategies have been used to place other enigmatic but clearly monophyletic taxa (e.g. Bakker et al., 1998; Littlewood et al., 1998). Three out-group taxa were included, from the related orders Boraginales, Gentianales and Solanales (APG, 1998; Olmstead et al., 2000; Albach et al., 2001a; Bremer et al., 2002; APGII, 2003). Further analyses of Lamiales were conducted on a dataset of 37 taxa, spanning the root node of each family but without the dense sampling of Acanthaceae.

Molecular sequencing. A matrix of molecular sequences was assembled for *rbcL* and *ndhF*. These genes have been sequenced for a broad range of Lamiales. *RbcL* has proved useful for phylogenetic analyses at family level and above; *ndhF* has undergone approximately twice the rate of nucleotide substitution and is most suitable for intergeneric analyses (Soltis & Soltis, 1998). Combined analyses of the two genes have provided high resolution and support in other interfamilial phylogenies with large numbers of taxa (e.g. Olmstead & Reeves, 1995; Oxelman et al., 1999; Spangler & Olmstead, 1999). New *rbcL* and *ndhF* sequences were generated for *Thomandersia hensii, T. laurifolia* and *Synapsis ilicifolia* (Schlegeliaceae). Methods of DNA extraction and sequencing are described in Appendices 2-8. The remainder of the matrix was assembled from Olmstead et al. (2001) with additional sequences from Scotland (unpubl. data), Olmstead and Reeves (unpubl. data) and GenBank (http://www.ncbi.nlm.nih.gov/entrez/). For a few genera, the two genes were

sequenced from different species; for *Martynia* only an *ndhF* sequence was available. Sequence alignment was achieved by eye; alignments (for the 37-taxon analyses) are shown in Appendices 9 and 10.

Phylogenetic analysis. Maximum parsimony analysis was conducted using PAUP* 4.0b10 for Macintosh (Swofford, 2002). Characters were unordered and equally-weighted. Gaps were coded as missing data, and zero-length branches collapsed so that only branches with unambiguous support were retained. Two search strategies were employed, in an attempt to find all most parsimonious (MP) trees and all possible islands (complying with Maddison, 1991; Maddison et al., 1992; Olmstead & Palmer, 1994; Catalan et al., 1997; Meerow & Snijman, 2001). The first strategy utilised 1,000 replicate heuristic searches, with tree-bisection-reconnection (TBR) branch-swapping and MULPARS on, saving all trees. The second strategy employed 10,000 searches, with TBR branch-swapping and MULPARS on, saving only two trees per replicate. Both strategies were then repeated while implementing a constraint tree to exclude topologies compatible with the strict consensus of the previous searches. Trees were rooted in a basal polytomy using the pre-defined out-group. The same search strategies were implemented while constraining *Thomandersia* to a number of positions in Acanthaceae, to test whether these were significantly less parsimonious (Olmstead et al., 2001).

Internal support was estimated by nonparametric bootstrap analysis (Felsenstein, 1985). Bootstrapping was conducted with 100 replicates of a 1,000-replicate heuristic search, with TBR branch-swapping, MULPARS off, saving all trees. Decay analysis was performed with searches of 1,000 replicates, TBR branch-swapping and random

addition, saving only two trees per replicate (Bremer, 1994; Morgan, 1997; Eriksson, 1998).

To assess whether the same or conflicting signals were present in the two datasets, the partition homogeneity test was conducted with 1,000 replicates of a 100-replicate heuristic search using a single random-order-entry starting tree, TBR branch-swapping, saving up to 10 trees per replicate (Farris et al., 1994; Cunningham, 1997; Dolphin et al., 2000).

Maximum likelihood analysis was conducted on the combined-gene dataset using a model and parameters selected by the hierarchical likelihood ratio test using MODELTEST (Posada & Crandall, 1998). The most complex models allowed by MODELTEST enable specification of a gamma distribution shape parameter, allowing for rate heterogeneity between sites and thus between partitions (Yang, 1996). Maximum likelihood analysis employed starting trees obtained by neighbour-joining, with TBR branch-swapping and MULPARS on. Likelihood bootstrap searches of 100 replicates were also conducted using PAUP*.

1.3.3 Results. Forty-one-taxon analysis. The *rbcL* and *ndhF* sequence dataset provided 3,584 aligned characters, of which 1,444 were variable and 821 were parsimony-informative. The hierarchical likelihood ratio test suggested the most likely model and parameters for evolution of these sequences was the transversional model, TVM+I+G (-lnL=25841.5449); with base frequencies A=0.2841, C=0.1671, G=0.1822, T=0.3666; substitution rate matrix A-C=1.8116, A-G=3.2045, A-

T=0.2711, C-G=1.8659, C-T=3.2045, G-T=1; proportion of invariable sites 0.4029; and gamma distribution shape parameter 0.8973.

The partition homogeneity test conducted on the *ndhF* and *rbcL* partitions gave the result p=0.011, suggesting there was no significant incongruence in signal between the datasets. They were therefore combined in a simultaneous analysis, which would provide greater statistical and explanatory power than analysing the two datasets separately (Farris, 1983; Hillis, 1987; Swofford, 1991; Nixon & Carpenter, 1996).

Parsimony analysis of the combined molecular dataset recovered 128 MP trees of 4,161 steps (the second search strategy found 76 of these trees). No new equally-parsimonious islands were found in the constrained analyses. The ensemble consistency index excluding uninformative characters (CI) was 0.41 and the ensemble retention index (RI) 0.35. The strict consensus of the MP trees is shown in Fig. 1.1.

The result of the maximum likelihood analysis, a tree with –lnL=25789, is shown in Fig. 1.2.

When *Thomandersia* was constrained to Acanthaceae, in parsimony analysis, the trees produced were 4,180 steps long, 19 steps longer than the MP unconstrained tree, and *Elytraria* (Acanthaceae) was sister to Pedaliaceae. When *Elytraria* and *Thomandersia* were both constrained to Acanthaceae, the trees were 20 steps longer than the MP tree. When *Thomandersia* was constrained to the retinaculate Acanthaceae (*Ruellia, Justicia, Barleria* and *Aphelandra*), the trees produced were 4,185 steps long, 24 steps longer than the MP tree, and Acanthaceae was polyphyletic,

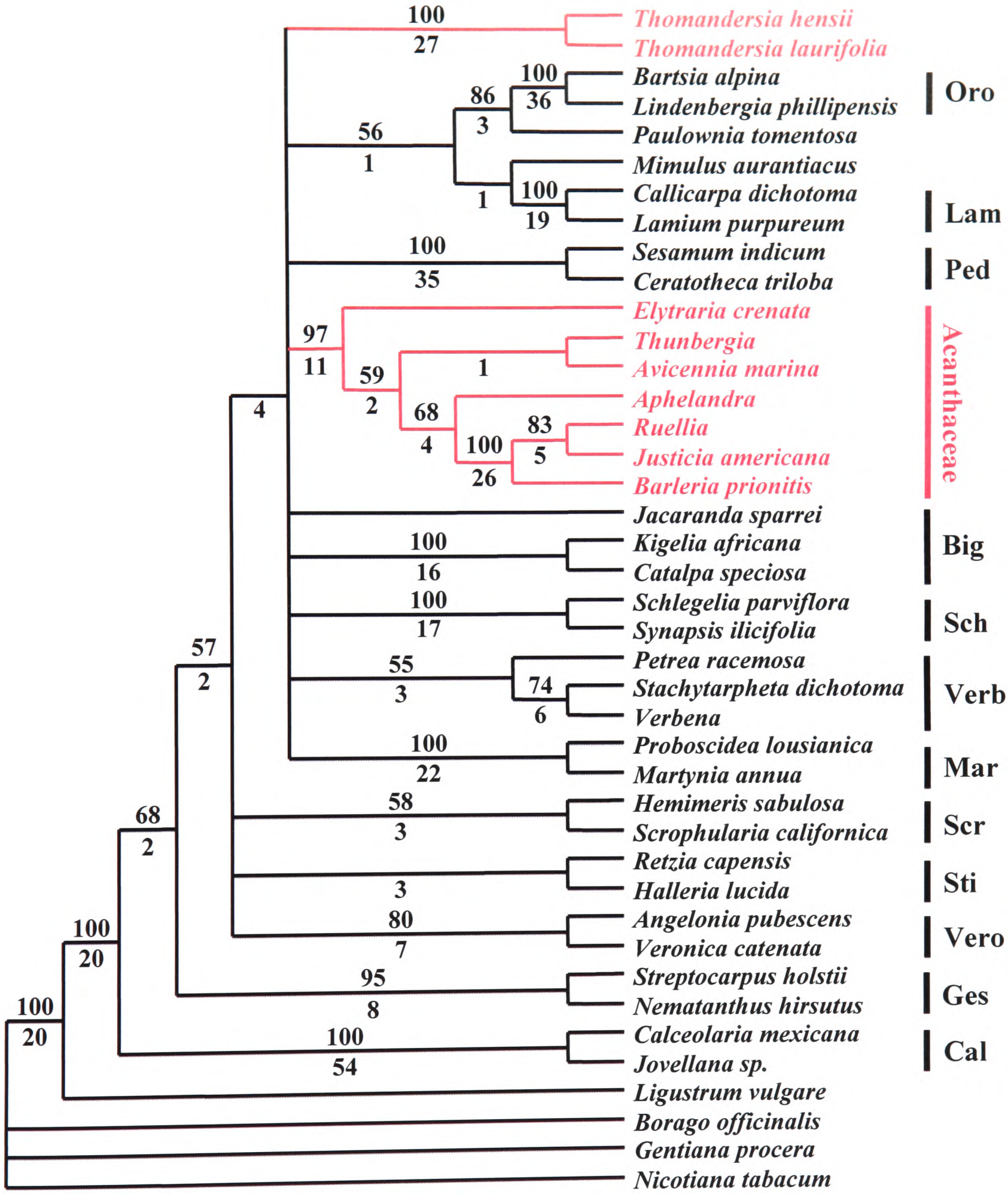


Figure 1.1 Strict consensus of 128 MP trees obtained from parsimony analysis of the two-gene molecular dataset for 41 taxa in Lamiales, focusing on Acanthaceae and *Thomandersia* (highlighted in red). Figures above branches are bootstrap values greater than 50%, those below are decay values. Families taken from Olmstead et al. (2001). Big, Bignoniaceae; Cal, Calceolariaceae; Ges, Gesneriaceae; Lam, Lamiaceae; Mar, Martyniaceae; Oro, Orobanchaceae; Ped, Pedaliaceae; Sch, Schlegeliaceae; Scr, Scrophulariaceae *s.s.*; Sti, Stilbaceae; Verb, Verbenaceae; Vero, Veronicaceae.

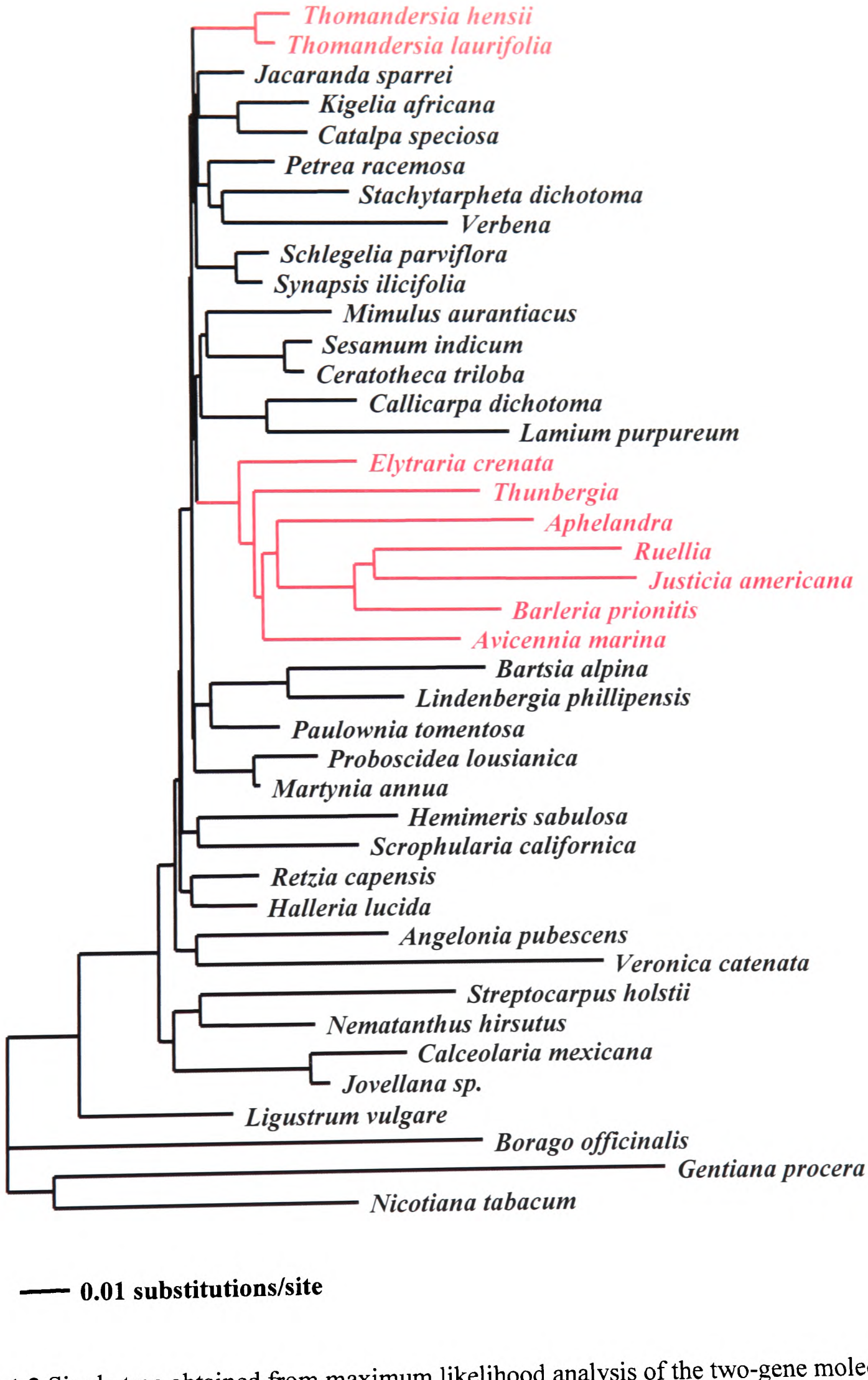


Figure 1.2 Single tree obtained from maximum likelihood analysis of the two-gene molecular dataset for 41 taxa in Lamiales, focusing on Acanthaceae and *Thomandersia* (highlighted in red). Branch lengths are proportional to the number of nucleotide changes inferred upon them.

with the remainder of the family sister to Pedaliaceae. When Acanthaceae were constrained to be monophyletic and *Thomandersia* constrained to Acanthoideae, an arrangement compatible with existing classifications (Bentham, 1876; Baillon, 1891; Lindau, 1894; 1897; Hutchinson & Dalziel, 1931; Heine, 1966b), the trees were 4,186 steps long, 25 steps longer than the MP unconstrained tree. In this tree, Acanthaceae were forced into an arrangement that is incongruent with most previous analyses of the family (Hedren et al., 1995; Scotland et al., 1995; McDade & Moody, 1999; McDade et al., 2000; Scotland & Vollesen, 2000), comprising two sister clades, one containing the non-retinaculate subfamilies and the other Acanthoideae and *Thomandersia*.

Thirty-seven-taxon analysis. The two-gene molecular dataset for 37 taxa contained 3,578 aligned characters, of which 1,371 were variable and 749 parsimony-informative. The hierarchical likelihood ratio test suggested the most likely model and parameters for evolution of these sequences was TVM+I+G (-lnL=23282.7012); with base frequencies A=0.2855, C=0.1667, G=0.1847, T=0.3631; substitution rate matrix A-C=1.9484, A-G=3.1575, A-T=0.2730, C-G=1.8342, C-T=3.1575, G-T=1; proportion of invariable sites 0.3971; and gamma distribution shape parameter 0.8584.

The partition homogeneity test conducted on the *ndhF* and *rbcL* partitions for these taxa gave the result p=0.01, indicating significant incongruence between the two datasets. Because of this, the datasets were first analysed separately (Bull et al., 1993; Ballard, 1996). Parsimony analysis of *ndhF* sequences produced seven MP trees 2,760 steps long, with CI 0.44 and RI 0.56. A strict consensus tree is shown in Fig. 1.3A. Analysis of *rbcL* sequences produced 44 MP trees 826 steps long, with CI 0.43 and RI

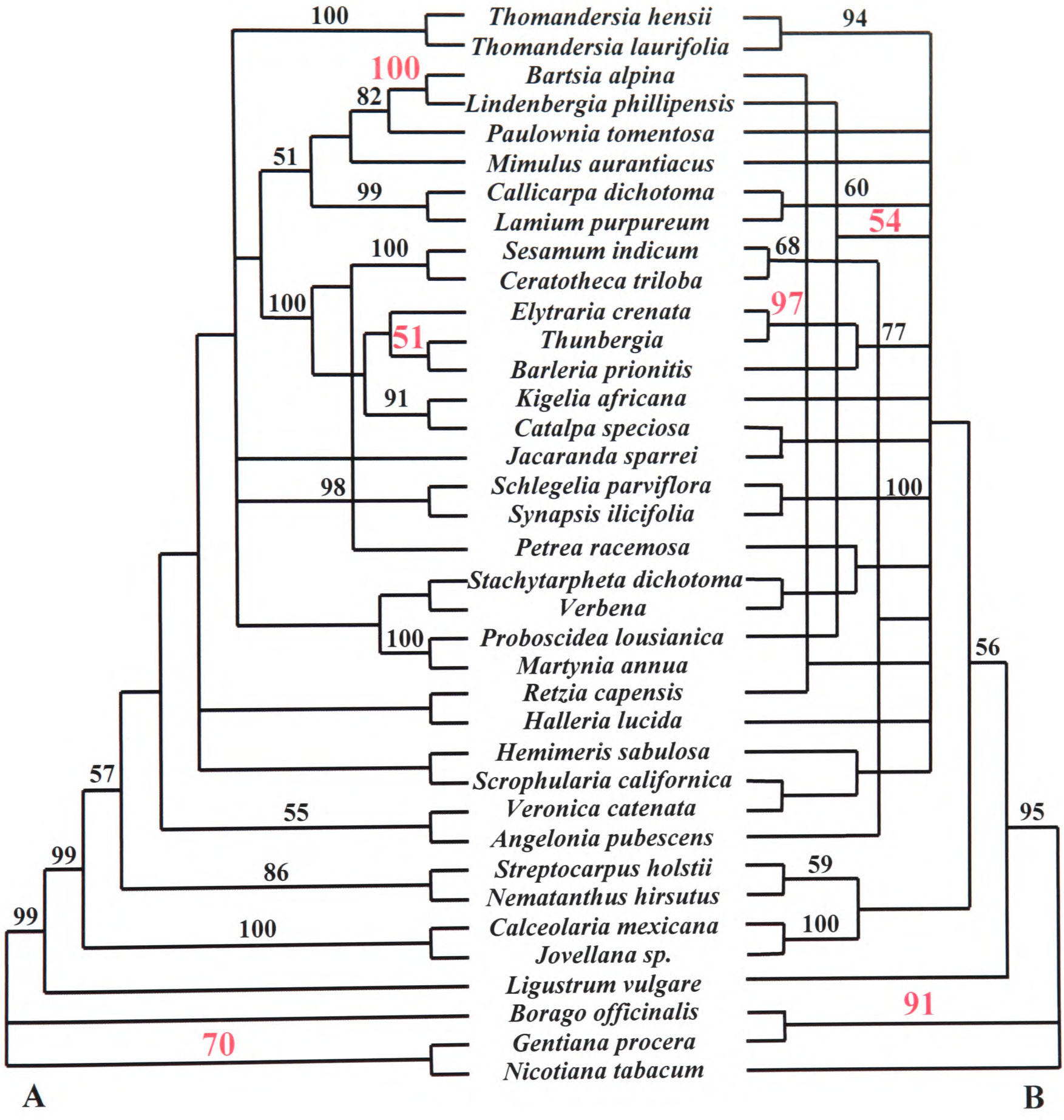


Figure 1.3 Strict consensus trees obtained from parsimony analysis of single-gene datasets for 37 taxa in Lamiales. Figures above branches are bootstrap values greater than 50%; those in red indicate "hard incongruencies" – clades that are incongruent in the two trees and supported by bootstrap values greater than 50%. A. *ndhF* (strict consensus of seven MP trees). B. *rbcL* (strict consensus of 44 MP trees).

0.57. A strict consensus tree is shown in Fig. 1.3B. Few differences in topology between the *ndhF* and *rbcL* trees were well supported: in only three instances were incongruent clades supported by a bootstrap value greater than 50% (Fig. 1.3). These were *Lindenbergia-Proboscidea* (54% in the *rbcL* tree) and *Lindenbergia-Bartsia* (100% in the *ndhF* tree); *Elytraria-Thunbergia* (97% *rbcL*) and *Barleria-Thunbergia* (51% *ndhF*); and *Borago-Nicotiana* (91% *rbcL*) and *Gentiana-Nicotiana* (70% *ndhF*). In all three cases, one topology was better supported than the other. This suggested there was no "hard incongruence" (Farris et al., 1994; 1995; Wendel & Doyle, 1998; Barker & Lutzoni, 2002) between the *rbcL* and *ndhF* datasets and they were therefore combined into a single dataset for further analysis.

Parsimony analysis of the combined two-gene molecular dataset recovered 31 MP trees of 3,620 steps (the second search strategy found 28 of these trees). No new equally-parsimonious islands were found in the constrained analyses. The CI was 0.43 and the RI 0.35. A strict consensus of the MP trees is shown in Fig. 1.4. The result of the maximum likelihood analysis, a tree with –lnL=23233, is shown in Fig. 1.5.

1.3.4 Discussion. Classifications should be based on monophyly (Hennig, 1966; Backlund & Bremer, 1998; Carine & Scotland, 2002). In many cases, the monophyletic groups recovered in molecular phylogenetic analysis are in agreement with past morphological classifications (Swofford, 1991; Patterson et al., 1993; Smith & Sytsma, 1994; Endress, 2000; Hillis & Wiens, 2000; APGII, 2003; Scotland et al., 2003). However for some taxa, molecular phylogenies have suggested new relationships or discounted previous hypotheses. For example, Scrophulariaceae *sensu lato* and Flacourtiaceae are thought to be polyphyletic (Olmstead & Reeves, 1995;

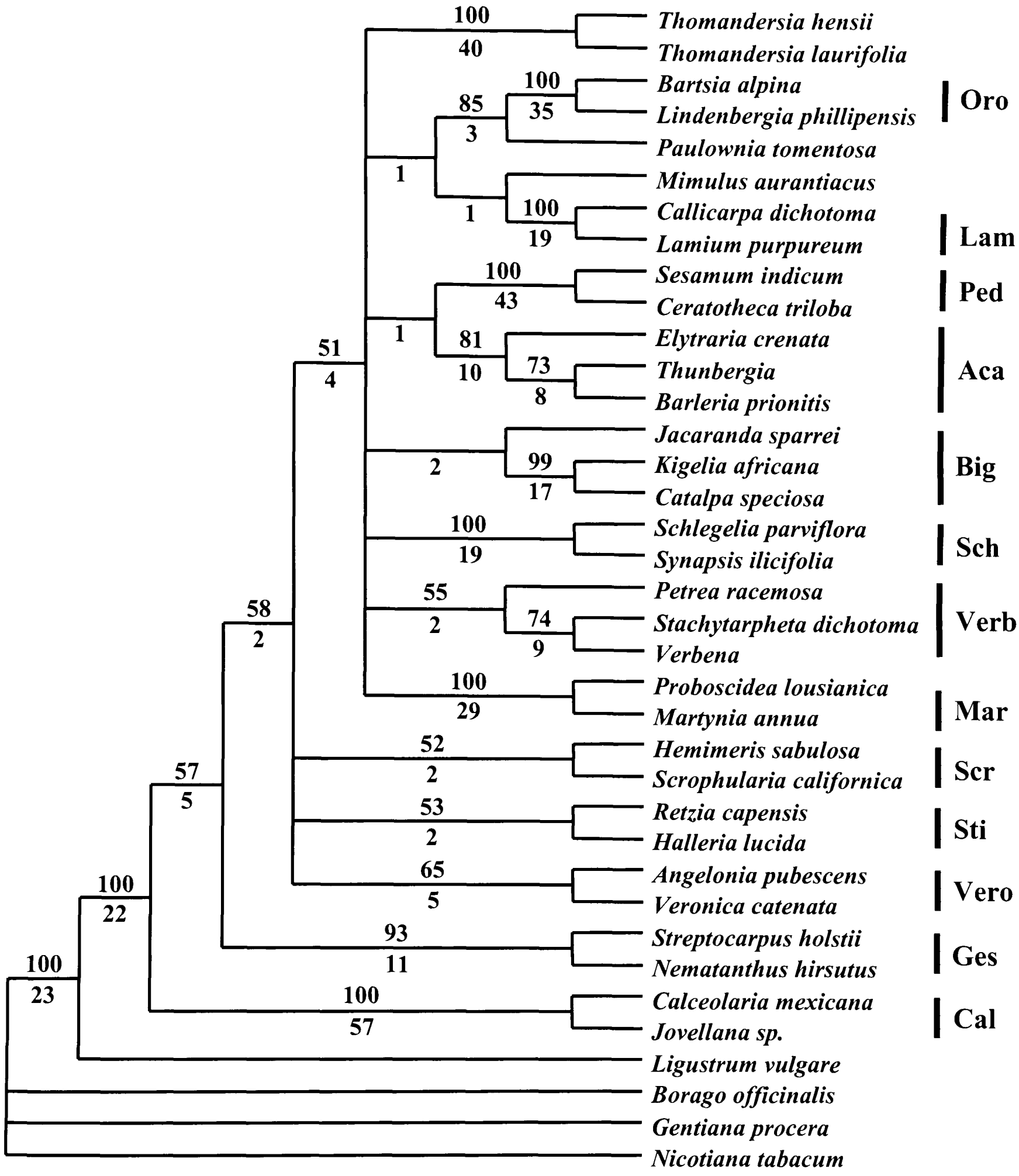


Figure 1.4 Strict consensus of 31 MP trees obtained from parsimony analysis of the two-gene molecular dataset for 37 taxa in Lamiales. Figures above branches are bootstrap values greater than 50%, those below are decay values. Families taken from Olmstead et al. (2001). Aca, Acanthaceae; Big, Bignoniaceae; Cal, Calceolariaceae; Ges, Gesneriaceae; Lam, Lamiaceae; Mar, Martyniaceae; Oro, Orobanchaceae; Ped, Pedaliaceae; Sch, Schlegeliaceae; Scr, Scrophulariaceae *s.s.*; Sti, Stilbaceae; Verb, Verbenaceae; Vero, Veronicaceae.

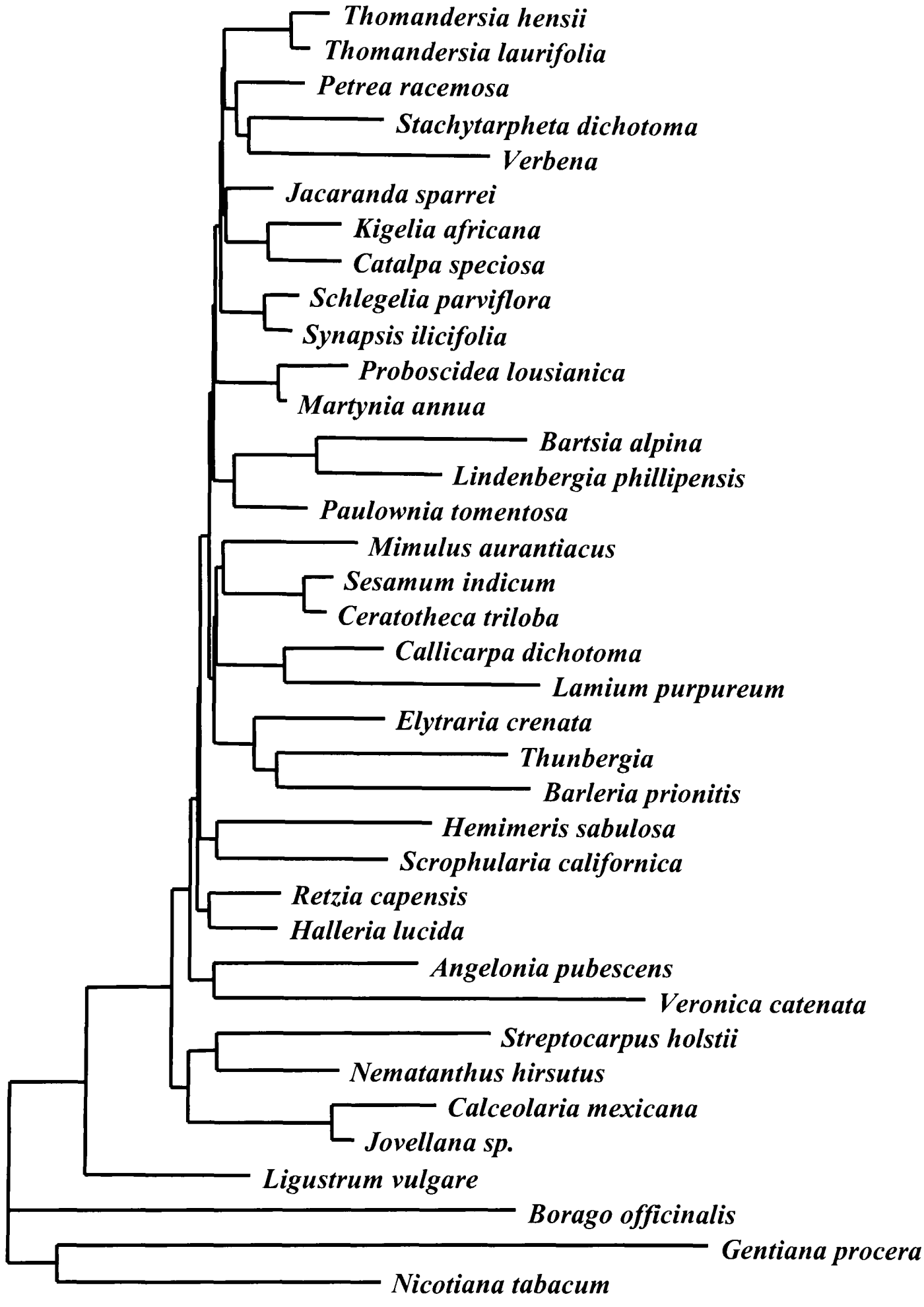


Figure 1.5 Single tree obtained from maximum likelihood analysis of the two-gene molecular dataset for 37 taxa in Lamiales. Branch lengths are proportional to the number of nucleotide changes implied upon them.

Olmstead et al., 2001; Chase et al., 2002), while *Platanus* is now considered to be a member of Proteaceae (APGII, 2003). The analyses conducted here suggest *Thomandersia* may be another case of misclassification.

Strong support for the in-group in all analyses (e.g. Figs. 1.1, 1.4) confirms *Thomandersia* belongs in Lamiales. However, its relationships to other groups within the order are more problematic. In contrast to previous morphological classifications (e.g. Bentham, 1876; Baillon, 1891; Lindau, 1894; 1897; Heine, 1966a), both parsimony and likelihood analyses found Acanthaceae to be monophyletic excluding *Thomandersia* (Figs. 1.1, 1.2). The topology recovered is unlikely to be due to artefacts such as long-branch attraction causing *Thomandersia* to associate with highly-divergent taxa (Felsenstein, 1978), because both methods of analysis agreed on the result, and because when *Thomandersia* was constrained to Acanthaceae it did not lie on a branch significantly longer than any other taxon.

Acanthaceae were supported by bootstrap values of 88% in maximum likelihood analysis and 97% in parsimony analysis, with a Bremer support (decay) value of 11 steps. Acanthoideae, in which *Thomandersia* has often been classified, was poorly supported (41%), partly due to the inclusion of *Avicennia*, a taxon of unstable position (Sanderson & Shaffer, 2002; Schwarzbach & McDade, 2002). When *Avicennia* was excluded, Acanthaceae were monophyletic with 98% bootstrap support, and Acanthoideae with increased support at 66%.

The inclusion of *Thomandersia* in a monophyletic Acanthaceae was 20 steps less parsimonious than the MP trees. Including *Thomandersia* in a monophyletic

Acanthoideae required 25 additional steps, i.e. this was not a parsimonious solution. Furthermore, enforcing this constraint caused a rearrangement of relationships within Acanthaceae to form a topology unlike any previously proposed (Hedren et al., 1995; Scotland et al., 1995; McDade & Moody, 1999; McDade et al., 2000; Scotland & Vollesen, 2000; Manktelow et al., 2001).

The 128 MP trees differed, among other respects, in the phylogenetic relationships of both *Thomandersia* and Acanthaceae. Thus neither the sister group of Acanthaceae nor that of *Thomandersia* was resolved in the strict consensus tree (Fig. 1.1). In maximum likelihood analysis, *Thomandersia* was resolved within a clade comprising Verbenaceae, Bignoniaceae and Schlegeliaceae, although bootstrap support for this group was low (Fig. 1.2). Acanthaceae were sister to a clade comprising Pedaliaceae, *Mimulus* and Lamiaceae, but also with little support.

Molecular data therefore provide little evidence that *Thomandersia* is related to Acanthaceae, challenging the homology of retinacula at this level (Wilson, 1960; Patterson, 1982; De Pinna, 1991; Rieppel, 1996). However, they provide no consistent, supported alternative hypothesis for the sister group of *Thomandersia* in Lamiales.

In the remainder of this thesis, the phylogenetic relationships of *Thomandersia* are investigated using a 37-taxon dataset, including up to three representative exemplars of each major family in Lamiales. Maximum parsimony analysis of this dataset generated a phylogeny (Fig. 1.4) entirely congruent with that from the denser sample of Acanthaceae (Fig. 1.1). In maximum likelihood analysis (Figs. 1.2, 1.5), the two

taxon sets also generated very similar phylogenetic trees. This suggested that differences in taxon-sampling within Acanthaceae do not affect the phylogenetic relationships inferred, and therefore justified employing a smaller sample of Acanthaceae in further analyses.

Phylogenies inferred using separate *ndhF* and *rbcL* sequence datasets showed a number of topological differences. Well supported conflict between clades may reflect real differences in evolutionary signal between datasets (Barker & Lutzoni, 2002; Rokas et al., 2003; Hipp et al., 2004); poorly-supported (soft) incongruence is thought to result from stochastic sampling error (Farris et al., 1994; 1995; Barker & Lutzoni, 2002; Hipp et al., 2004). The *ndhF* and *rbcL* trees conflicted over only three clades receiving greater than 50% bootstrap support (Fig. 1.3), and in two of these cases the less-conventional relationship was not very well supported (51% for the relationship of *Thunbergia* and *Barleria* in Acanthaceae in the *ndhF* tree; 54% for that of *Lindenbergia* and *Proboscidea* in the *rbcL* tree). The remaining incongruence involved only out-group taxa, which may result from the sequences being unsuitable to accurately resolve relationships among distantly-related groups. Thus, the two chloroplast markers contained broadly congruent signals, and were subsequently combined in a single analysis to provide greater statistical and explanatory power (Hillis, 1987; Swofford, 1991; Nixon & Carpenter, 1996; Kluge, 1998; Farris, 2000).

Simultaneous analysis is likely to provide the most robust hypothesis of the phylogenetic relationships of *Thomandersia* and the best starting point for reassessing its anatomical characters. However, the strict consensus of the MP trees from this analysis failed to resolve the sister group of *Thomandersia* (Fig. 1.4), and did not

contain any well-supported interfamilial clades within a large group of "core Lamiales". The analysis indicated, in agreement with previous studies, that Oleaceae, Calceolariaceae and Gesneriaceae form successive basal branches of Lamiales (Olmstead et al., 2001; Bremer et al., 2002; APGII, 2003), but did not suggest any other well-supported phylogenetic relationships between families of Lamiales.

Maximum likelihood analysis of the combined-gene dataset suggested *Thomandersia* was most closely related to Verbenaceae, Bignoniaceae and Schlegeliaceae respectively (Fig. 1.5), but without significant bootstrap support (less than 5% for each of these branches).

In summary, the current molecular-based phylogenetic relationships of *Thomandersia* are at odds with the prevailing morphology-based classification. Phylogenies based on *rbcL* and *ndhF* sequence data are inconclusive regarding the sister group of *Thomandersia*. Bignoniaceae, Schlegeliaceae and Verbenaceae are implicated as the most likely candidates.

These preliminary analyses highlight two problems of molecular-based phylogenetic inference in Lamiales. Firstly, there are a number of isolated ("rogue") taxa (mostly recognised at the level of genus) with unstable and poorly-supported phylogenetic relationships. Examples include *Jacaranda, Mimulus,* Schlegeliaceae, Stilbaceae and *Thomandersia.* Secondly, while family-level clades tend to be consistently resolved and supported, the relationships between them are unstable and poorly supported. This results from a preponderance of short interfamilial branches towards the base of the tree, where few nucleotide substitutions separate the divergence of major clades (e.g.

Fig. 1.5), regardless of whether *rbcL, ndhF* or both genes are used, and whether parsimony or likelihood methods are employed. The same pattern is confirmed in other recent analyses of Lamiales (Olmstead et al., 2001; Bremer et al., 2002).

1.4 Outline of the thesis

This investigation into the systematics of *Thomandersia* builds upon the existing taxonomic account (Heine, 1966a), traditional morphological classification (Bentham, 1876; Baillon, 1891; Lindau, 1894; 1897; Hutchinson & Dalziel, 1931; Heine, 1966b) and the preliminary molecular phylogeny of Lamiales described above.

In terms of infrageneric taxonomy, a new account for *Thomandersia* is presented in Chapter 2. Although all species are easily distinguished when fertile, collections continue to be misidentified. In addition, a number of new collections have been made since the 1960s, which extend the geographical range and phenotypic diversity of the species of *Thomandersia* and raise new taxonomic questions. Thus a taxonomic revision of *Thomandersia* is needed, including an improved key to species, species descriptions and distribution data.

The thesis will also reconsider the morphological characters of *Thomandersia* in the light of the molecular phylogeny. In particular, the homology of the retinacula in *Thomandersia* and Acanthaceae requires thorough investigation. In Chapter 3, a detailed investigation is conducted into the retinacula of *Thomandersia* and Acanthaceae, assessing their primary homology by similarity (Remane, 1952; Rieppel, 1996; Rieppel & Kearney, 2002). A range of other anatomical features of *Thomandersia* and Lamiales are considered, with the aim of objectively searching for

possible morphological synapomorphies that may have been overlooked in previous classifications.

Finally, building on the preliminary molecular sequence-based phylogeny presented here, Chapters 4 and 5 investigate the methods and data available for improving the inference of Lamiales phylogeny. These chapters focus on overcoming some of the problems encountered so far in determining relationships in Lamiales and identifying the sister-group of *Thomandersia,* and may help to suggest solutions to similar problems of phylogenetic inference in other groups.

The two difficulties highlighted in this preliminary phylogeny of Lamiales are widespread throughout angiosperms. One problem is the presence of rogue genera, with uncertain familial relationships, which are often small clades in an isolated position (Smith et al., 1993; Bremer et al., 2002). Some rogue groups have long been controversial in morphological classifications, displaying a combination of characters diagnostic of different families, e.g. *Emblingia, Hydrostachys, Paulownia*, *Schlegelia, Siparuna* and *Trichostephanus* (Armstrong, 1985; Renner et al., 1997; Rosales, 1997; Chandler & Bayer, 2000; Albach et al., 2001b). Others were traditionally classified in a family to which molecular data have now refuted a relationship, such as *Mimulus,* once a member of Scrophulariaceae (Beardsley & Olmstead, 2002). In some cases, intensive molecular studies have successfully determined the probable phylogenetic relationships of rogue genera, for example *Cyrtandromoea* (Gesneriaceae), *Halesia* (Styracaceae), *Peridiscus* (Saxifragales), *Polypremum* (Tetrachondraceae) and *Tepuianthus,* sister to Thymeleaceae (Smith et al., 1997a; APGII, 2003; Davis & Chase, 2004). However a number of rogue taxa, for example *Hydrostachys,*

Pentaphylax, Paracryphiaceae, Polyosomaceae and Sphenostemonaceae (Bremer et al., 2002; APGII, 2003), remain unplaced. There is an especially large number of these taxa in Lamiales (Bremer et al., 2002; APGII, 2003; Olmstead, in progress).

Secondly, Lamiales is a well-supported monophyletic order containing generally well-supported families, but notable for its intractable interfamilial relationships. These are poorly resolved and supported in analyses of as many as six molecular partitions (Bremer et al., 2002). In the preliminary analysis presented here, only one interfamilial group (Orobanchaceae plus *Paulownia*) was relatively well supported. Low support and poor resolution of interfamilial relationships are problems shared with many other angiosperm groups, including the large asterid orders Asterales and Ericales (Anderberg et al., 2002; Bremer et al., 2002; Lundberg & Bremer, 2003).

Several factors have been invoked to explain the presence of short, unstable internal branches in phylogenies reconstructed with molecular sequence data. These include poor taxon sampling, lack of phylogenetic signal or conflicting signal in different partitions (Renner & Chanderbali, 2000), the presence of rogue taxa (Sanderson & Shaffer, 2002), or the historical signal of a rapid radiation (Fishbein et al., 2001). All except the latter are limitations of the datasets and/or methods used, rather than the intrinsic nature of the phylogeny itself, so are ultimately solvable and may benefit from investigation. In Lamiales there is little scope to improve taxon sampling: each family has already been sampled to span its root node (Olmstead et al., 2001; Prendini, 2001). Therefore the major part of this thesis investigates aspects of the impact of character sampling.

Chapters 4 and 5 consider various aspects of character sampling; in particular the most suitable type, quantity and selection of characters for effective reconstruction of phylogenies such as Lamiales. Firstly, morphological characters are considered, in the context of the debate between morphological and molecular evidence in phylogeny reconstruction. The usefulness of morphological data is tested for a range of empirical datasets, and the specific impact of morphological characters on phylogenetic analysis in Lamiales is investigated.

Morphological datasets are of finite size, and there is a trade-off between the number of characters that can be included and the likelihood that they will mislead phylogenetic inference (Poe & Wiens, 2000; Scotland et al., 2003). Molecular datasets, on the other hand, are in principle limited only by the size of the genome (Daly et al., 2001; Herniou et al., 2001; Matsuoka et al., 2002; Freudenstein et al., 2003; Rokas et al., 2003). Chapter 5 uses simulated sequence data to investigate the question of how much molecular data would be needed to provide acceptable support and accurate resolution of interfamilial relationships in Lamiales.

1.5 Conclusions and aims

No taxon can be studied in isolation, but requires a consideration of the phylogeny of the larger hierarchical group to which it belongs. The isolated and uncertain position of *Thomandersia* necessitates a broad investigation of interfamilial relationships in Lamiales, one of the largest angiosperm orders (Stevens, 2001; Bremer et al., 2002; Olmstead, in progress).

Phylogenetic analysis aims to reconstruct accurate, well-resolved relationships with robust, well-supported clades. These factors are all lacking in the phylogeny of Lamiales, which suffers from two problems described above: unplaced, isolated genera, and short internal branches in molecular phylogenetic analysis. Additional investigation, using a wider range of datasets and methodological approaches, is essential for resolving relationships in the order (APGII, 2003). Lamiales is an ideal group in which to test how the application of different datasets may help solve such problems. Determining the best approach to solving the twin problems of rogue genera and unstable interfamilial relationships in Lamiales may in turn suggest fruitful methods for resolving similar problems in other parts of the angiosperm tree.

In summary, this study of the systematics of *Thomandersia* develops from an existing taxonomic account, morphological classification, and two-gene molecular phylogeny, and aims to:

1. Present a new taxonomic account for *Thomandersia*, including an improved key to species, descriptions and distribution data.
2. Describe the floral and wood anatomy of *Thomandersia* in the context of Lamiales and ascertain the underlying homology of its characters, in particular the distinctive extrafloral nectaries and retinacula.
3. Investigate the use of morphological datasets in phylogenetic analysis and explore the potential of morphological data to reconstruct the phylogeny of Lamiales.
4. Ascertain whether a supported, accurate phylogeny of Lamiales is resolvable with molecular data, how much data would be needed to achieve this, and estimate the specific impact of sequencing an additional molecular marker.

5. Determine the most appropriate overall strategy for resolving the phylogeny of Lamiales and the sister group of *Thomandersia* with maximum accuracy and support.
6. Contribute to the debate surrounding the use of different data types in phylogenetic analysis, the systematics of other poorly-resolved angiosperm orders, and the phylogenetic relationships and taxonomic status of rogue genera such as *Thomandersia*.

Chapter 2. A taxonomic account of *Thomandersia*

2.1 Introduction

Thomandersia has been considered monophyletic since it was described in the late nineteenth century. A distinct combination of morphological characters unites the species within the genus and distinguishes them from other Lamiales. *Thomandersia* was last revised in 1966, when six species were recognised (Heine, 1966a). Several new collections have been made since that time (Fig. 2.1), which extend the geographic range and phenotypic diversity of the species and raise new taxonomic questions.

In this chapter the taxonomic history of *Thomandersia* is described. Some underlying concepts for the delimitation of infrageneric taxa are discussed, including the rationale for describing species and subspecies and how this applies to *Thomandersia*. The existence of possible new species, subspecies and hybrid specimens is considered. The conclusions are implemented in a new taxonomic account of *Thomandersia*, including an improved key to species, species descriptions, distribution maps and illustrations.

2.2 Delimiting and diagnosing infrageneric taxa

There is a longstanding debate surrounding the biological reality and recognition of species (e.g. Du Rietz, 1930; Mayr, 1942; Sokal, 1973; Van Valen, 1976; Cronquist, 1978; Wiley, 1978; Templeton, 1989; Andersson, 1990; Davis, 1995; Olmstead, 1995). No consensus has been reached on the definition of a species, or on whether species truly exist as independent evolutionary units (Winston, 1999). There have

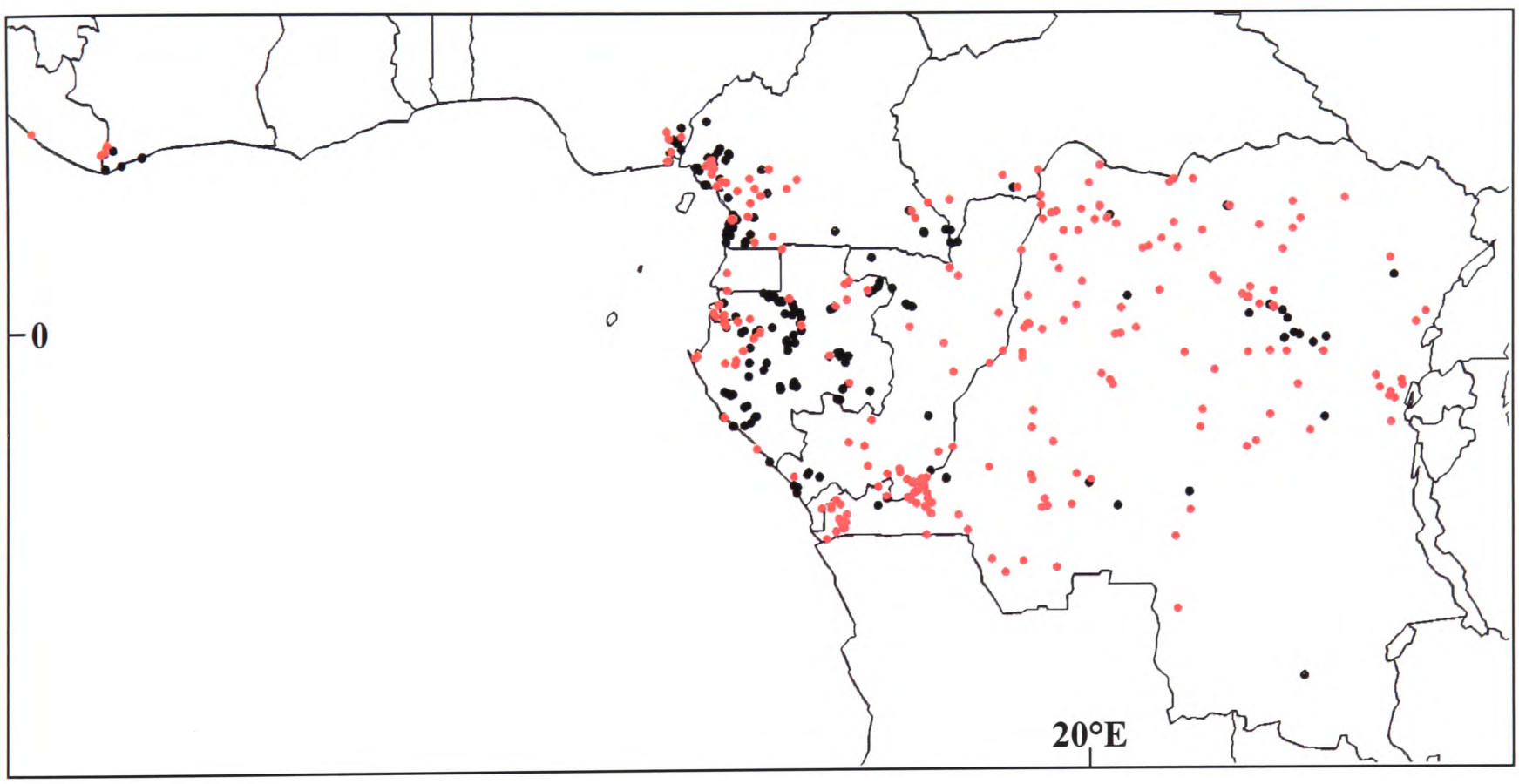


Figure 2.1 Map of western Africa to show distribution of *Thomandersia* collections made prior, and subsequent, to the most recent revision of the genus (Heine 1966a). Red points, collections taken up to and including 1965; black points, collections taken in 1966 and thereafter.

been a variety of attempts to conceptualise species, including the biological, cohesion, ecological, evolutionary, phenetic, and phylogenetic species concepts (Table 2.1); each has advantages and disadvantages in different situations. In plants, hybridisation, polymorphism within species and ongoing speciation can result in differing species circumscriptions depending upon which concept is employed.

Table 2.1 Species concepts.

Concept	Definition of species	Selected references	Usual application
Biological	A group of interbreeding natural populations that is reproductively isolated from other groups	Mayr (1942), May & Ashlock (1991)	Solitary, sexually-reproducing organisms (e.g. vertebrates)
Cohesion	The most inclusive population of individuals with the potential to limit its boundaries by basic micro-evolutionary forces (gene flow, natural selection and genetic drift)	Templeton (1989)	Asexually-reproducing organisms
Ecological	A lineage occupying a minimally different adaptive zone to other lineages	Van Valen (1976), Andersson (1990)	Ecology
Evolutionary	A single lineage of ancestor-descendant populations, which maintains its identity from other such lineages and has its own evolutionary tendencies and historical fate	Simpson (1961), Wiley (1978; 1981)	Palaeontology
Phenetic	The smallest group that is consistently distinguishable from other such groups	Du Rietz (1930), Sokal (1973), Doyen & Slobodchikoff (1974), Cronquist (1978)	Practical taxonomy
Phylogenetic	A cluster of organisms that is diagnosably distinct from other such clusters; the smallest monophyletic group of common ancestry	Cracraft (1989), De Queiroz & Donoghue (1990)	Phylogenetic systematics

Perhaps the most commonly applied are the biological, phenetic and phylogenetic species concepts. In most circumstances, the species delimited using these different concepts are very similar. For instance, groups that are so different that they cannot together produce fertile offspring (biological species) usually also differ in distinct phenotypic characters (phenetic species) and those characters are usually traits derived by descent (phylogenetic species).

In practice, the first and definitive step in operational species discovery is often the recognition of distinct phenetic groupings (Doyen & Slobodchikoff, 1974). Phenetically-similar groups can also be recognised as species on the basis of geographic, reproductive or ecological criteria (Winston, 1999) or genetic divergence (Palumbi & Cipriano, 1998; Wiens & Penkrot, 2001; Tautz et al., 2002; Hebert et al., 2003). In *Thomandersia,* the vast majority of specimens fall into distinct phenetic groups that have long been recognised as species (De Wildemann & Durand, 1898-1902; Durand & De Wildemann, 1899b; De Wildemann, 1905; 1906; Heine, 1966a). These are predominantly distinguished on gross floral morphological characters. Vegetative features may also be diagnostic, for instance in *T. butayei,* which has distinctively emarginate leaves (De Wildemann, 1906).

Describing infraspecific taxa is a more ambiguous process than describing species (Stace, 1986; Hamilton & Reichard, 1992). To be objective and consistent, infraspecific taxa should be based on a strong understanding of evolutionary processes within the species (Winston, 1999). Observed practice and suggested protocols for naming infraspecific taxa (Stuessy, 1990; Hamilton & Reichard, 1992) indicate that subspecies and varieties should be characterised by several conspicuous morphological differences and geographical, ecological or phylogenetic integrity. Morphological distinctness is a primary consideration in describing both subspecies and varieties (Hamilton & Reichard, 1992), but varieties tend to be less geographically or ecologically distinct than subspecies. Forms are also characterised by a distinct phenotype but may show no permanent geographic, ecological or phylogenetic separation from the rest of the species.

2.3 Taxonomic history of *Thomandersia*

2.3.1 Nomenclature. Collections now determined as *Thomandersia* were first described as a new genus in Acanthaceae in 1876 (Bentham, 1876). They were named *Scytanthus*, from a note made on one specimen (*Mann* 2321) by the collector Thomas Anderson. In 1891 *Scytanthus* was renamed *Thomandersia* because another genus, *Skytanthus* Meyen (Apocynaceae), already existed with which it could easily be confused (Baillon, 1891). In fact *Scytanthus* T.Anderson ex Benth. was also a later homonym of *Scytanthus* Hook (Rafflesiaceae), which was overlooked by Baillon (Heine, 1966a).

2.3.2 Suprageneric classification. Following Bentham, Lindau (1894; 1897) classified *Thomandersia* in Asystasieae (Acanthoideae, Imbricatae, Acanthaceae). In *Flora of Tropical Africa* it was transferred to Tetrandeae (Burkill & Clarke, 1899). The genus remained in Acanthaceae (e.g. Hutchinson & Dalziel, 1931) until Bremekamp, and later Raj, argued it should be transferred to Pedaliaceae, primarily on the basis of pollen morphology (Bremekamp, 1942; Raj, 1961).

In *Flora of Tropical West Africa, Flora of Gabon* and a revision of the genus, Heine (1963; 1966a; 1966b) maintained *Thomandersia* as a member of Acanthaceae. Sreemadhaven (1976; 1977) subsequently elevated *Thomandersia* to family status, largely on the basis of leaf anatomy. Sreemadhaven (1976) separated Acanthaceae into a number of smaller families including Justiciaceae, Meyeniaceae, Thunbergiaceae, Nelsoniaceae and Acanthaceae *sensu stricto,* which are all usually synonymised with Acanthaceae (e.g. Scotland & Vollesen, 2000).

2.3.3 Infrageneric classification. When it was first described, *Scytanthus* contained a single species, *S. laurifolius* T.Anderson ex. Benth., from tropical West Africa (Bentham, 1876; 1877). Bentham noted that there were two forms of *S. laurifolius*: a smaller one from Nigeria and a larger one, with less acuminate and more coriaceous leaves, from Cameroon (Bentham, 1877). In 1899 two new species, *T. congolana* De Wild. & T.Durand and *T. hensii* De Wild. & T.Durand, were described in Durand and De Wildemann's work for *Flore du Congo* (De Wildemann & Durand, 1898-1902; Durand & De Wildemann, 1899a). *T. congolana* was distinguished from the type species by its smaller size, characteristic habit and distinctively-patterned corolla (Durand & De Wildemann, 1899a). *T. hensii* differed from *T. laurifolia* by having pure white flowers and seeds with a scaly surface. However, it was noted that *T. hensii* and *T. laurifolia* were each very variable, and also very similar to one another, in their range of vegetative characters (Durand & De Wildemann, 1899a). In 1909 Durand treated *T. hensii* as conspecific with a more widely-circumscribed *T. laurifolia*, including two varieties (described below), under (Durand & Durand, 1909).

In 1905 De Wildemann described *T. laurentii* De Wild., which differed from existing species by its hairier leaves and velvety gynoecium and capsule (De Wildemann, 1905: 180). The following year he described a fifth species, *T. butayei* De Wild., primarily distinguished by its emarginate leaf apices (De Wildemann, 1906). The final species described to date was *T. anachoreta* Heine (1966a), with a disjunct distribution in west Africa and differing from the other species in having two calycine nectaries rather than one (Heine, 1966a).

In 1905 three infraspecific taxa were described (De Wildemann, 1905). *T. congolana* var. *grandifolia* De Wild. was described as having larger leaves than the typical *T. congolana.* However, even in the protologue, De Wildemann noted collections intermediate between the small- and large-leaved forms. *T. hensii* var. *longipetiolata* De Wild. was described as having larger leaves and longer petioles than other *T. hensii*, and *T. hensii* var. *latifolia* as having "leaves bigger in all proportions" (De Wildemann, 1905: 180). These two varieties are so similar, and leaf size in *T. hensii* so variable, that the varieties are usually "sunk" into the species (e.g. Heine, 1966a).

Most recently, Champluvier (pers. comm.) is proposing a new species, *T. collina* Champl., comprising material from the Democratic Republic of Congo, previously ascribed to *T. hensii* but inhabiting a different altitudinal zone to the rest of the species (*Bequaert* 49, 6670, *Cambridge Congo Expedition* 286, 351, *Christiaensen* 1877, *Gutzwiller* 1465, 2064, 2127, 2258, 2329, 2414, 2532, 3773, *Lebrun* 5618, *Leonard* 1689, 1873, 2896, *Pierlot* 627, 825, 2413, 2609, 3266, *Troupin* 2476, 3471, 3514, 3846, 4030, 4281, 4312, 9323). In habit and foliage characters these specimens are hard to distinguish from other *T. hensii.* Champluvier (pers. comm.) suggests they may be diagnosed by their more or less glabrous calyx and inflorescence rachis, pedicellate (rather than subsessile) flowers, non-overlapping corolla lobes, and glabrous corolla tube. Examination of *T. hensii* collections across the whole phenotypic and geographic range suggests that only the latter is a consistent, diagnostic character of the material. In other respects the specimens fall within the phenotypic range of other, lowland *T. hensii*, which is a very widespread and variable species. The main reason for distinguishing these collections is on the basis of its habitat, Afromontane and Piedmont forest, rather than the Guineo-Congolian lowland

rainforest where *T. hensii* is typically found. Therefore there does not seem to be unequivocal evidence for this new taxon, since a new species or subspecies should display several fixed morphological differences from existing taxa, especially where its distribution is parapatric with existing species (Stuessy, 1990; Hamilton & Reichard, 1992; Winston, 1999). A few collections (*Goossens* 4685, *Lebrun* 525, *Lejoly* 1610, 1686, 1774, 1780, 1831, 1833, *Lisowski* 41071, 44139) are intermediate in both character and locality between typical *T. hensii* and the proposed *T. collina*, indicating genetic and geographic overlap between the forms.

2.3.4 Discussion of the taxonomic account. The taxonomic account is based upon examination of 800 collections made available by the herbaria of BM, BR, FHO, K, MO, P, SCA, WAG and YA, in addition to field-based observations in Cameroon.

Thomandersia has proven to be a "good genus" – small, cohesive and easy to diagnose. The six species are easy to distinguish from one another, but many collections continue to be wrongly identified. For many years, all specimens were assigned to *T. laurifolia*, the first described species, or *T. hensii*, the most widespread. This is particularly true in the field, where vernacular names tend to be applied indiscriminately across all species (Bouquet, 1969). Additional confusion resulted from the widely-used *Flora of West Tropical Africa*, which included only a single species, *T. laurifolia*, and description which encompassed all characteristics of all species (Hutchinson & Dalziel, 1931).

Several ambiguous collections may represent hybridisation events or possible new species. The difficulty in classifying these specimens stems from the variable nature

of the most widespread species, *T. hensii,* and the overlap between its characters and those of other species, particularly *T. laurifolia.* To thoroughly investigate the extent of hybridisation in *Thomandersia* a genetic study should be conducted, for which sufficient material was not available. Based on phenotypic characters it seems that hybridisation between species is relatively rare, perhaps because the species generally inhabit separate habitats. However, a few collections (*De Wilde* 380, 9400, *Dowsett-Lemaire* 1371, *LaCroix* 4984, *Lejoly* 86/331, *Le Testu* 1213) display characters that appear intermediate between species, and come from regions where the species' distributions do overlap (Fig. 2.2), so are therefore considered possible hybrids.

One particular group of specimens (*Breteler* 6735, 6851, 12492, *Thollon* 212), from south-eastern Gabon, are all morphologically similar, having leaves with an obtuse-cuneate base and very prominent tertiary veins beneath, long, robust, densely-packed inflorescences, a pubescent rachis and calyx, corolla glabrous except at the edges of the lobes and large (up to 2 cm long), oblong, relatively thin-walled fruits, containing 4-6 seeds bearing roundish warts. These collections may be hybrids of *T. congolana* and *T. laurifolia* (unequivocal collections of both species exist from the same area), but could also represent a new species; there is not sufficient material to confirm or deny this here.

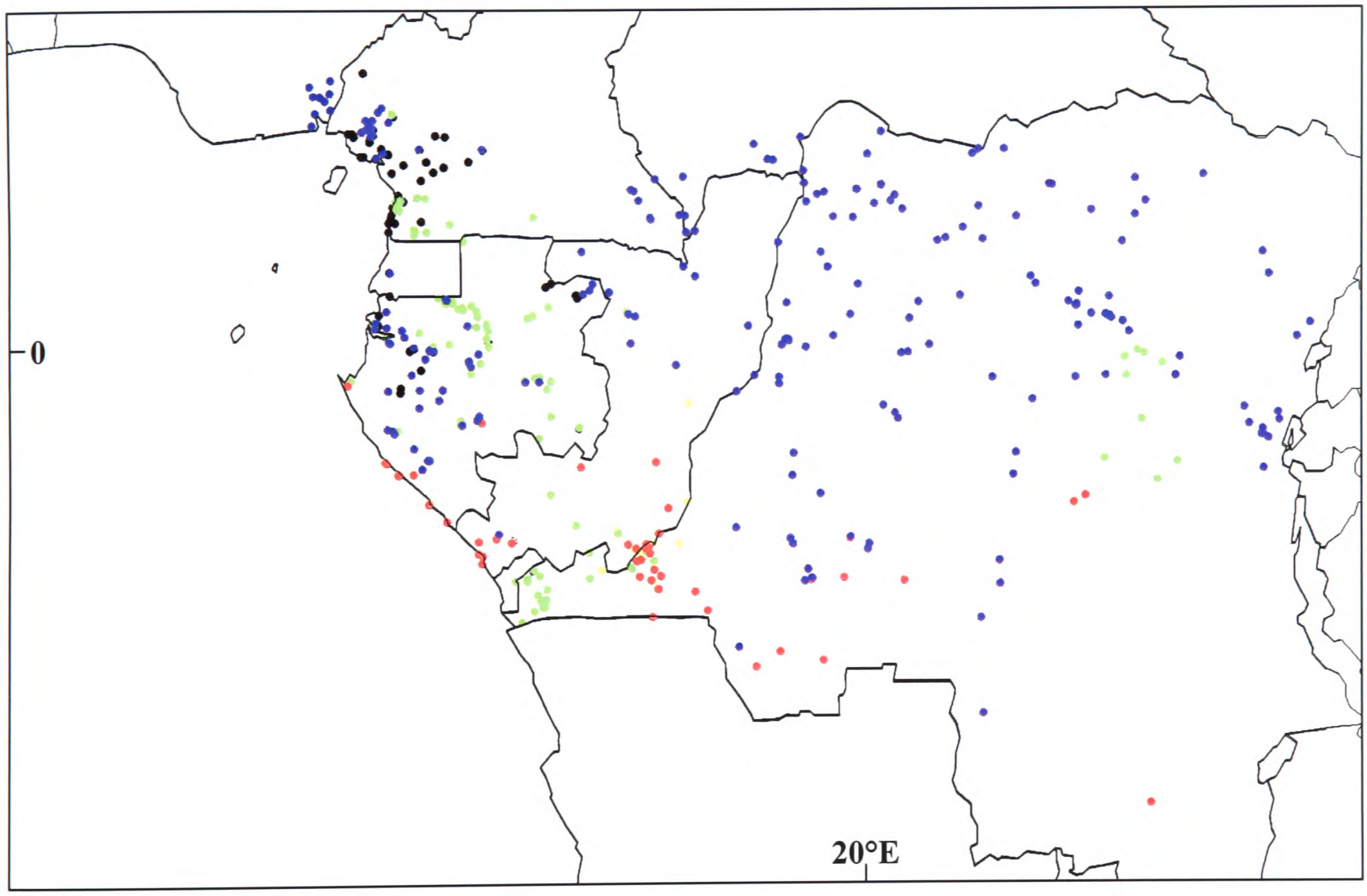


Figure 2.2 Map of western central Africa to show distribution of five species of *Thomandersia*, and overlap between their distributions. Red points, *T. butayei*; green points, *T. congolana*; blue points, *T. hensii*; yellow points, *T. laurentii*; black points, *T. laurifolia.*

In this taxonomic account I recognise the same six species as Heine (1966a). Each is distinguished by unique morphological characters. Despite this, forming a key to species was problematic because there are very few morphological characters that define groups of species within the genus. For this reason the key presented here relies on "chipping" species off one by one, rather than being evenly-dichotomous. This means the user must, on average, consult a greater number of couplets than in a more balanced key, but for only six species this is not a significant drawback. Since each species is quite distinctive the key can be based on more clear-cut characters, is easier to use and contains more information than one which groups the species first into pairs or threes.

To reduce as far as possible the amount of time spent interrogating the key, the most likely species to be encountered (due to their relative distribution and abundance) are presented first. It is recommended that the hardest species to distinguish be placed at the end of a key, reducing the probability of incorrect identifications (Metcalf, 1954; Tilling, 1984; Winston, 1999). For *Thomandersia* the two most similar species, when sterile, are *T. hensii* and *T. laurifolia*. However, these are also the two most common species and are very easy to distinguish when in fruit or flower, which is generally the case for most of the year, so they have been placed near the beginning of the key.

2.4 Taxonomic account

Thomandersia Baill., Hist. Pl., 10: 456 (1891); Lindau in Engl., Bot. Jahrb. Syst. 18: 55 (1894); Lindau in Engl. & Prantl, Nat. Pflanzenfam. 4(3b): 325 (1895); Burkill & C.B.Clarke in This.-Dyer, Fl. Trop. Afr., 5: 119 (1899); tom. cit. 512 (1900); Hutch. & Dalz., Fl. West Trop. Afr., ed. 1, 2: 255 (1931); Bremek., Rec. Trav. Bot. Néerl.,

39: 166 (1942); Raj, Grana Palyn., 3: 97-101 (1961); Heine in Hutch. & Dalz., Fl. W. Trop. Afr., ed. 2, 2: 413 (1963); Heine in Fl. Gabon., 13: 143-155 (1966); Heine, Bull. Jard. Bot. Etat, 36: 207-248 (1966); Sreem., University of South Florida Ph.D. thesis (1976); Sreem., Phytologia 37: 413-416 (1977). Type species: *T. laurifolia* (T.Anderson ex Benth.) Baill., Hist. Pl., 10: 456 (1891).

Scytanthus T.Anderson ex Benth. in Benth. & Hook.f., Gen. Pl. 2: 1093 (1876); Benth., in Hooker's Icon. Pl., 13: 8 (1877).

Slender shrubs, small trees or occasionally lianas, 0.5-15 m in height; without latex. *Stems* terete, glabrous or downy when young; nodes flattened, without stipules or articulation. *Petioles* bent and swollen at base and tip, becoming constricted on drying. *Leaves* opposite, decussate, simple, usually markedly petiolate, occasionally subsessile, more or less anisophyllous; lamina oblong-elliptic, obovate, occasionally orbicular or lanceolate, acuminate or emarginate at tip and cuneate-rounded at base, the smaller leaf of each pair sometimes cordate, more or less leathery, dotted irregularly with non-pellucid glands, the two surfaces discolorous, upper surface glabrous or with scattered hairs, lower surface glabrous or downy, particularly on veins; margin entire or with a few blunt teeth, usually towards the apex; midrib prominent below and impressed above, secondary venation pinnate, tertiary venation reticulate; tuft domatia often present in the axils between the secondary veins and midrib. *Inflorescences* erect terminal or axillary racemes, dense and pyramidal or more or less lax. *Flowers* subsessile-pedicellate, arranged irregularly, opposite or in whorls of three. *Bracts* small (c.1 mm), triangular-subulate, hairy. *Bracteoles* minute, subulate, ciliate, borne on the calyx. *Calyx* campanulate, with five short, triangular

lobes, green, purple or brown, fleshy, glabrous or hairy, margin ciliate, persistent and accrescent, abaxially swollen into one or two conspicuous bumps each with one or two more or less obvious ostioles. *Corolla* 10-20 mm long, zygomorphic, bilabiate, greenish, white, yellow, orange, red, pink or purple, sometimes with darker stripes, slightly leathery; tube glabrous or pubescent, adaxially slightly gibbous at base; upper lip two-lobed, lower lip three-lobed, more or less concave or with the lateral lobes spreading; lobes glandular-hairy with entire, sometimes undulate margin. *Staminode* single, adaxial, minute, subulate. *Stamens* four, didynamous, alternating with the corolla lobes, included or slightly exserted; filaments fused to the corolla tube, glabrous, glandular or hairy; anthers bithecate, dorsifixed, dehiscence extrorse via longitudinal slits. *Pollen* ellipsoid-spheroid, flattened at poles, punctate, penta- or hexacolpate. *Style* single, apical, solid. *Stigma* cylindrical or bilobed. *Gynoecium* superior, eusyncarpous, bilocular, median, sessile, swollen with nectariferous tissue at base (but annular disk absent), glabrous, glandular or with appressed hairs; placentation axile; ovules 2-3 per locule, surrounded by an expanded placenta. *Capsule* subglobular-ellipsoid, conical or ovate, dry, woody, brown-black when mature, glabrous, glandular or downy, subtended by the accrescent calyx, with walls up to 1.5 mm thick, dehiscing loculicidally into two valves, each containing 1-3 seeds. *Seeds* black, brown, orange, yellow or cream, globular or ovate-ellipsoid, 2-5 mm in diameter; testa covered with triangular scales, or spirally-arranged, rounded warts, subtended by a flattened, elongated, extension of the funicle (retinaculum), to 2 mm wide and 5 mm long.

Distinguished from other Lamiales, except Acanthaceae, by large abaxial calycine nectaries and retinacula; from Acanthaceae by stems not articulated, lack of cystoliths and acicular crystals, bracts and bracteoles minute, capsule dehiscence not explosive.

Distribution. Western and central Africa: southern Côte d'Ivoire and Liberia, south-eastern Nigeria, southern Cameroon, southern Central African Republic, Equatorial Guinea, Gabon, Republic of Congo, Democratic Republic of Congo.

Habitat. Understorey of primary or secondary evergreen, semi-deciduous, mixed, wet, dry, gallery, riverine, coastal, flood-plain, plateau and upland forest or forest remnants, especially at forest edges, in clearings, disturbed areas and by roadsides (Fig. 2.3). Also on coasts, mountains or ravines, by rivers or in periodically-flooded places, on fallow land, shady savannah or scrub. On loamy, rocky, lateritic, clayish, sandy, limestone, granite or iron-rich soils. Altitude: 0-1,000 m.

Notes. The habit of *Thomandersia* is very variable, including small trees, shrubs and lianas, even within the same species (Fig. 2.3). *Thomandersia* can be distinguished when sterile by its strongly anisophyllous leaves borne on long petioles (except *T. congolana*) and stems with characteristically long, leafless internodes (up to 0.5 m in some specimens). In damp areas it may also produce small stilt roots (*Wortley* 3).

Thomandersia is a significant component of the flora of western and central Africa. It is often abundant where found, sometimes forming dense clumps or thickets by adventitious rooting from nodes and sprouting from damaged stumps (Fig. 2.3C). *Thomandersia* is selectively consumed by bongos (*Tragelaphus eurycerus*) in the Central African Republic (Klaus-Hugi et al., 1999), and *T. hensii* (often misidentified



Figure 2.3 Variation in habit and habitats of *Thomandersia.* A. *T. c.f. laurifolia* shrublet, primary forest understorey (Cameroon). B. *T. hensii* tree, edge of forest (Central Africa Republic; image supplied by D. Harris). C. *T. hensii* re-sprouting from fallen stems, old felling area (Cameroon). D. *T. laurifolia* treelet, old rubber plantation (Cameroon).

as *T. laurifolia*; D. Harris, pers. comm.) is the most commonly recorded species in the diet of forest elephants (*Loxodonta cyclotis*) in the Republic of Congo (Blake, 2003). Chimpanzees (*Pan troglodytes troglodytes*) in the Republic of Congo use branches or young saplings of *T. hensii*, in preference to any other plant species, to perforate termite mounds before "fishing" for termites (Suzuki et al., 1995).

Ants have been observed visiting the distinctive calycine nectary (*Bates* 324, *Wortley* 4; Fig. 2.4A). Nectar secretion begins when flower buds are quite immature, and continues for some time. The corolla tube of *T. hensii* often bears punctures suggestive of nectar-robbing (e.g. *Troupin* 9323, *Wortley* 5; Fig. 2.4B) (Inouye, 1980). The effect of nectar robbing on reproductive success is debatable and varies between species (Maloof & Inouye, 2000; Irwin & Maloof, 2002). Its effect in *Thomandersia* has not been assessed, but it is notable that aborted ovules are common and seed set often low, the majority of inflorescences bearing large numbers of small, empty fruits and a few ripe, fertile ones.

Uses. Although two collectors (*Fleury* 33368, in Cameroon; *Montchal* 192, in Democratic Republic of Congo) report that *Thomandersia* is not used by humans, numerous uses have been recorded by other collectors, most often relating to *T. hensii.* The wood of this species is very hard, suitable for poles, stakes and screens (Burkill, 1985), and is used for building construction in the Democratic Republic of Congo (*Malchair* 67). *T. hensii* is also planted in villages as an ornamental (*Jespersen* 24), and because it is believed to provide the village chief with protection and success in hunting leopards (*Briemeyer* 30). A branch is often hung inside huts to bring fortune in hunting (Heine, 1966a; Bouquet, 1969; Burkill, 1985). In the Republic of

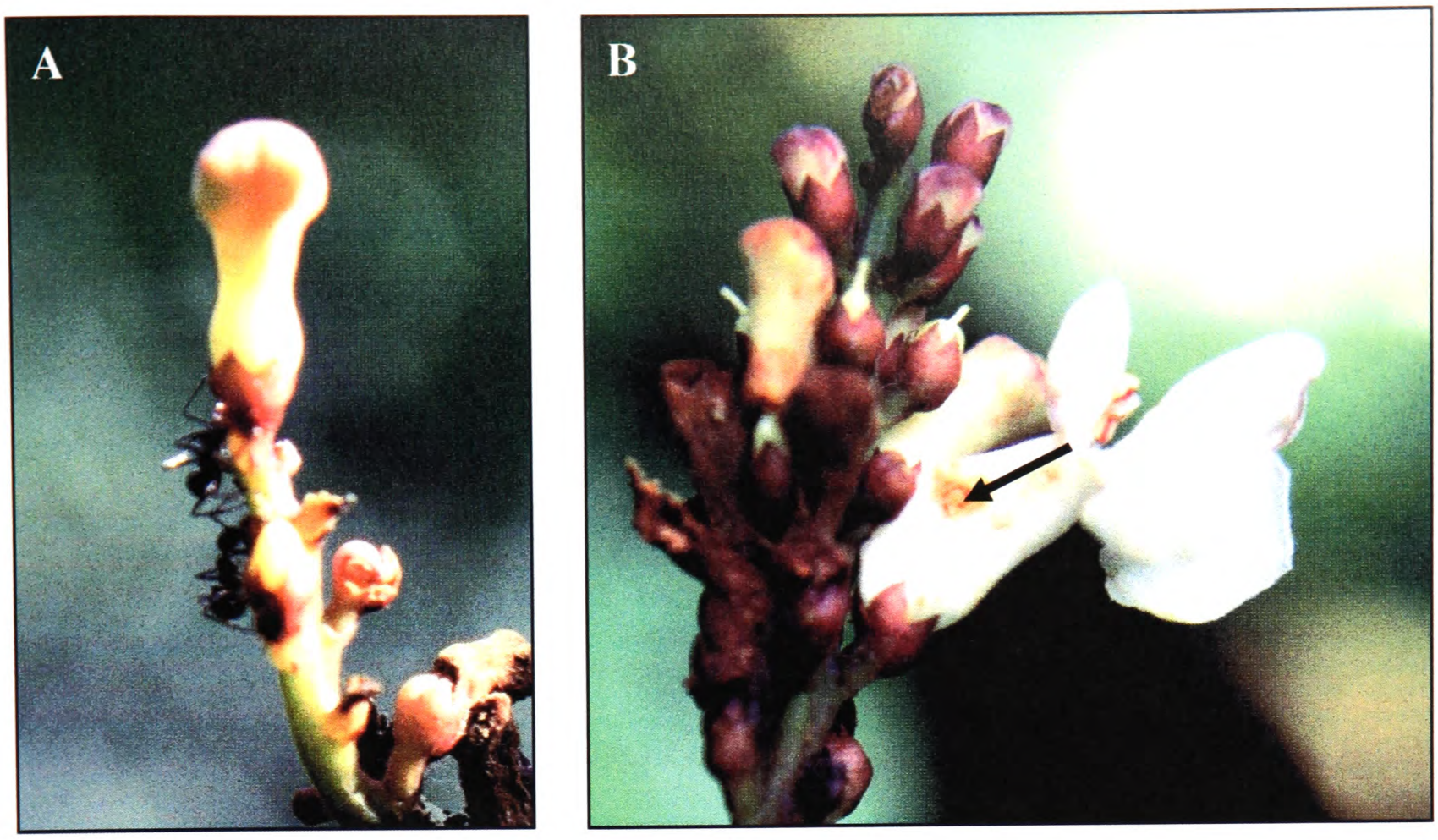


Figure 2.4 Ecological interactions of *Thomandersia hensii*. A. nectar-seeking ants on calyx. B. corolla, showing hole pierced by nectar robbing insect (arrowed).



Figure 2.5 Postage stamps released in Gabon, 1972. A. *T. hensii.* B. *T. laurifolia* ( ).

Congo, the tree is believed to discourage demons, and drinking the sap is thought to exorcise evil spirits and heal illnesses caused by sorcery (Bouquet, 1969; Burkill, 1985). Stems of *T. hensii* are also used in ritual ceremonies (Bahuchet, 1993). In Gabon, the importance of *T. laurifolia* and *T. hensii* was recognised in 1972 with the release of two postage stamps bearing their illustrations (Fig. 2.5).

T. hensii is reported to produce sap that is very irritant on contact with the skin (*Daramola* 93); nonetheless *Thomandersia* has numerous traditional medicinal uses throughout its range. Walker's collecting notes from Gabon suggest that no distinction is made between *T. hensii* and *T. congolana* in traditional medicine. Bouquet (Bouquet, 1969) also states that all native species of *Thomandersia* are employed in medicine and sorcery in the Democratic Republic of Congo, and are all known by the same vernacular names. Since they are interchangeable, the medicinal uses recorded for all species are here described together.

Principle medicinal applications of *Thomandersia* include the treatment of female infertility, infectious genito-urinary diseases, dysentery and intestinal parasites (Bouquet, 1969). The leaf sap is believed to be particularly effective as a vermifuge when mixed with that of *Haumania danckelmaniana* (Marantaceae) and *Solanum nigrum* (Solanaceae) (Bouquet, 1969).

In the Democratic Republic of Congo, *Thomandersia* is widely used as a tonic for general fatigue (Bouquet, 1969). The leaves are thought to contain an antivenin to counteract snakebite (Szafranski et al., 1991), and are used to treat colic (*De Giorgi* 59, 1026, 1206, 1325), constipation (*De Giorgi* 1483), diabetes and heart disease

(Szafranski et al., 1991), rheumatism (*De Giorgi* 571), scabies (Szafranski et al., 1991), syphilis (*Couteaux* 150), sterility (Szafranski et al., 1991), worms (*De Giorgi* 442, *De Wulf* 399, *Reygaert* 34) and other abdominal pains and disorders (*Kitembo* 65, *Pynaert* 911). The leaf sap is used externally to treat inflammation (Burkill, 1985), boils, abscesses (*Louis* 1978), fungal infections, venereal ulcers and syphilis sores (Heine, 1966a; Bouquet, 1969; Szafranski et al., 1991). The root sap is indicated in the treatment of eye and ear infections (Bouquet, 1969; Szafranski et al., 1991). The leaves, bark and roots are used in the Central African Republic (Szafranski et al., 1991) against intestinal worms (*Leeuwenberg* 7059), smallpox, and as an enema to treat diarrhoea (Heine, 1966a). In Cameroon, *Thomandersia* leaves are applied as a massage for rheumatism (*Fleury* 33345) and to treat fevers (FAO, 1999). In Gabon *Thomandersia* is indicated in the treatment of yaws (*Walker* s.n.).

Thomandersia leaves are considered to have antiseptic properties (Heine, 1966a). Bouquet (1969) records that, in the Democratic Republic of Congo, open sores can be treated first by packing with crushed, dried leaves and then by washing the wound with a decoction of the whole plant. Heine (1966a) considered that the secondary chemical properties of *Thomandersia* were worth investigating and this has proved to be the case. Four previously-known terpenoid compounds, plus a new terpenoid, thomandertriol, have been isolated from the stems of *T. laurifolia*. Two new alkaloids, thomandersine and isothomandersine, were also found in the leaves (Ngadjui et al., 1994; Ngadjui, 1995). In a study of plants from the Democratic Republic of Congo, *T. hensii* leaves were found to have the highest chemical deterrent activity against insects, of all species tested (Szafranski et al., 1991).

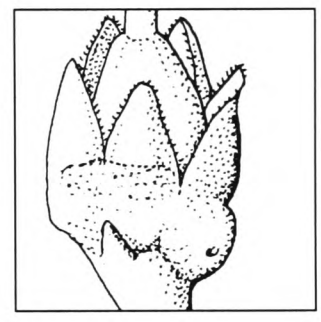
Key to species

1a. Two abaxial glandular swellings at base of calyx (see drawing); capsule slightly downy; seeds creamy yellow; Liberia or Côte d'Ivoire.....*T. anachoreta*

1b. Single abaxial glandular swelling at base of calyx (see drawing); capsule glabrous or with appressed yellow hairs; seeds yellow-orange, brown or black; not Liberia or Côte d'Ivoire.....2



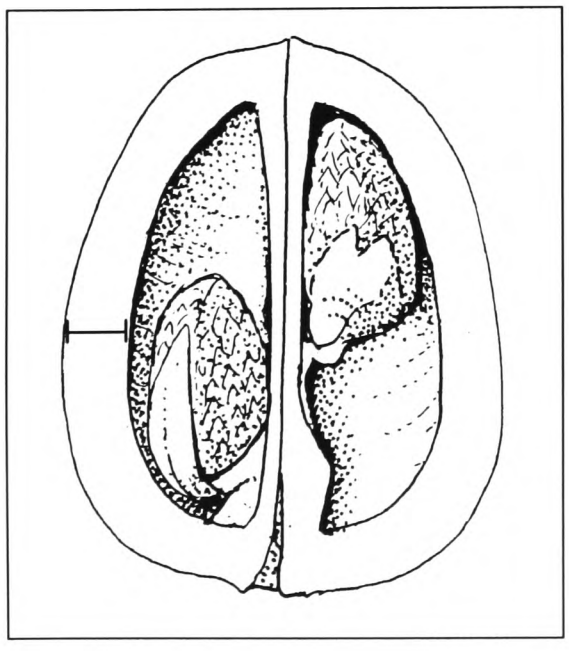
1a



1b

2a. Corolla lobes white; capsule wall at least 1 mm thick (see drawing); inflorescence rachis at least 2 mm thick at widest point; leaves large (often at least 20 cm long) and inflorescence dense, often pyramidal at tip.....*T. hensii*

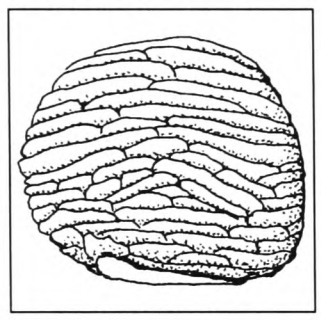
2b. Corolla lobes yellow, green, pinkish, reddish or purplish; capsule wall up to 1 mm thick; inflorescence rachis up to 2 mm thick at widest point; if leaves greater than 20 cm long, inflorescence lax and not pyramidal.....3



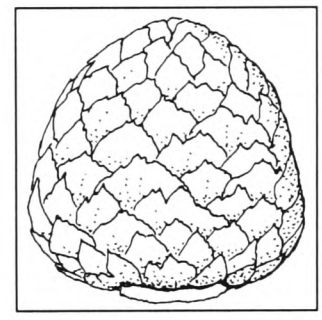
measurement of capsule wall thickness

3a. Inflorescence up to 45 cm long, lax and elongate; seeds with spiral pattern of warts (see drawing), black or dark brown; leaves often large (sometimes at least 20 cm long).....*T. laurifolia*

3b. Inflorescence not more than 16 cm long, more or less dense; seeds with triangular scales (see drawing), yellow-brown; leaves up to 20 cm long.....4



3a



3b

4a. Corolla striped brown-red inside; petiole not more than 20 mm long; gynoecium with glandular trichomes.....*T. congolana*

4b. Corolla lobes not striped; larger leaf of each pair with petiole 10-50 mm long; gynoecium glabrous or with appressed yellow pubescence.....5

5a. Leaf apex emarginate; gynoecium glabrous; capsule glabrous; leaves up to 8 cm long; capsule wall at least 0.5 mm thick.....*T. butayei*

5b. Leaf apex cuspidate; gynoecium covered with short, furry yellow hairs; capsule with short, yellow, appressed hairs; leaves usually more than 5 cm long; capsule wall less than 0.5 mm thick.....*T. laurentii*

Thomandersia anachoreta Heine, Bull. Jard. Bot. Etat 36: 239-241 (1966). Type: Côte d'Ivoire, San Pédro, 20 February 1955, *Aké Assi* 2823 (holotype P, isotype ABI).

Treelet, shrublet or scrambling shrub, 0.5-15 m in height. *Stems* brownish or purplish-green, with dense, short, pale pubescence when young, becoming glabrous. *Petioles*

densely pubescent when young, that of larger leaves 15-30 mm long, of smaller leaves 2-5 mm. *Leaves* markedly anisophyllous; lamina, of the larger of each pair 110-160 x 20-70 mm, oblong-elliptic, ovate or obovate, apex long-acuminate, cuspidate, base cuneate-rounded-obtuse, with 4-6 pairs of lateral veins, of the smaller of each pair 20-80 x 15-40 mm, ovate-cordiform-suborbicular, apex long-acuminate, cuspidate, base rounded-(sub)cordate, with 3-4 pairs of lateral veins, leathery, with a few round glands (1 mm or more in diameter), especially towards the midrib, bright green above, paler below, glabrous or with short, sparse, pale hairs on veins and margin above, glabrous or with a few scattered longer hairs below, veins above and below with short, pale pubescence; margin entire, occasionally slightly undulate or with large, well-spaced, cuspidate serrations, concentrated towards the apex; midrib embedded above, prominent beneath, tertiary venation reticulate; tuft domatia occasionally present in the axils between the lateral veins and midrib beneath. *Inflorescence* terminal, occasionally axillary, 80-150 mm long, dense, robust and elongate, sometimes slightly curved; rachis and pedicels greenish-brown with dense, pale pubescence. *Bracts* tiny (1-2 mm), ovate-triangular-lanceolate, often a little backward-curving, hairy. *Bracteoles* minute, lanceolate, hairy. *Flowers* arranged irregularly, in whorls or occasionally opposite, pedicellate-subsessile; pedicels 1-2.5 mm long; buds brown-purplish. *Calyx* 2-3 mm long, campanulate-urceolate, greenish-brown-black, bearing conspicuous paired abaxial glands, 1 mm in diameter, black, in round, more or less distinct bumps, with visible ostioles; lobes 0.5-1 mm long, ovate-triangular, pointed, overlapping, downy, with darkly ciliate margin. *Corolla* thick and leathery, bilabiate; tube 12-15 mm long, yellow, with many long, spreading hairs inside; lobes yellowish, greenish or pinkish, 7-8 mm long, with dense, short, glandular hairs inside and out, lateral lobes spreading, margin entire or slightly crinkled, ciliate.

Staminode tiny (<2 mm), awn-shaped, inserted c.2 mm from base of corolla tube, yellow. *Stamen* filaments inserted c.4 mm from the base of the tube, the shorter pair 4 mm long, the longer pair 5 mm, with long, spreading hairs at base and short, glandular hairs towards the top; anther locules equal, 1-1.5 mm long. *Style* 4-6 mm long, 0.5 mm in diameter, yellow, glabrous, slightly glandular, soon caducous. *Stigma* cylindrical, cup-shaped, slightly broader than the style. *Gynoecium* 2.5-4 mm long, conical-pyramidal, slightly flattened or pointed and glandular at tip, pale brown-orange, glabrous, fleshy. *Capsule* 10-20 x 6-8 x 2-3 mm, ellipsoid-ovoid, sometimes made slightly pear-shaped by irregular constrictions, brown, slightly downy, with thin (<0.5 mm) wall and pointed retinacula; subtended by the accrescent calyx, 4 mm diameter, star-shaped, the adaxial lobe larger than the rest. *Seeds* two per valve, 4 x 3 x 3 mm, irregularly spheroidal-subcuboidal, cream-yellow, covered with rounded-triangular, overlapping scales or warts (Illustration: Fig. 2.6).

Distribution. Southern Côte d'Ivoire and Liberia (Fig. 2.7).

All other collections seen. **Côte d'Ivoire.** Bereby, 11 October 1973, *De Koning* 2364 (WAG); Grabo, 10 November 1964, *Aké Assi* s.n. (MO), 22 May 1968, *Aké Assi* 10143 (P); Mahino, 4 March 1965, *Aké Assi* 7924 (P); Monogaga, 2 April 1968, *Geerling & Bokdam* 2436 (BR, K, MO), 12 April 1973, *De Koning* 1413 (WAG); San Pedro, 5 December 1997, *Jongkind* 4154 (WAG); Sassandra, 21 August 1956, *De Wilde* 323 (WAG); Tabou, 5 May 1999, *Jongkind & students of the University de Cocody, Abidjan* 4504 (WAG), 11 April 2000, *Jongkind & Assi-Yapo* 5065 (WAG). **Liberia.** Cestos River, 9 March 1948, *Baldwin* 11264 (K, MO); Nyaake, 23 June 1947, *Baldwin* 6151 (K).



Figure 2.6 *Thomandersia anachoreta.* A. habit and inflorescence. B. underside of leaf. C. bud. D. floral dissection. E. calyx. F. fruit and seed. G. seed. (A - E from *Jongkind* 4504; F, G from *Aké Assi* 2823).

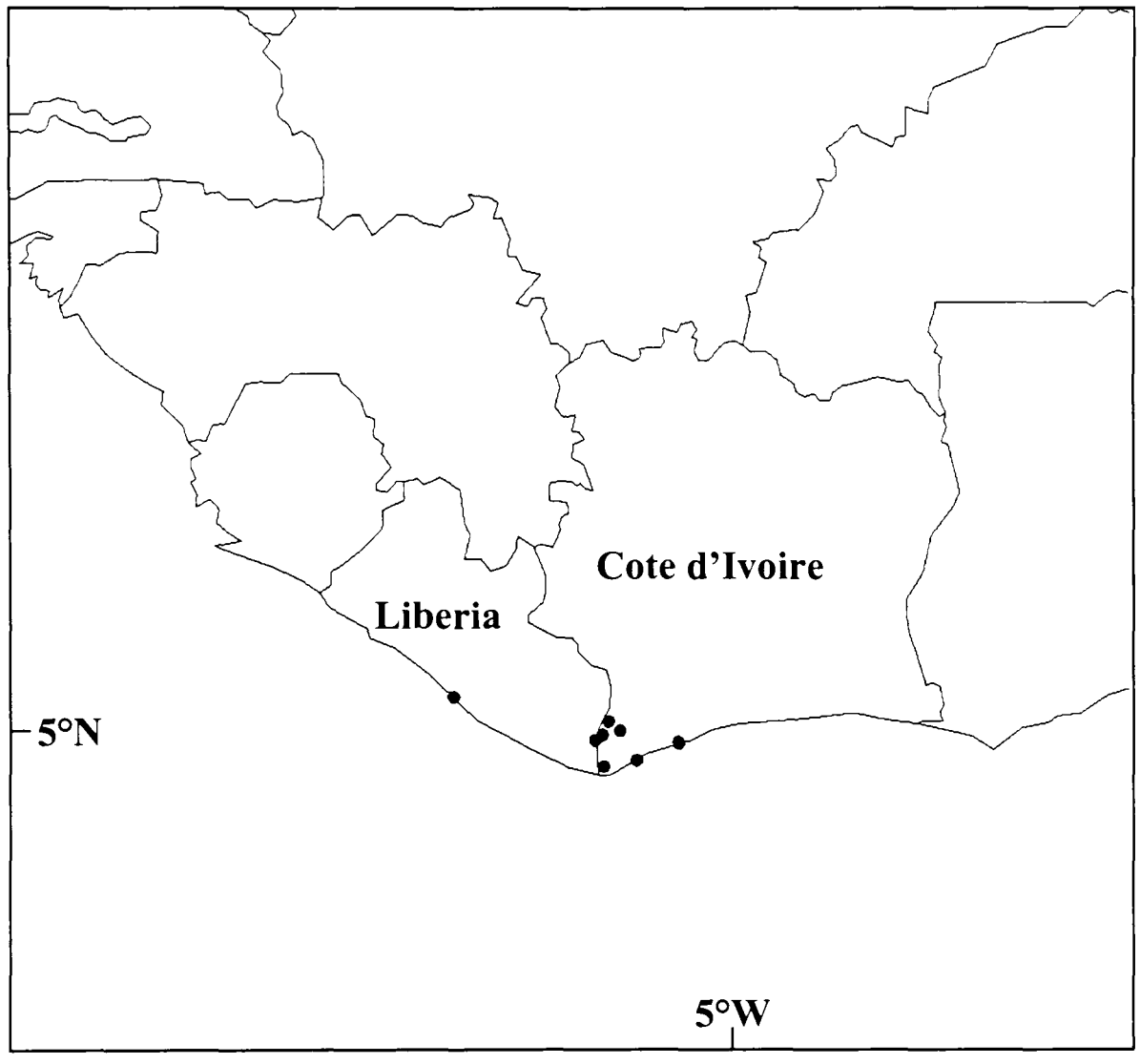


Figure 2.7 Map of West Africa to show distribution of collections of *Thomandersia anachoreta.*

Habitat. Coastal, wet and disturbed forests and forest edges, on loamy or rocky soils.

Thomandersia butayei De Wild., Ann. Mus. Congo, ser. 5, Bot. 1: 312 (1906); op. cit. ser. 3, 3: 272 (1910); Hutch. & Dalz., Fl. W. Trop. Afr., 2: 255 (1931); Heine in Fl. Gabon., 13: 143-155 (1966); Heine, Bull. Jard. Bot. Etat, 36: 207-248 (1966). Type: Democratic Republic of Congo, between Kisantu and Kwango, September 1900, *Butaye* in *Gillet* 1480 (holotype BR).

Shrub or small tree, 1-5 m in height. *Stems* slender, pale brown, with dense, short, pale pubescence when young, becoming glabrous; bark brown, shiny, with irregular flakes. *Petioles* with dense, pale pubescence, that of larger leaves 10-35 mm long, of smaller leaves 3-10 mm. *Leaves* anisophyllous; lamina, of the larger of each pair 25-80 x 15-40 mm, ovate-elliptic, apex emarginate, base cuneate-rounded or subcordate, with 2-4 pairs of distinctly anastomose lateral veins, of the smaller of each pair 10-40 x 8-25 mm, ovate-cordiform-orbicular, apex emarginate, base rounded-cordate, with 1-3 pairs of lateral veins, papery, with small (<0.5 mm diameter) round glands, glossy green above, paler below, glabrous or with a few sparse hairs, especially on midrib above, glabrous or with scattered pale hairs below, the veins below usually pubescent, sometimes glabrous; margin entire or with 4-6 large, emarginate lobes, almost as deep as the undivided part of the lamina, spaced around the leaf margin, with a vein reaching the margin at the tip; midrib embedded above, prominent beneath, lateral venation embedded below, tertiary venation reticulate; leaves often folded up along midrib when dry; tuft domatia very pronounced, pale brown-white, in the axils of the lateral veins and sometimes the smaller veins beneath. *Inflorescence* occasionally terminal, usually axillary, 30-100 mm long, lax and curvy, sometimes dense but not

pyramidal; rachis and pedicels red-brown, with dense pubescence. *Bracts* tiny (<1 mm), triangular-lanceolate, hairy, bent slightly upwards or held at a right-angle to the rachis. *Bracteoles* minute, triangular-ovate, appressed against the calyx, ciliate at edge. *Flowers* arranged irregularly, opposite or alternate, pedicellate; pedicels 1.5-3 mm long; buds pale. *Calyx* 1.5-2.5 mm long, campanulate, green, bearing a single, shiny, glandular swelling up to 0.5 mm deep; lobes 0.5-1.5 mm long, oblong-elliptic, rounded, overlapping, slightly glandular-pubescent, margin ciliate. *Corolla* bilabiate; tube 6-8 mm long, greenish or yellowish-white, sometimes tinged pink or purple, glabrous outside; lobes similar in colour, 4-6 mm long, lateral lobes spreading when fully open, margin undulate or crinkled, with pale pubescence; lobes and inside of the tube covered with glandular hairs, especially in bud. *Staminode* tiny (1 mm), subulate, inserted c.2 mm from base of corolla tube, glabrous. *Stamen* filaments inserted c.5 mm from the base of the tube, more or less equal, 3.5-4 mm long, with a few glandular hairs at base; anther locules equal, 1-1.5 mm long. *Style* c.6 mm long, 0.2 mm in diameter, slightly hairy. *Stigma* cylindrical, slightly hairy. *Gynoecium* c.3 mm long, ovoid, glabrous, fleshy. *Capsule* 8-12 x 5-8 x 3-8 mm, ovoid-ellipsoid or subconical, greenish-brown or black, glabrous, with fairly thick (0.7-1 mm) wall; subtended by the accrescent calyx, to 5 mm diameter, star-shaped, ribbed, spreading. *Seeds* 1-2 per valve, 2-4 x 2-4 x 1-3 mm, almost spheroidal, flattened at base, yellow-orange or light brown, covered with dense, sharp, triangular scales (Illustration: Fig. 2.8).

Distribution. Gabon, Democratic Republic of Congo, Republic of Congo (Fig. 2.9).

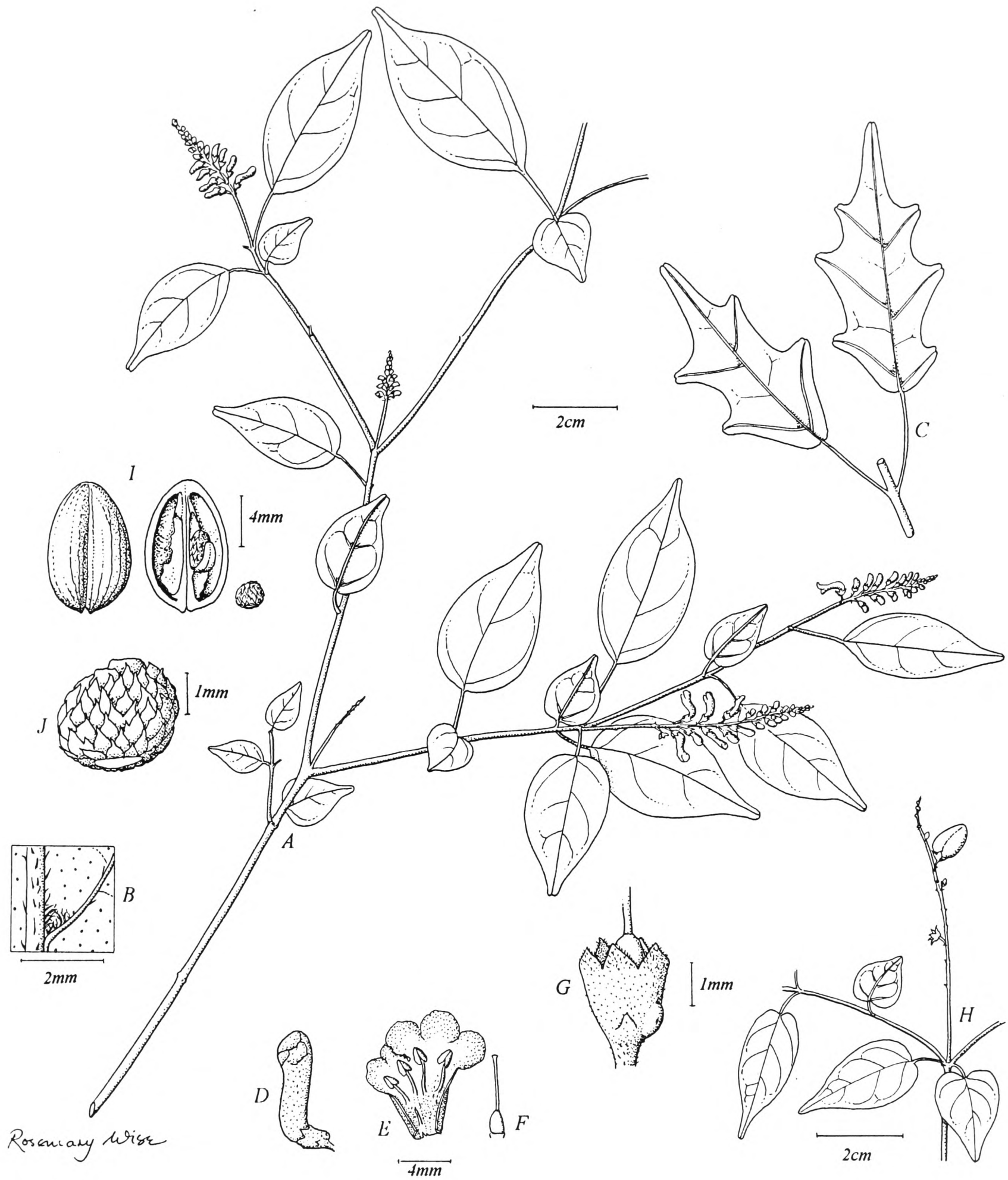


Figure 2.8 *Thomandersia butayei.* A. habit and inflorescence. B. underside of leaf. C. foliage showing most extreme form of toothed leaves. D. bud. E. floral dissection. F. gynoecium. G. calyx. H. infructescence. I. fruit and seed. J. seed. (A, B, D – H from *Moutsambote* 4520; C from *Lisowski* B8013; I, J from *McPherson* 16790).

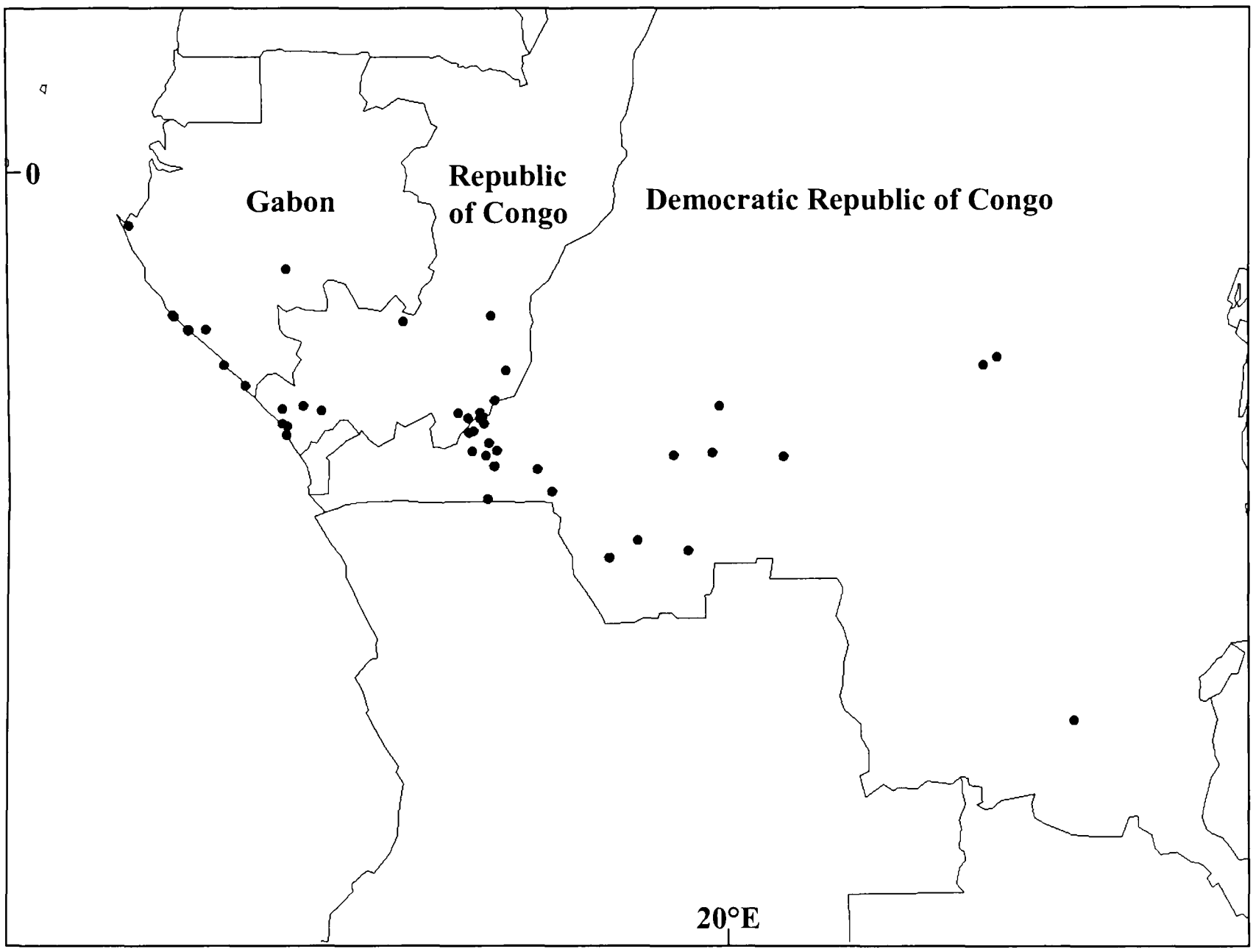


Figure 2.9 Map of western central Africa to show distribution of collections of *Thomandersia butayei.*

All other collections seen. **Democratic Republic of Congo.** Babunda, March 1921, *Vanderyst* 8958 (BR); Banga-Boma, May 1932, *Vanderyst* 31502 (BR); Bas-Congo, September 1932, *Vanderyst* 34739 (BR), 14 April 1944, *Germain* 2073 (BR, K, P), 8 April 1959, *Pauwels* 2210 (BR), *Vanderyst* 17692 (BR); Benga, *Vanderyst* 15437, 19184 (BR); Haute-Mele, January 1925, *Vanderyst* 14318 (BR); Ile des Mimosas, 5 June 1966, *Breyne* 135 (BR, MO); Ipamu, July 1921, *Vanderyst* 9958, 9722 (BR); Kasanguli, 24 July 1996, *Pohl* ZRE100 (BR); Katako-Kombe, 22 March 1937, *Gillardin* 235 (BR, K); Kayo, 1 September 1990, *Moutsambote & Dowsett-Lemaire* 4520 (BR, K, MO); Kebama, 25 August 1959, *Pauwels* 4269 (BR, MO); Kidima, 19 February 1932, *Callens* 3478 (BR); Kimuisi, June 1954, *Callens* 4601 (BR); Kinbongo, 28 October 1958, *Pauwels* 456 (BR); Kinkasi, 20 October 1952, *Callens* 3723 (BR); Kinkoko, 20 April 1960, *Compere* 1925 (BR); Kinkombo, 20 April 1960, *Compere* 1928 (K); Kinshasa, 3 April 1915, *Bequaert* 7252 (BR); Lac de ma Vallee, 14 July 1970, *Breyne* 774 (BR); Lodja, July 1932, *Lebrun* 6168 (BR); Lufuna, 3 August 1944, *Germain* 2601 (BR, K); Luvanium, 10 June 1967, *Breyne* 461 (BR); Maynda-Mukoko, 10 January 1926, *Gillet* s.n. (BR); Mikope, 12 May 1978, *Dumont* 188 (BR); Mofinu, 18 December 1969, *Breyne* 706 (BR); Mpese, December 1948, *Callens* 1983 (BR); Ndjili, 5 March 1965, *Pauwels* 4914 (BR); Palmeraie, 10 August 1991, *Masens* 857 (BR); Selembao, 31 July 1996, *Pohl* ZRE274 (BR); Yubi, June 1920, *Pobeguin* 238 (P), 10 August 1947, *s.c.* s.n. (P). **Gabon.** Bame, 12 May 2001, *Stone, Walters, Nzabi & Mboumbore* 3270 (BR); Gamba, 23 September 1968, *Breteler & Van Raalte* 5663 (BR, MO, WAG), 15 June 1992, *Wieringa* 1170 (WAG), 21 November 1994, *De Wilde & De Wilde-Bakhuizen* 11134 (WAG); Mayumba, 13 November 1907, *Le Testu* 981 (P); Mbigou, 5 December 2001, *Wieringa, Nzabi & Boussiengui* 4603 (WAG); Mondorobe, June 1921, *Pobeguin* 59 (P); Rembo, 23

March 1991, *Van Nek* 763 (WAG); Sette Cama, 3 February 1894, *Dybowski* 75 (P), 22 April 1997, *McPherson* 16790 (MO); Yenzi, 19 February 1994, *Van der Burgt* 36 (WAG). **Republic of Congo.** Bas-Kouilou, 25 May 1986, *De Foresta* HF994 (BR, P); Boungoulo, 6 November 1965, *Bouquet* 1931 (P); Boutsouka-Tonkama, June 1964, *Bouquet* 31 (P); Brazzaville, 11 February 1960, *Sita* 188 (P); Dimonika, 25 November 1978, *Cusset* 547 (BR, P); Djili, 22 May 1963, *De Nere* 1228 (MO, P); Kakamoeka, 11 October 1990, *Lisowski* B8013 (BR, MO, WAG); Kiougna, 18 December 1922, *Sargos* 168 (BR, P); Lefini, 12 October 1991, *Thomas & Harris* 8528 (MO); Mbamou, 3 August 1912, *Chevalier* 27664 (P); Mbanzanguedi, 20 July 1964, *Bouquet* 251 (P); Pointe Indienne, 3 February 1966, *Farron* 4939 (P); Pool, s.d, *Pobeguin,* s.n. (P); Reneville, 31 July 1912, *Chevalier* 27427 (BR, P); Tchissanga, 3 October 1990, *Lisowski* B6966 (BR, MO, WAG); Tchisseka, 17 November 1965, *Bouquet* 1966 (P); locality unknown, 17 October 1962, *Makany* 298 (P).

Habitat. Fallow land, shady savannah or scrub, secondary forest or forest edges, often on coasts or flood-plains, on sandy soils.

Vernacular names. Democratic Republic of Congo: Nkaka ba ngulu, Ntendi (Kiyaka), Ntungu.

Thomandersia congolana De Wild. & T.Durand in T.Durand & De Wild., Bull. Soc. Roy. Bot. Belgique., 38: 107 (1899); Burkill & C.B.Clarke in This.-Dyer, Fl. Trop. Afr., 5: 512 (1900); De Wild. & T.Durand, Ann. Mus. Congo, ser. 1, Bot. 1: 147 (1901); tom. cit. 179 (1901); De Wild., Miss. Em. Laurent, 179 (1905); De Wild., Ann. Mus. Congo, ser. 5, Bot. 1: 313 (1906); Mildbr., Wiss. Erg. Zweit. Deut. Zentr.-Afr.-Exp., Bot. 2: 99 (1922); Heine in Fl. Gabon., 13: 143-155 (1966); Heine, Bull.

Jard. Bot. Etat, 36: 207-248 (1966). Types: Democratic Republic of Congo, between Selembao & Leopoldville, 24 November 1895, *Dewevre* 472 (lectotype BR, designated Heine, Bull. Jard. Bot. Etat, 36: 207-248, 1966); Democratic Republic of Congo, Mayombe, 1897, *Cabra* 35 (syntype BR).

T. congolana De Wild. & T.Durand var. *grandifolia* De Wild., Miss. Em. Laurent, 179 (1905); T. & H.Durand, Syll. Fl. Cong. 427 (1909). Types: Democratic Republic of Congo, Kimuenza, May 1907, *Gillet* 2192 (holotype BR); Democratic Republic of Congo, Sanga, November 1903, *Oddon* in *Gillet* 2/3576 (syntype BR); Democratic Republic of Congo, Ganga-Kisanga, *Dewevre* 1082a (syntype BR).

Shrub or small treelet, 0.5-2.5(-4) m in height, leaves sometimes clustered at tips of branches. *Stems* terete, greyish or brownish green, with dense, erect, pale to reddish hairs, becoming glabrous; bark pale brown. *Petioles* dark green-brown, with dense, pale or reddish pubescence, that of larger leaves 4-20 mm long, of smaller leaves 1-3 mm (subsessile). *Leaves* extremely anisophyllous; lamina, of the larger of each pair 60-200 x 20-90 mm, elliptic-oblong, oblanceolate or obovate, apex abruptly long-acuminate, cuspidate, base acute-cuneate, slightly decurrent, with 5-7 pairs of lateral veins, of the smaller of each pair 10-60 x 10-40 mm, elliptic-obovate, orbicular or cordiform, apex acuminate, cuspidate, occasionally slightly emarginate, base cuneate-cordate, with 3-5 pairs of lateral veins, leathery-papery, dark, glossy, often discoloured by fungal growths and deformed by galls, with round glands, especially towards the base near midrib, and many tiny dark glands on the under-surface of young leaves, green above, paler below, glabrous or with a few sparse hairs above and below, midrib below with fairly dense, long, pale, erect hairs; margin entire or

occasionally (particularly of smaller leaves) with a few blunt, cuspidate teeth towards the apex; midrib embedded above, prominent beneath, lateral venation slightly channelled above, tertiary venation scalariform-reticulate; tuft domatia often present, in the axils between the lateral veins and midrib. *Inflorescence* terminal or axillary, 30-140 mm long, very dense, compacted into a pyramid; rachis robust and rigid, pedicels dark brown, hairy. *Bracts* 1-2 mm long, triangular-lanceolate, hairy. *Bracteoles* up to 1 mm long, subulate and slightly carinate, hairy. *Flowers* arranged irregularly, opposite or in whorls, pedicellate; pedicels 2-3 mm long, 5 mm when accrescent, then very stout; buds pale yellowish-green, with greyish, downy pubescence. *Calyx* 1-2.5 mm long, campanulate-cupular, brownish-green, bearing a single conspicuous glandular swelling, up to 0.5 mm deep, with one or two ostioles; lobes 0.5-1 mm long, triangular, spreading (not overlapping), with very short, glandular hairs and ciliate margin. *Corolla* fleshy-leathery, bilabiate; tube 5-8 mm long, pinkish-orange-brown outside, yellowish inside, with long hairs inside towards the base; lobes 3-8 mm long, yellowish-cream, the lateral ones darker, striped with brown or red, spreading, glandular outside and glandular-hairy inside, margin crinkled. *Staminode* minute, hidden by the corolla hairs, inserted 1 mm from base of corolla. *Stamen* filaments inserted at least 4 mm from base of tube, strongly didynamous, the shorter pair c.3 mm long, the longer c.6 mm, whitish, covered with small glands; anther locules held slightly one above the other, 1-1.5 mm long. *Style* 5-7 mm long, 0.2-0.3 mm diameter, white, glabrous. *Stigma* cupular and slightly bilobed, broader than style, glabrous. *Gynoecium* c.2 mm long, ovoid-ellipsoid, dark brown, with glandular trichomes, fleshy, especially at base. *Capsule* 12-14 x 8-10 x 4-8 mm, ovoid-conical-oblong, with pointed apex, green, becoming dark brown-black, glabrous and shiny, with thin (0.4-0.8 mm) wall; subtended by the accrescent calyx, 3-

4 mm diameter, open, rotate. *Seeds* 1-2 per valve, 3-5 x 2-3 x 1-2 mm, irregular-almost spheroidal, yellow-orange or brown, covered with irregular, overlapping, long triangular-lanceolate scales, sometimes lacking from the very tip of the seed (Illustration: Fig. 2.10).

Distribution. Cameroon, Democratic Republic of Congo, Gabon, Republic of Congo (Fig. 2.11).

All other collections seen. **Cameroon.** Avobengono, 6 November 1966, *Letouzey* 8294 (P, YA); Bipindi, *Zenker* 1780 (K, P), 2063 (WAG), 3085 (BR, K, MO, P, WAG), 3085a (BR, K), 4452, (K, MO), s.n. (BR, MO), s.n. (BR, P); Ebianemeyong, 15 April 2001, *Van Andel, Atou'ou & Van der Hoeven* TVA3310 (WAG); Ebone, 22 December 1967, *Bamps* 1531 (BR); Kienke Forest Reserve, 5 January 1968, *Bamps* 1678 (BR); Kienke River, 28 April 1969, *Bos* 4394 (BR, K, MO, P, YA); Lolodorf, 27 May 1969, *Bos* 4644 (P, WAG), 8 July 1970, *Bos* 7057 (P, WAG); Ma'an, 5 March 1963, *Raynal, J. & A.* 10224 (P, YA); Mdjivini, October 2002, *Tchouto* 2734 (YA); Mekoassi, 21 February 1963, *Raynal, J. & A.* 9937 (P); Meyo Ntem, 27 November 1979, *Letouzey* 15228 (P, YA); Mont d'Elephant, 10 May 2001, *Van Andel, Maas, P. & H. & Mva* TVA3566 (WAG); Mvila, 8 May 1995, *Van Gemerden* 168 (YA); Nyabizan, 26 August 2001, *Van Andel, Mva & Evina* TVA4050 (WAG). **Democratic Republic of Congo.** Boma, *Claessens* s.n. (BR); Ganda-Sundi, *De Briey* 1027 (BR); Kalima, February 1981, *Kitembo* 65 (BR, WAG); Kibuli Forest Reserve, 15 April 1959, *Gaillez* 230 (BR); Kiobo, 4 April 1940, *Donis* 15 (BR); Kirundu, 6 February 1915, *Bequaert* 6852 (BR); Kisangani, 6 December 1981, *Lejoly* 81/600 (BR); Kizu, 28 January 1923, *Wellens* 263 (BR); Luali, 25 August 1913, *Bequaert* 625 (BR);



Figure 2.10 *Thomandersia congolana.* A. habit and inflorescence. B. underside of leaf. C. bud. D. floral dissection. E. gynoecium. F. calyx. G. fruit and seed. H. seed. (A – F from *Breteler* 6716; G, H from *McPherson* 15757).

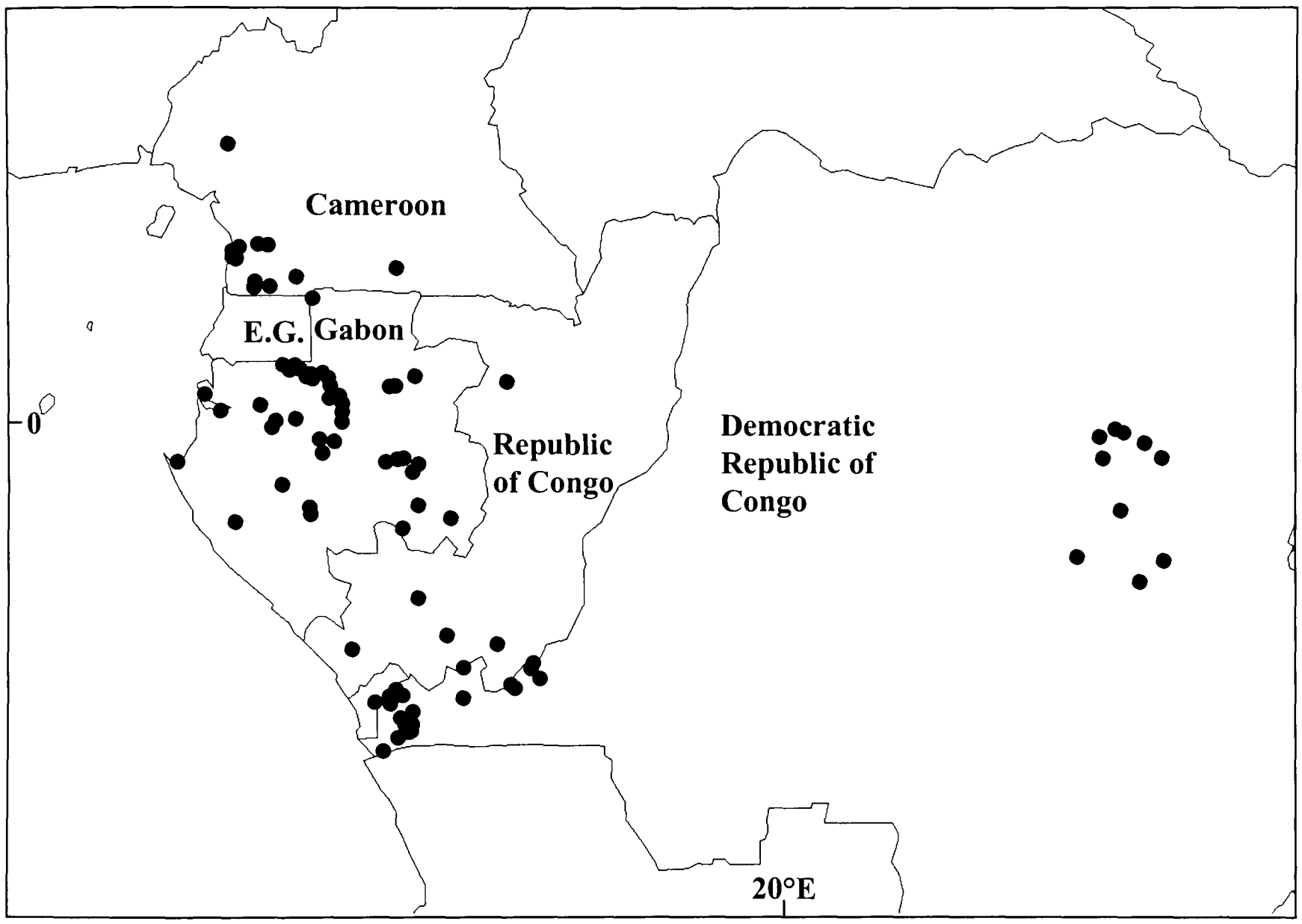


Figure 2.11 Map of western central Africa to show distribution of collections of *Thomandersia congolana*. E.G., Equatorial Guinea.

Lubutu, 27 January 1915, *Bequaert* 6757 (BR); Luki, 24 March 1947, *Toussaint* 2225 (BR, FHO), 16 July 1948, *Maudoux* 27 (BR, WAG), 19 July 1948, *Donis* 1929 (BR), 23 February 1949, *Donis* 2423 (BR), 23 January 1950, *Maudoux* 281 (BR, WAG), 16 February 1959, *Wagemans* 2169 (BR), *Mahieu* 357, (BR); Lukula, 13 March 1948, *Donis* 1739 (BR), 24 August 1959, *Compere* 166 (BR, K); Mayombe, *De Briey* s.n. (BR), *Cabra* 14 (BR); Mbola, 30 August 1957, *Wagemans* 1689a (BR, K), 1689b, (BR); Mengwe, 5 December 1981, *Lisowski* 80186 (BR), 6 December 1981, *Lejoly* 81/567 (BR); Moyengo, August 1959, *Leonard* 5887 (BR, K); Ngongo, 24 February 1970, *s.c.* 331 (WAG); Odzala, 17 February 1995, *Champluvier* 5425 (BR); Pene-Tungu, 5 December 1981, *Lejoly* 81/484 (BR); Sanga, 16 November 1952, *Callens* 3751 (BR); Temvo, 10 February 1919, *Vermoesen* 1809 (BR), February 1919, *Vermoesen* 1690 (BR, K), 1 March 1919, *Vermoesen* 1724 (BR); Tsanga-Ndelo, 28 April 1930, *Vanderyst* 26980 (BR); Ubundu, 10 March 1978, *Lisowski* 48138 (BR, K), 11 March 1978, *Lejoly* 2867 (BR); Vaku, December 1923, *Wellens* 472 (BR); Yailanga, 17 April 1959, *Bamps* 526 (BR); Zongo, February 1954, *Callens* 4534 (BR); locality unknown, 20 October 1930, *Vanderyst* 26471 (BR), *Berger* s.n. (BR).

Gabon. Abanga River, 2 June 1963, *Hallé* 2131 (P), 7 July 1963, *Hallé* 2328 (P), 16 September 1985, *Leeuwenberg & Persoon* 13554 (BR, K, MO); Bambidie, 16 October 1994, *Breteler, Nzabi & Wieringa* 13327 (WAG); Booue, 30 June 1966, *Hallé & Le Thomas* 212 (P); Djidji, 17 May 1987, *Wilks* 1532 (MO); Ebimengha, 22 August 1912, *Fleury* 26417 (P), 26 August 1912, *Fleury* 26439 (P); Koumameyong, 13 February 1987, *Wilks* 1333 (MO), 10 April 1988, *Breteler, Jongkind & Dibata* 8564 (BR, P, WAG); Lambarene, 24 September 1994, *Breteler, F.J. & B.J.M - Klein Breteler* 12988 (WAG); Lara, 6 September 1978, *Breteler & De Wilde* 451 (BR, MO, P, WAG); Lastoursville, 21 March 1929, *Le Testu* 7348 (BR, P), 18 April 1932, *Le*

Testu s.n. (FHO, MO); Leroy Forest Exploitation, 19 January 1983, *De Wilde, Arends, Louis & Karper* 80 (BR, MO, P, WAG), 8 July 1985, *Bos, Van der Laan & Nzabi* 10507 (MO, WAG); Lope-Okanda, 20 March 1989, *McPherson* 13774 (MO), 15 May 1992, *McPherson* 15757 (K, MO), 31 October 1995, *White* 1511 (MO); Makokou, 2 February 1961, *Hallé* 1039 (P), 25 June 1970, *Farron* 7517 (P), 2 November 1977, *Leeuwenberg* 11407 (BR, P, WAG), 29 June 1981, *Gentry* 33028 (MO, WAG), 12 May 1985, *Dorr & Barnett* 4233 (K, MO, P, WAG); Massaa, 25 February 1961, *Hallé* 1333 (P), 1 October 1971, *Hladik* 1563 (P), November 1971, *Hladik* 1599 (P), 28 January 1975, *Hladik* 2451 (P), 4 April 1977, *Florence* 151 (P); Missanga, 13 November 1991, *Breteler & Jongkind* 10436 (WAG); Mitzic, 18 July 1957, *Jeffrey* 77 (K, P); Moanda, 1 October 1970, *Breteler* 6716 (BR, K, MO, P, WAG), 16 October 1970, *Breteler* 6942 (BR, K, MO, P, WAG); Mouila, 30 November 1984, *Arends, De Wilde & Louis* 543 (WAG), 19 September 1986, *Breteler, Lemmens & Nzabi* 8001 (BR, MO, WAG); Ndjole, 25 April 1992, *Breteler, Jongkind & Wieringa* 11106 (WAG); Ngoko, 11 August 1912, *Fleury* in *Chevalier* 26346 (K, P); Nke, 4 October 1983, *Floret & Louis* 1603 (WAG); Nkollessong, 22 March 1910, *Tessman* 58 (K); Ogooue-Ivindo 27 November 1995, *De Wilde, Van der Maesen, Bourobou & Moussavou* 11475 (WAG), 19 May 1987, *Reitsma, J.M. & B., Wilks & Nzabi* 3486 (WAG); Ogooue-Lolo, 22 November 1993, *Breteler, F.J. & B.J.M - Klein Breteler* 12271 (WAG), 11 April 1990, *Breteler, Jongkind, Wieringa & Moussavou* 9838 (WAG); Okano River, 14 March 1961, *Hallé* 1473 (K, P), 18 April 1988, *Breteler, Jongkind & Dibata* 8779 (BR, MO, P, WAG); Oveng, 8 November 1983, *Louis, Breteler & De Bruijn* 486 (BR, MO, P, WAG), 7 May 1985, *Reitsma, J.M. & B., Breteler & Louis* 863 (MO, WAG), 7 May 1986, *Reitsma, J.M. & B.* 2163 (MO, WAG); Oveng-Mitzic, 30 March 1985, *Reitsma, J.M. & B.* 733 (MO, WAG);

12 July 1985, *Bos, Van der Laan & Nzabi* 10618 (WAG); Rabi-Kounga, 14 November 1991, *Schoenmaker* 146 (WAG); Saint Martin, 30 November 1937, *Walker* s.n. (BR, K, MO, P, WAG), 23 December 1975, *Walker* s.n. (P); Sebe River, 15 December 1993, *Breteler, F.J. & B.J.M - Klein Breteler* 12642 (WAG); Sibang, 6 January 2000, *Simons & Westerduijn* 597 (WAG); Waka River, 25 November 1984, *Arends, Louis & De Wilde* 454 (BR, MO, P, WAG); locality unknown, 13 January 1968, *Hallé & Villiers* 4384 (P). **Republic of Congo.** Bnagou, December 1956, *Koechlin* 3911/7259 (P); Djoumouna, 15 October 1965, *Farron* 4672 (P), 30 October 1967, *Sita* 1900 (P), 3 December 1969, *Makany* 1138 (P), 11 December 1969, *Hallé* 1702 (P), 16 December 1969, *Makany* 1233 (P), 25 January 1975, *Bitsindou* 13 (P), 10 December 1976, *Bitsindou* 454 (P); Kakamoeka, 11 October 1990, *Lisowski* B8048 (BR, MO); Kimpila, 6 November 1964, *Bouquet* 648 (P); Komono, 17 January 965, *Bouquet* 954 (P); Kuilila, 26 November 1978, *Cusset* 613 (P); Louboto, 8 November 1964, *Bouquet* 675 (P); Mongo River, 11 January 1916, *Gossweiler* 6128 (BM). **Country unknown.** 11 December 1895, *Lecomte* s.n. (P); 20 March 1935, *s.c.* 425 (BR).

Habitat. Wet primary forest or forest remnants, gallery forest or old secondary forest, often along rivers or in periodically-flooded places, on lateritic soils or granite.

Vernacular names. Democratic Republic of Congo: Kinube, Kitsalala (Kiumbe), Lelekese (Kingengele), Lutidi, Mbesebese (Kirega), Pangi-ya-lutidi; Gabon: Ekaku (Mpongwe), Eko (also used in Equatorial Guinea), Mukuka (Eshira, Bauungu).

Notes. T. congolana has a disjunct distribution; it is entirely absent from the central part of the Democratic Republic of Congo (Fig. 2.11).

Thomandersia hensii De Wild. & T.Durand in T.Durand & De Wild., Bull. Soc. Roy. Bot. Belgique, 38: 108 (1899); De Wild. & T.Durand, Ann. Mus. Congo, ser. 1, Bot. 1: 153 (1901); tom. cit. 179 (1901); De Wild., Miss. Em. Laurent, 179 (1905); De Wild., Ann. Mus. Congo, ser. 5, Bot. 1: 313 (1906); op. cit. 2: 200 (1907); Heine in Fl. Gabon., 13: 143-155 (1966); Heine, Bull. Jard. Bot. Etat, 36: 207-248 (1966). Types: Democratic Republic of Congo, Coquilhatville, 5 February 1896, *Dewevre* 683 (lectotype BR, designated Heine, Bull. Jard. Bot. Etat, 36: 207-248, 1966); locality unknown, 28 May 1888, *Hens* C113 (syntype BR, isosyntype P).

T. hensii De Wild. & T.Durand var. *longipetiolata* De Wild., Miss. Em. Laurent, 179 (1905). Types: Democratic Republic of Congo, Kapinga, 23 November 1903, *Laurent E. & M.* s.n. (holotype BR); Democratic Republic of Congo, Dundusana, *Laurent* 130 (syntype BR).

T. hensii De Wild. & T.Durand var. *latifolia* De Wild., tom. cit., 180 (1905). Type: Democratic Republic of Congo, Djuma, July 1902, *Gillet* 2759 (holotype BR).

T. laurifolia (T.Anderson ex Benth.) Baill. var. *longipetiolata* (De Wild.) T. & H.Durand, Syll. Fl. Cong., 427 (1909).

T. laurifolia (T.Anderson ex Benth.) Baill. var. *latifolia* (De Wild.) T. & H.Durand, loc. cit. (1909).

Much-branched, straggling shrub or many-stemmed small tree (Fig. 2.3B), often forming clumps or thickets, 1.5-9 m in height, up to 5 cm diameter at breast height. *Stems* long, slender, flattened at nodes, dark brown-purplish when young (Fig. 2.12A), glabrous or slightly downy; bark becoming pale, flaky, sometimes corky; slash thin, greenish-white, with irritant sap. *Petioles* brownish or purplish-green, slightly downy,

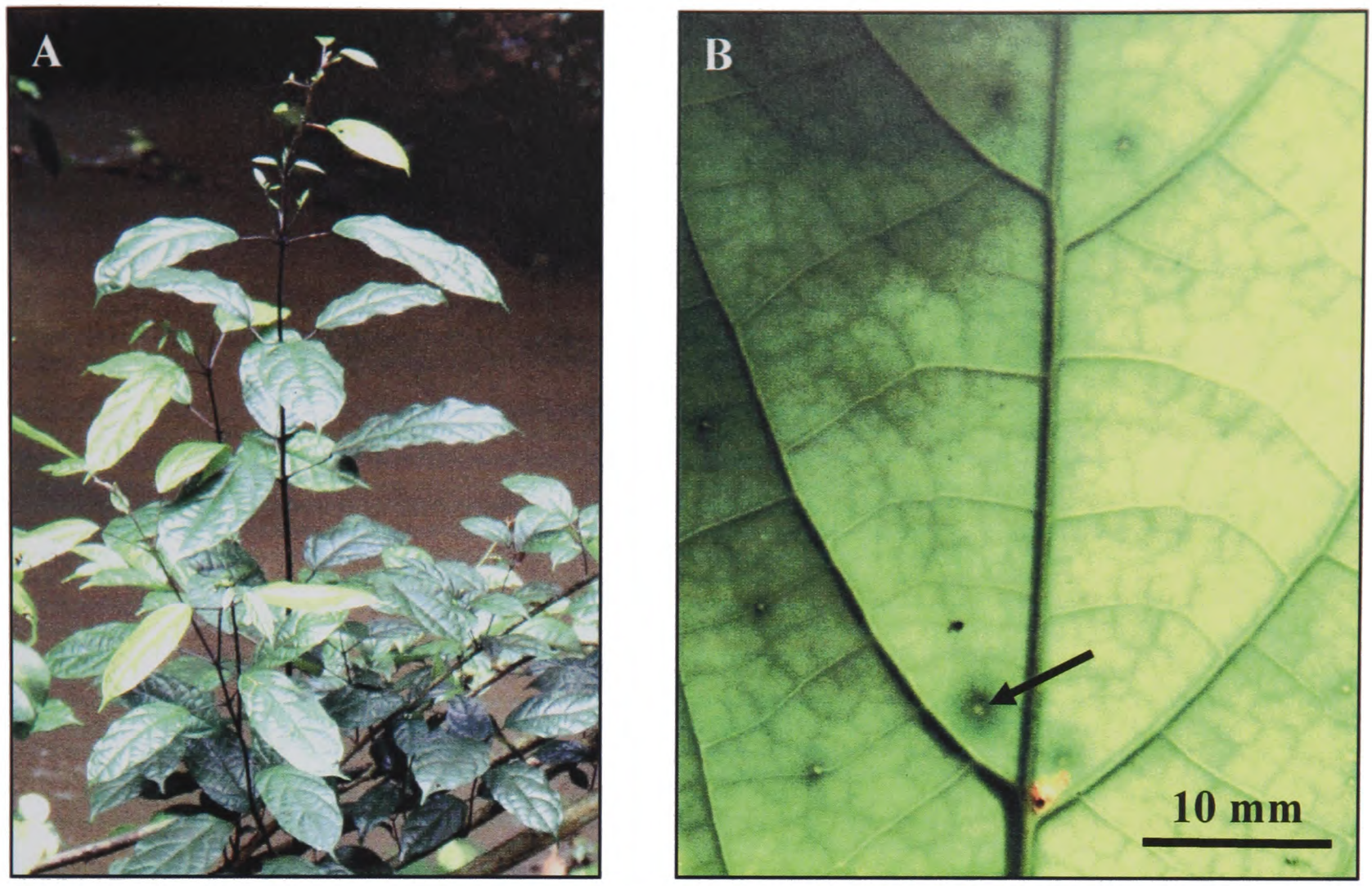


Figure 2.12 *Thomandersia hensii.* A. young shoot, showing blackish stem and decussate leaf arrangement. B. detail of leaf, showing glands (arrowed). C. inflorescence, showing calyx and corolla colour, and gynoecium. D. inflorescence, showing open corolla with androecium (image supplied by D. Harris).

becoming glabrous, both ends swollen, becoming twisted, wrinkled or invaginated when dry, that of larger leaves 15-70 mm long, of smaller leaves 3-10 mm. *Leaves* anisophyllous; lamina, of the larger of each pair 80-250 x 25-140 mm, elliptic or lanceolate to broadly obovate, apex abruptly and narrowly acuminate, slightly cuspidate, base cuneate-rounded, with 5-6 pairs of lateral veins, of the smaller of each pair 20-100 x 15-50 mm, elliptic-ovate or cordiform, apex acute-acuminate, cuspidate, base rounded-cordate, with 3-4 pairs of lateral veins, leathery-papery, often with round galls and insect damage, with glands up to 1 mm diameter, especially towards the midrib at base (Fig. 2.12B), dark green above, paler below, glabrous or with scattered short pale hairs, especially on midrib above, more or less glabrous or with a few scattered hairs below, veins above and below with more or less scattered hairs; margin entire or occasionally very slightly dentate; midrib embedded above, prominent beneath, lateral veins embedded above, prominent beneath, anastomose, tertiary venation lax scalariform-reticulate; tuft domatia sometimes present in the axils between the lateral veins and midrib. *Inflorescence* usually terminal, 40-200 mm long, dense, pyramid-shaped, erect, occasionally branched; rachis robust and rigid, with irregular longitudinal ridges, brownish-green, with soft, short, yellowish pubescence. *Bracts* minute (<1 mm long), triangular, downy. *Bracteoles* minute (<1 mm), triangular-lanceolate, hairy. *Flowers* sweetly fragrant, arranged regularly, opposite or in whorls of 3-4, subsessile-pedicellate; pedicels 1-3 mm long, squat; buds pinkish or purplish-yellow, darker at apex (Fig. 2.12C). *Calyx* 1.5-3 mm long, campanulate, brownish or purplish-green, bearing a single conspicuous, shiny, often black, glandular bulge, 1 mm deep, with one (occasionally two) ostiole(s); lobes 1-2 mm long, ovate-triangular, spreading (not overlapping), downy-glabrescent, ciliate at margin. *Corolla* fleshy-leathery, bilabiate; tube 8-10 mm long, pinkish or brownish-

white, downy-glabrescent; lobes white inside, yellowish outside, purplish or pinkish-tinged, 6-10 mm long, spreading, with very short glandular hairs all over, most conspicuous on the outside of the lobes in bud, margin slightly undulate, crinkled or frilled, ciliate. *Staminode* up to 1 mm long, inserted 3 mm from base of corolla. *Stamen* filaments inserted c.7 mm from base of the tube, the shorter filaments c.2 mm long, the longer c.4 mm, white, the free part glabrous or with very tiny glandular hairs; anther locules equal, 1-1.5 mm long, bright pale yellow-green (Fig. 2.12D). *Style* 8-10 mm long, c.0.3 mm diameter, curved, white-yellow, glabrous. *Stigma* broad, cylindrical-cupular, asymmetric but not lobed. *Gynoecium* 1.5-2 mm long, ovoid-ellipsoid, pale yellow, turning brownish, glabrous or very slightly glandular, fleshy. *Capsule* 10-18 x 10-15 x 6-10 mm, globular-ovoid or oblong, rounded, purplish-green, becoming brown-black, glabrous and matt, with a more or less obvious line corresponding to the septum, along which it sometimes splits a little at top and bottom as well as dehiscing loculicidally, with very thick (1-2.5 mm) wall; subtended by the accrescent calyx, 4-6 mm diameter, cupular or star-shaped, the adaxial lobe longer than the others, retinacula raggedly blunt-tipped. *Seeds* 1-2 per valve, 4-5 x 4-5 x 3-4 mm, spheroidal-irregular conical, yellowish or reddish-brown, covered with overlapping, triangular-lanceolate or rounded scales (Illustration: Fig. 2.13).

Distribution. Cameroon, Central African Republic, Democratic Republic of Congo, Equatorial Guinea, Gabon, Nigeria, Republic of Congo (Fig. 2.14).

All other collections seen. **Cameroon.** Alen, 24 September 1897, *Bates* 586 (P); Badekok, 14 July 1965, *Leeuwenberg* 6099 (BR, MO, P, WAG, YA); Bakole-

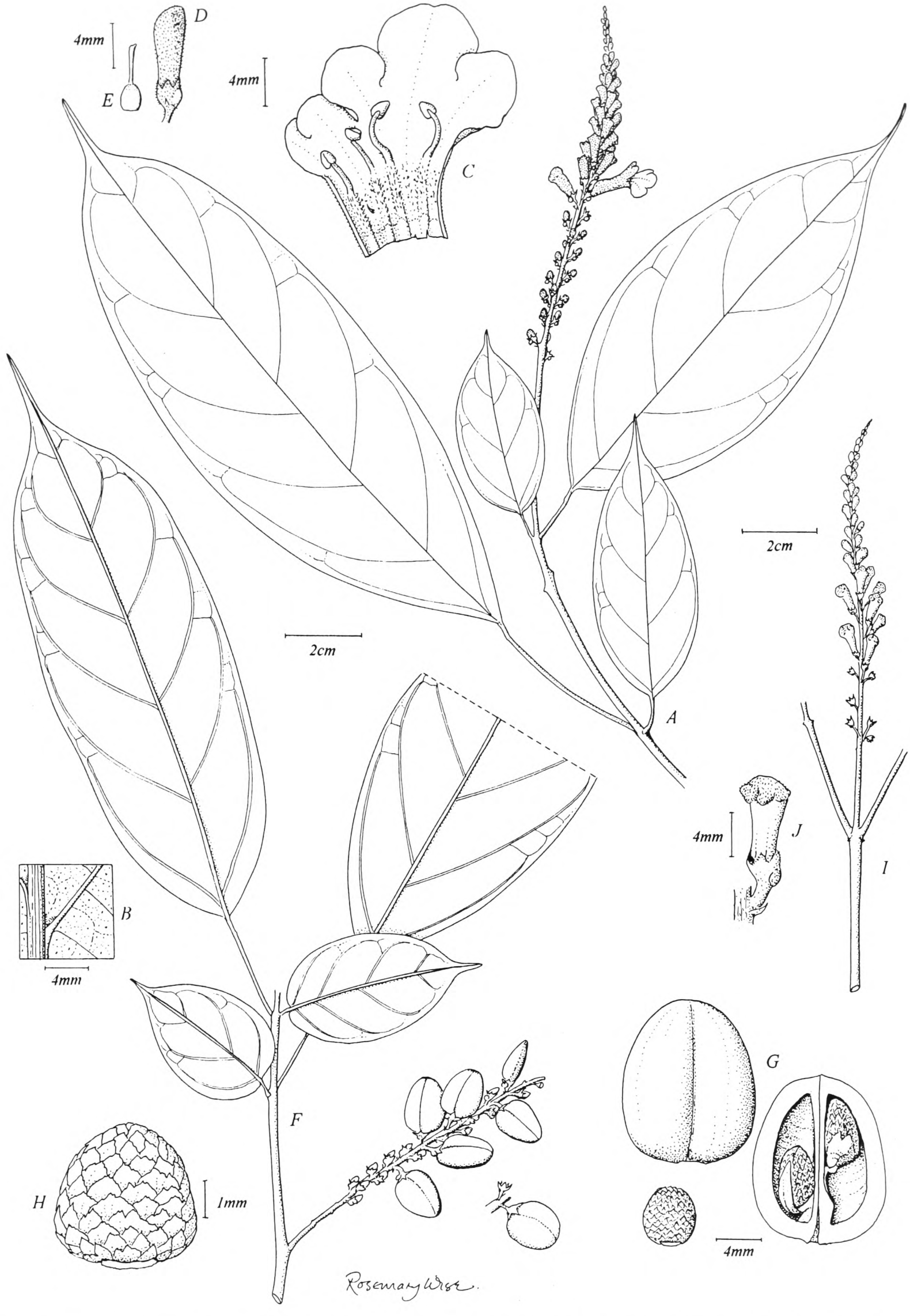


Figure 2.13 *Thomandersia hensii.* A. habit and inflorescence. B. underside of leaf. C. floral dissection. D. bud. E. gynoecium. F. infructescence. G. fruit and seed. H. seed. I. inflorescence of more glabrous specimen from Afromontane and Piedmont forest. J. bud of more glabrous specimen. (A – H from *Etuge* 145; I, J from *Troupin* 3995).

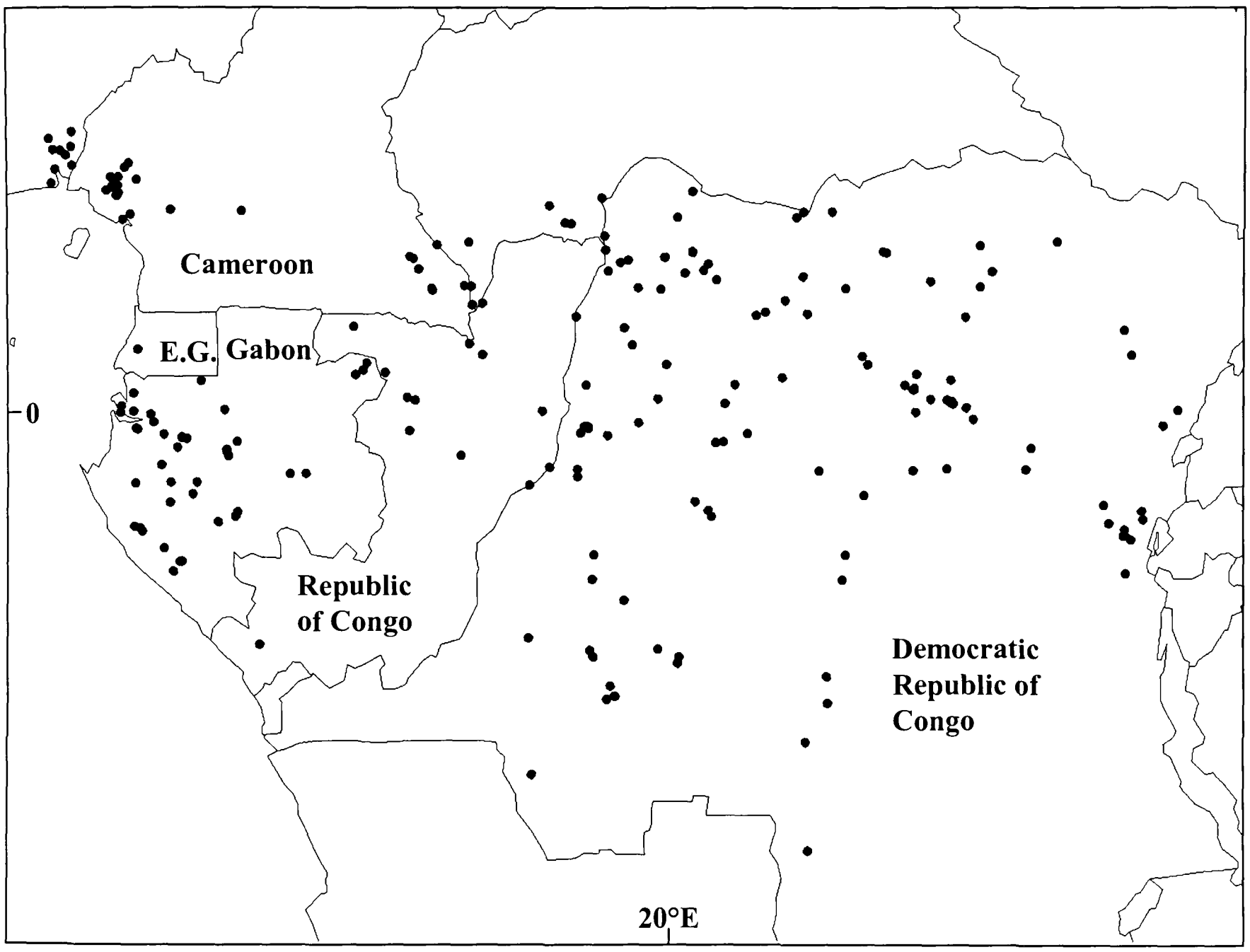


Figure 2.14 Map of western central Africa to show distribution of collections of *Thomandersia hensii*. E.G., Equatorial Guinea.

Bakossi, 24 May 1986, *Etuge & Thomas* 145 (MO, P, WAG, YA); Bakundu, 11 August 1951, *Olorunfemi* 30702 (K), 20 April 1960, *Adebusuyi* FHI44018 (BR); Banga, 20 March 1948, *Onochie* 9314 (BR), 9314/2 (FHO, K, P); Bange, 21 May 1963, *Letouzey* 5104 (K, P, YA); Barombi Station, 16 August 1983, *Thomas* 2482 (MO, P, WAG, YA), *Preuss* 339 (BM); Bela, 1 December 1982, *De Kruif* 799 (WAG, YA); Bombe, 17 April 1959, *Daramola* 41036 (K, YA), 12 May 1987, *Manning* 1795 (K, MO, WAG), 25 October 2002, *Wortley* 5 (K, SCA, YA); Cameroun River, *Mann* 718 (P); Dibombe, 21 April 1972, *Leeuwenberg* 9676 (BR, K, MO, P, YA); Douala, 31 December 1949, *Baldwin* 13977 (K, MO); Eseka, 18 July 1955, *Mbarga* 90 (YA); Etam, 22 March 1986, *Etuge & Thomas* 83 (BR, MO, P, WAG, YA); Ikiliwindi, 4 March 1987, *Etuge & Thomas* 503 (MO); Kendongi, 17 December 1959, *Latilo* 43813 (FHO, YA), 15 May 1985, *Thomas* 4810 (K, MO, P, YA); Kese, 2 September 1945, *Aubreville* 79 (P); Kinsasa, 4 March 1971, *Letouzey & Villiers* 10508 (P, YA); Konye, 21 April 1986, *Nemba & Thomas* 4 (MO, P, WAG, YA); Loum, 20 December 1957, *De Wit* 373/7765 (WAG); Masok, 30 March 1965, *Leeuwenberg* 5274 (BR, K, MO, P, YA); Mboye, 14 May 1963, *Letouzey* 5037 (BR, P, YA); Moloundou, 28 February 1983, *De Namur* 2384 (YA); Mongo, June 1917, *Fleury* 33299a, 33386 (P); Ndagnan, 21 July 1987, *Thomas, Fay & Harris* 7212 (K, MO, WAG, YA); Ngato, 31 March 1973, *Letouzey* 12217 (K, P, YA); Pete, 15 August 1945, *Okeke* 8371 (FHO); Welele, 22 March 1987, *Manning* 1627 (MO, YA); Welende, 19 March 1987, *Manning* 1591 (MO); Yelfoume, June 1917, *Fleury* 33368 (P); locality unknown, 10 October 1937, *Hedin* 1832 (P). **Central African Republic.** Bambari, 6 March 1921, *Tisserant* 289 (BR, P), March 1921, *Tisserant* 52 (K), 19 January 1923, *Tisserant* 1405 (P), 23 December 1963, *Descoings* 11925 (P); Bangandon, 20 June 1961, *s.c.* 2193 (P); Bangui, 18 December 1903, *Chevalier* 10886, 10911 (P), 29 March 1963,

Descoings 10501 (P); Lidjombo, 5 July 1988, *Fay & Harris* 8598 (MO); Maboke, 1 February 1974, *Mazade* 12 (P); Mbaiki, December 1926, *Fidao* s.n. (P); Ndakan, 10 May 1988, *Gentry, Thomas, Fay, Harris & Bromley* 62672 (MO); Nola, 27 November 1965, *Leeuwenberg* 7059 (P, WAG), 28 November 1965, *Leeuwenberg* 7072 (WAG); locality unknown, *Boukoko* s.n. (P), *Viangin* s.n. (P). **Democratic Republic of Congo.** Aluta, 1 June 1977, *Lejoly* 1610 (BR), 2 June 1977, *Lejoly* 1686 (BR); Bafwaboli, *Claessens* 321 (BR); Bagonde-Ikendo, 9 October 1957, *Evrard* 2627 (BR); Bambesa, April 1934, *Bredo* 1111, 1185 (BR), 24 June 1942, *Dubois* 245 (BR), 8 May 1952, *Gerard* 179 (BR), 2 July 1953, *Gerard* 661 (BR), 5 May 1958, *Gerard* 3813 (BR), 31 January 1961, *Gerard* 4824 (BR, K), *Bredo* s.n. (BR), *Pittery* 112, 131 (BR); Bandundu, July 1907, *Sapin* s.n. (BR); Bankaie, 11 July 1953, *Gilbert* 14401 (BR), 4 October 1953, *Gilbert* 14901 (BR); Bari, July 1938, *Gilbert* 1601 (BR, FHO, WAG); Barumbu, 10 November 1913, *Bequaert* 1142 (BR); Basankusu, November 1906, *Bruneel* s.n. (BR); Basoko, May 1921, *Goossens* 2878 (BR); Bena-Tshadi, November 1981, *Casier* 183 (BR); Bene-Dibele, 21 April 1906, *Flamigni* 153a (BR); Bengamisa, 2 June 1936, *Gilbert* 2358 (BR); Bikoro, 26 July 1983, *Nsola* 216 (BR), *Broun* s.n. (BR); Boboua RCA, 8 November 1967, *Breyne* 1103 (BR); Boende, *Hulstaert* 24 (BR); Bokala, 13 November 1946, *Leonard* 1006, (BR, WAG); Bokolo, November 1980, *Dechamps* 8030 (BR); Bokoro, 15 November 1947, *Jans* 624 (BR); Bokuma, 26 February 1941, *Hulstaert* 74 (BR); Bomandja, 12 June 1958, *Evrard* 4229 (BR); Bongabo I, 3 June 1971, *Breyne* 1645 (BR); Bonkula, September 1913, *De Giorgi* 1325 (BR); Booke, May 1958, *Robin* 12 (BR); Bosodula, *Bilderling* 53 (BR); Budjala, 17 July 1938, *Briemeyer* 30 (BR), May 1924, *Goossens* 4685 (BR), May 1925, *Goossens* 4112, 4113 (BR); Bumba, 29 December 1926, *Linder* 1822 (K, P); Buna, *Flamigni* 6374 (BR); Bunyakiri, 24 September 1917, *Gutzwiller* 1465 (BR),

19 October 1957, *Gutzwiller* 2329 (BR, K), 24 September 1957, *Gutzwiller* 2064 (BR), 1 October 1957, *Gutzwiller* 2127 (BR), 3 October 1957, *Gutzwiller* 2166 (BR), 8 October 1957, *Gutzwiller* 2258 (BR), 2 November 1957, *Gutzwiller* 2414 (BR), 3 January 1958, *Gutzwiller* 2532 (BR), 2 June 1958, *Gutzwiller* 3773 (BR), 8 February 1959, *Leonard* 2896 (BR); Buta, 15 October 1952, *Gerard* 297 (BR); Bwemba Forest, 25 October 1958, *Pierlot* 2609 (BR); Djilingi de Likote, 13 May 1937, *Collart* 15 (BR); Djoa-Bolima, 16 October 1958, *Evrard* 5035 (BR); Dongo, *Sapin* s.n. (BR); Djuma, *Gentil* s.n. (BR); Dundusana, February 1913, *Reygaert* 34 (BR), June 1913, *De Giorgi* 1026 (BR), *Mortehan* 233 (BR), *Vermoesen* 210 (BR); Eala, 31 May 1905, *Laurent* 841 (BR), June 1905, *Laurent* 1412 (BR), 30 July 1905, *Laurent* 1263 (BR), 22 January 1907, *Pynaert* 911 (BR), 1 February 1907, *Pynaert* 1090 (BR), 18 August 1912, *Chevalier* 28715 (P), 12 May 1919, *Vermoesen* 2132 (BR), 15 May 1919, *Vermoesen* 2245 (BR), 7 September 1925, *Robyns* 273 (BR), 23 September 1925, *Robyns* 703 (BR, K, WAG), May 1930, *Lebrun* 248 (BR, K), June 1930, *Lebrun* 525 (BR), August 1930, *Staner* 930 (BR), November 1930, *Staner* 1526 (BR, K, MO), 1534 (BR, P), 11 May 1932, *Corbisier-Baland* 1291 (BR), January 1933, *Lebrun* 6822 (BR), 25 April 1933, *Corbisier-Baland* 1780 (BR), 20 May 1936, *Louis* 1978 (BR, MO), 13 July 1937, *Couteaux* 254 (BR), 5 February 1957, *Couteaux* 150 (BR), 10 December 1957, *Evrard* 3104 (BR), *Corbisier* 1291 (MO, P), *Laurent* 208 (BR), *Leemans* 53 (BR), *Mayne* 2616 (BR), V*ermoesen* 2616 (BR); Ekuta, *Sapin* s.n. (BR, FHO); Epulu, 25 April 1987, *Hart* 731 (MO); Equateur, *Sapin* s.n. (BR, K), *Pynaert* 284 (BR); Fausse, May 1899, *Trilles* 18 (P); Gemena, May 1924, *Goossens* 4101, 4858 (BR); Gombe, 18 March 1915, *Bequaert* 7110 (BR); Guanda, *Jespersen* 19 (BR); Hyaet, August 1920, *Vanderyst* 8342, 8348 (BR); Ile Kongolo, 26 March 1977, *Lejoly* 1117 (BR), 24 May 1977, *Lejoly* 1529 (BR), 22 November 1982, *Mosango* 544

(BR); Indjolo I, *Ledoux & Huyghe* 35 (BR); Kabondo, 6 April 1976, *Lisowski* 42152 (BR); Ipamu, September 1920, *Vanderyst* 10826 (BR), May 1921, *Vanderyst* 9421, 9440 (BR); Irangi, 2 July 1955, *Pierlot* 627 (BR), 7 September 1956, *Troupin* 2476 (BR, FHO, K), 12 November 1956, *Christiaensen* 1877 (BR), 23 May 1957, *Troupin* 3471 (BR, K), 1 June 1957, *Troupin* 3514 (BR, K), 20 July 1957, *Troupin* 3846 (BR, FHO, K), 16 August 1957, *Troupin* 4030 (BR), 30 August 1957, *Troupin* 4281 (BR), 4 September 1957, *Troupin* 4312 (BR), 26 November 1958, *Leonard* 1873 (BR, P); Kala, May 1924, *Goossens* 4354 (BR); Kandolo, 1 June 1985, *Szafranski* 1185 (BR, WAG); Karambi Plain, 21 December 1958, *Troupin* 9323 (BR), 16 August 1959, *Cambridge Congo Expedition 1959* 286, 351 (BM, BR); Karawa, 26 March 1924, *Goossens* 4024 (BR), April 1924, *Goossens* 4253, 4800, 6348 (BR); Kikivit, February 1917, *Gillet* s.n. (P); Kikwit, 20 July 1904, *Lescrauwaet* 148 (BR), January 1914, *Vanderyst* 2864, 2964, 3078, 3205 (BR); Kisangani, 19 January 1973, *Lisowski* 16530 (BR), 25 April 1977, *Lejoly* 1402 (BR), 29 June 1979, *Lisowski* 52639 (BR, WAG), 12 January 1982, *Mandango* 3010 (BR); Kukuluba, 13 September 1953, *Callens* 4258 (BR); Kwanga, 25 October 1955, *Devred* 2733 (BR, MO); Lac des II, October 1932, *Lebrun* 6331 (BR), *Claessens* 281 (BR); Leverville, *Vanderyst* 6162, 6269, 6408 (BR); Libanza, April 1924, *Goossens* 4749 (BR); Libenge, *Sapin* s.n. (BR); Likati, 22 March 1956, *Gerard* 2210 (BR), 30 December 1977, *Lisowski* 46543 (K), 31 December 1977, *Lisowski* 46518 (BR); Likimi, 15 February 1910, *Malchair* 67 (BR), 2 January 1913, *De Giorgi* 59 (BR), January 1913, *De Giorgi* 136 (BR), November 1913, *De Giorgi* 1483 (BR), March 1924, *Goossens* 3243 (BR), May 1924, *Goossens* 4107 (BR), May 1938, *Gilbert* 1502 (BR); Likimi-Karawa, March 1924, *Goossens* 6322 (BR); Likofu, 9 June 1957, *Evrard* 2312 (BR); Lindi, 17 November 1982, *Pauwels* 6571 (BR, WAG); Liwea, 16 November 1925, *Robyns* 995 (BR); Lubimbe,

20 August 1958, *Pierlot* 2413 (BR); Lubuc-Loange, October 1922, *Vanderyst* 12543 (BR); Lubutu, 8 January 1977, *Lisowski* 44139 (BR); Lukolela, *Claessens* 779 (BR); Lula, 17 February 1921, *Claessens* 175 (BR), *Claessens* 160 (BR); Luluabourg, *Sparano* 120 (BR); Madabu, 27 April 1957, *Gerard* 2831 (BR); Madibi, 2 July 1906, *Sapin* s.n. (BR), May 1907, *Sapin* s.n. (BR); Magima, 14 April 1987, *Apema* 223 (BR); Maiko, 2 June 1977, *Lejoly* 1774, 1780, 1831, 1833 (BR); Malanga, September 1938, *Gillardin* 438 (BR); Mapangu, 6 June 1978, *Dumont* 279 (BR); Maringa-Lopori, *Van Gils* s.n. (BR); Masako, 23 June 1988, *Kalanda* 530 (BR); Masingu, 17 November 1958, *Leonard* 1689 (BR, K); Mingazi, 6 September 1955, *Pierlot* 825 (BR); Mobwasa, 15 May 1913, *Lemaire* 146 (BR), May 1913, *De Giorgi* 802 (BR), December 1913, *Reygaert* 1455 (BR), *Reygaert* 630, 1275 (BR); Monga, 1 November 1953, *Gerard* 778 (BR); Mongo, 20 September 1935, *Louis* 129 (BR, P); Monsole, July 1923, *Goossens* 4195 (BR); Mosibasiba, 26 March 1975, *Lisowski* 40504 (BR); Mukumari, June 1939, *Gillardin* 609 (BR); Musa, August 1913, *De Giorgi* 1206 (BR); Mutenge, *Sapin* s.n. (BR); Ngasi, 14 November 1935, *Louis* 619 (BR, MO), *Claessens* 663 (BR); Nouvelle-Anvers, July 1908, *De Giorgi* 173 (BR), 24 May 1909, *Pynaert* 8 (BR), March 1913, *De Giorgi* 442, 485, 571 (BR), April 1913, *De Giorgi* 604 (BR); Nyamilera, 25 November 1959, *Pierlot* 3266 (BR); Odzala, 1 February 1995, *Champluvier* 5352 (BR), 9 February 1995, *Champluvier* 5379 (BR), 14 February 1995, *Champluvier* 5407 (BR), 23 November 1996, *Lejoly* 96/812 (BR); Orientale, 13 January 1904, *Laurent, E. & M.* s.n. (BR), 9 November 1934, *De Wulf* 399 (BR), *Vandergucht* 10 (BR); Palmeraie, 21 November 1990, *Masens* 510 (BR); Pelenda, 21 February 1952, *Callens* 3553 (BR, K); Romee, 24 January 1896, *Laurent E. & M.* s.n. (BR); Stanleyville, 29 September 1957, *Croockewit* 708 (WAG), *Kohl* 55 (BR), *Schwetz* 58 (BR); Tshopo, 22 November 1972, *Lisowski* 15119 (BR), 7 May

1973, *Lisowski* 18504, (BR, K); Tshuapa, May 1910, *Jespersen* 24 (BR), November 1910, *Jespersen* s.n. (BR); Tumba Lake, 29 October 1957, *Thonet* 107 (BR); Upoto, 14 November 1925, *Robyns* 972 (BR); Urega, June 1932, *Lebrun* 5618 (BR, K); Wafania, November 1933, *Dubois* 150 (BR); Walikele-Lubutu, 19 October 1910, *Bequaert* 49 (BR), 18 January 1915, *Bequaert* 6670 (BR); Wanie-Rukula, 13 December 1972, *Lisowski* 16084 (BR), 5 January 1973, *Lisowski* 15756 (BR), 29 June 1979, *Lejoly* 5267 (BR); Wendji, July 1930, *Lebrun* 733 (BR), August 1930, *Lebrun* 1147 (BR); Yakoma, 27 February 1909, *Thonner* 253 (BR); Yambata, October 1913, *De Giorgi* 1371 (BR), *Montchal* 192 (BR); Yangambi, 24 October 1935, *Louis* 382 (BR), 27 March 1936, *Louis* 1549 (BR), 8 May 1936, *Louis* 1835 (BR, FHO), May 1937, *Gilbert* 25 (BR), 30 November 1937, *Louis* 818 (BR), 15 December 1937, *Louis* 7023 (BR), 27 April 1938, *Louis* 9106 (BR, MO), 19 May 1938, *Louis* 9452 (BR, P), May 1938, *Gilbert* 1155 (BR), June 1938, *Gilbert* 1249 (BR), 1 October 1938, *Louis* 11528 (BR, FHO, P), 7 October 1938, *Louis* 11591 (BR), 14 October 1938, *Louis* 11753 (BR), 19 September 1950, *Donis* 2769 (BR), 28 September 1950, *Donis* 2828 (BR), 7 October 1950, *Donis* 2883 (BR), 17 November 1954, *Gutzwiller* 469 (BR), 7 July 1958, *Leonard* 855 (BR), 16 February 1961, *Bolema* 402 (BR), January 1974, *Gaudreau* 8 (BR, MO, WAG), 9 February 1979, *Breyne* 3570 (BR), *Gilbert* 7543, 8015, 8208, 9822 (BR); Yanonge, 20 April 1975, *Ntore* 70 (P); Yatolema, 1 July 1975, *Lisowski* 41071 (BR, K); Yongo, 5 May 1959, *Evrard* 6247 (BR); Zobia, 12 May 1907, *Seret* 881 (BR); locality unknown, *Goossens* 2781 (BR), *Sapin* s.n. (BR). **Equatorial Guinea.** Como River, July 1896, *Bates* 491 (BM, P). **Gabon.** Abanga River, 3 February 1968, *Hallé & Villiers* 4901 (P); Assoukou, 2 October 1912, *Chevalier* 26852 (BR, P); Ayem Lake, 7 April 1963, *Hallé* 1573 (P), 22 April 1963, *Hallé* 1803 (P); Doudou Mountains, 20 April 1987, *Reitsma, J.M & B.*

& Wilks 3373 (WAG); Doussala, 16 May 1985, *Reitsma, J.M & B., Breteler & Louis* 967 (WAG); Etouk, 2 November 1983, *Louis, Breteler & De Bruijn* 296 (BR, WAG); Gopoma, 30 December 2000, *Wieringa, Jongkind & Schoonhoven* 3704 (WAG); Ikobey, 1 December 2001, *Wieringa, Nzabi & Boussiengui* 4534 (WAG); Kango, 1 October 1986, *Breteler & Lemmens* 8284 (BR, MO, P, WAG); Koumounabouali Mountains, 5 December 1996, *De Wilde & De Wilde-Bakhuizen* 11662 (WAG); Lambarene, 26 September 1968, *Breteler* 5691 (BR, K, MO, WAG), 24 September 1994, *Breteler, F.J. & B.J.M - Klein Breteler* 12986 (WAG); Lara, 7 September 1978, *Breteler & De Wilde* 473 (MO, WAG); Lastoursville, 23 September 1970, *Breteler* 6585 (BR, K, MO, P, WAG), 21 November 1993, *Breteler, F.J & B.J.M - Klein Breteler* 12224 (WAG); L'evicou, 12 April 1930, *Le Testu* 8015 (BR, P); Libange, September 1884, *Buttner* 352a (P); Lope-Okanda, 2 November 1982, *Leeuwenberg & Louis* 12429 (BR, MO, P, WAG), 23 June 1986, *Reitsma, J.M. & B.* 2333 (MO, WAG), 10 March 1989, *McPherson* 13750 (MO), 27 September 1989, *White* 1/158 (MO); Mbilangone, 24 August 1938, *Thomson* 11 (K, P); Mekambo, 5 August 1966, *Hallé & Le Thomas* 338 (BR, K, P); Mimongo, 4 December 2001, *Wieringa, Nzabi & Boussiengui* 4564 (WAG); Moukalaba, 5 December 1984, *Arends, Louis & De Wilde* 647 (BR, MO, WAG); Mviadi, 20 September 1983, *Floret & Louis* 1334 (WAG); Ndjole, February 1887, *Thollon* 735 (P), 6 November 1982, *Leeuwenberg* 12462 (BR, MO, WAG), *Thollon* 99 (P); Ninge-Ninge, 3 October 1912, *Chevalier* 26856 (P); Nkoulounga, 23 June 1959, *Touzet* 155 (P), 24 June 1959, *Hallé* 647 (P); Pahoun, 6 February 1959, *Touzet* 130 (P); Petsyalango, 4 October 1932, *Le Testu* 8933 (BR, P); Rabi-Kounga, 8 November 1991, *Schoenmaker* 109 (WAG); Rembo-Rabi, 23 November 1989, *De Wilde, Arends, Louis & Wieringa* 9682 (WAG), 25 January 1993, *De Wilde & Van der Maesen* 10970 (WAG); Remboue, 23 October 1991,

McPherson 15421 (BR, K, WAG); Saint Martin, 23 December 1939, *Walker* s.n. (P); Sibang, 7 December 1983, *Van der Maesen, Louis & De Bruijn* 5376 (WAG); Waka, 25 November 1984, *Arends, Louis & De Wilde* 455 (BR, MO, P, WAG), 22 September 1985, *Leeuwenberg & Persoon* 13624 (BR, K, MO, P, WAG), 13625, (BR, K, P, WAG), 23 September 1986, *Breteler, Lemmens & Nzabi* 8126 (BR, K, MO, P, WAG); Zile, 12 September 1947, *s.c.* s.n. (BR, P, WAG); Zoula, 26 December 2000, *Wieringa, Jongkind, Schoonhoven & Mbombet* 3612 (WAG); locality unknown, 6 May 1902, *Klaine* 3160 (P), 17 December 1902, *Klaine* 3189 (P), *Trilles* 36 (P). **Nigeria.** Awi Forest Reserve, 14 August 1974, *Okeke, Van der Laan & Nzabi* FHI72685 (MO); Calabar, 16 April 1971, *Van Meer* 1325 (WAG); Ekimita River Forest Reserve, 26 April 1971, *Van Meer* 1487 (WAG); Ndealiche, 21 May 1953, *Onochie* 33225 (K, WAG); Oban Forest Reserve, 7 May 1952, *Ujor* 31785 (K), 24 April 1971, *Van Meer* 1438 (WAG), 11 March 1973, *Latilo & Oguntayo* 70555 (K, WAG), 14 October 1973, *Daramola & Adebusuyi* FHI72410 (MO); Obubu-Umuariaga Valley, 20 August 1964, *Tuley, Ariwodo & Opara* 717 (K); Unyene, 15 May 1953, *Onochie* 33179 (K); Uwet, 24 July 1959, *Binuyo* 41410 (K, YA).

Republic of Congo. Besse, 9 November 1991, *Thomas, Harris, Moutsambote & Gereau* 8778 (MO); Epena, 24 February 1963, *De Nere* 990 (P); Etoumbi, 12 June 1961, *Descoings* 7336 (P), 5 August 1961, *Descoings* 8869 (P); Fort Rousset, 14 June 1961, *Descoings* 7530 (P); Imese, *Sapin* s.n. (BR); Nesbemokee, March 1920, *Pobeguin* 121 (P); Olouma, 1 February 1995, *Champluvier* 5351 (BR, P), 2 February 1995, *Champluvier* 5366 (BR, P); Ouesso, 2 November 1961, *Descoings* 9297 (P); Pokola, 22 October 1965, *Sita* 754 (P); Sounda, 5 February 1966, *Farron* 4987 (P).

Country unknown. 27 January 1953, *De Nere* 1128 (P).

Habitat. Evergreen, semi-deciduous, mixed, wet, dry, riverine, coastal, flood-plain, plateau and upland forests, especially on forest edges, clearings, disturbed areas and roadsides, on lateritic, clay, sand, limestone or iron-rich soils.

Vernacular names. Cameroon: Assenan, Dibanja (Pete), Evenzegue (Pahouin), Mbere, Ngoka (Bibaya), Ngoko (Bibaya), Ofes-afan (Yaounde); Central African Republic: Debak, Ikoka, Ntemba (Mbimo; this name is also used for certain members of Acanthaceae; *Leeuwenberg* 7072), Temba; Democratic Republic of Congo: Amadandidandi, Amandandidandi, Bamalobida (Azande), Bofa-ampamba, Boloto, Debak (Bekwil), Dikoka (Enyele), Dinduli (Kitembo), Efukoi nkombe, Ekoko, Ekoku, Ekwanga, Gbanda, Gwanda, Ibesebese, Ibuni (Yuombe), Ikobele, Ikoka (Bôngili, Fur, Turumbu), Imbelempaka, Imbesimbese, Imelempaka (Bangala), Imenempaka (Bokuala, Kundu, Mongo), Ingwanda, Isumbolongi (Mongo), Kinna (Kinande), Kivun (Vili), Ligwanda, Likoka (Turumbu), Likoko, Linduli (Kitembo), Lingwanda (Mongala), Lintuli, Litindi, Lo (Azande), Lokua (Mbaamba), Lokwankema, Lonkwankema, Lukokoka, Malanamantumba, Mbesembese (Kirega), Mbesimbesi, Mekontogi (Mbimo), Mokoka (Songo), Mokokoka (Sribinza), Mokuka (Bondjo), Mokundu, Molungapo (Lolenga), Monkuka (Bokiba), Moteni, Mubesebese (Kitembo), Mubeya, Muela-Momontomba, Muele, Mukua (Mbaamba), Mukulamukulo (Laali, Ndasa, Nzabi, Tié, Yaa), Ngada, Ngbada, Ngbanda (Babua), Ngbanda motani, Ngbanda pembe, Ngbanda penza, Ngela (Babua), Ngele, Ngoka (Babinga), Nguada (Gombe), N'nuun ba kuyu (Laadi), N'nuungu wa saangi (Laadi), Ntemba (Mbimo), Nubeya (Kinyanga), Okoka (Mbôsi), Okuka (Mbaamba), Tembe hani (Sanga), Tshitumbula benzo; Gabon: Ekaku (Mpongwe, Ngoka), Eke-e (Pahoun), Ekese, Mukoka (Bavore, Ngoka), Mukuka (Bavungu, Eshira, Ngoka).

Notes. *T. hensii* includes material (*Bequaert* 49, 6670, *Cambridge Congo Expedition* 286, 351, *Christiaensen* 1877, *Gutzwiller* 1465, 2064, 2127, 2166, 2258, 2329, 2414, 2532, 3773, *Hart* 731, *Lebrun* 5618, *Leonard* 1689, 1873, 2896, *Pierlot* 627, 825, 2413, 2609, 3266, *Troupin* 2476, 3471, 3541, 3846, 3995, 4030, 4281, 4312, 9323) suggested to belong to a new species, *T. collina* Champl. (Champluvier, pers. comm.), not recognised here (see previous discussion; Fig. 2.13I,J).

Thomandersia laurentii De Wild., Miss. Em. Laurent, 180 (1905); De Wild., Ann. Mus. Congo, ser. 5, Bot. 1: 313 (1906); Mildbr., Wiss. Erg. Zweit. Deut. Zentr.-Afr.,-Exp., Bot., 2: 12 (1922); Heine in Fl. Gabon., 13: 143-155 (1966); Heine, Bull. Jard. Bot. Etat, 36: 207-248 (1966). Type: Democratic Republic of Congo, Kimuenza, October 1900, *Gillet* 1700 (lectotype BR, designated Heine, Bull. Jard. Bot. Etat, 36: 207-248, 1966).

Shrub, 0.5-5 m in height. *Stems* slender, dark brown, downy, becoming glabrous and shiny. *Petioles* narrow, with many short, dark hairs, that of larger leaves 10-50 mm long, of smaller leaves 5-10 mm. *Leaves* anisophyllous, the smaller of each pair often caducous; lamina, of the larger of each pair 50-180 x 20-80 mm, lanceolate-elliptic to ovate or obovate, apex gradually and very narrowly acuminate, the tip pointed, cuspidate, base rounded-cordate, with 3-5 pairs of lateral veins, of the smaller of each pair 10-40 x 10-25 mm, elliptic-ovate, orbicular or cordiform, apex acuminate, base rounded-cordate, with 2-4 pairs of lateral veins, thinly papery or slightly leathery, with a few large glands, visible as dark dots, especially towards the midrib, with sparse, scattered hairs above and scattered downy yellow hairs below, veins with dense downy hairs below and sparser hairs above; margin entire, slightly crinkled, or

with a few obscure teeth towards the apex, often damaged by insects; midrib embedded above, prominent beneath, lateral veins embedded above, prominent beneath, tertiary venation reticulate; tuft domatia few, generally on the smaller leaves, whitish. *Inflorescence* axillary or terminal, 30-160 mm long, slender but dense, sometimes curved; rachis dark brown with soft, downy, pale pubescence. *Bracts* 1-1.5 mm long, ovate-lanceolate. *Bracteoles* minute (<0.5 mm), triangular-ovate. *Flowers* usually opposite, pedicellate; pedicels 1-2 mm long, slender. *Calyx* 1-2 mm long, campanulate, purple-brownish, bearing a single small gland covered with pale hairs; lobes 0.5-1 mm long, ovate-triangular, rounded, spreading (not overlapping), glandular and downy, with very marked, pale, ciliate pubescence at margin. *Corolla* tough and leathery, bilabiate, yellowish-white to pinkish, reddish, brownish or purplish; tube 5-12 mm long, glabrous outside, with dense, long hairs inside; lobes 3-5 mm long, not spreading, with dense glandular hairs all over, margin entire or slightly crinkled and conspicuously yellowish-ciliate (furry). *Staminode* 2 mm long, acicular, hairy, inserted 4 mm from base of corolla. *Stamen* filaments inserted 5 mm (the smaller pair) or 6 mm (larger pair) from base of tube, arching inwards, didynamous, the shorter filaments c.4 mm long, the longer c.6 mm, with long, spreading hairs, especially towards the base; anther locules reniform, broader towards the apex, one slightly larger than the other, 1-1.2 mm long, pale. *Style* 12-13 mm long, c.0.2 mm diameter, subexserted, soon caducous, glabrous, slightly glandular at base. *Stigma* cylindrical, glabrous. *Gynoecium* c.1.5 mm long, conical-ovoid, yellow, densely covered with short, furry yellow hairs, fleshy. *Capsule* 8-10 x 5-7 x 3-4 mm, ovoid-conical, with a rounded point, green, becoming brown-black, with very short, yellow, appressed hairs, especially towards the base, becoming glabrous, with thin (0.2-0.5 mm) wall; subtended by the accrescent calyx, rotate-star-shaped, 4-5 mm in

diameter, the lobes spreading. *Seeds* one per valve, 2-3 mm diameter, spheroidal, yellow to dark orange-brown, covered with triangular-ovate overlapping scales (Illustration: Fig. 2.15).

Distribution. Western Democratic Republic of Congo, Republic of Congo (Fig. 2.16).

All other collections seen. **Democratic Republic of Congo.** Bateke, 9 December 1982, *Lejoly* 82/714 (BR); Bombo River, 27 May 1976, *Breyne* 2997 (BR); Djelo-Binza, 28 February 1947, *Jans* 410 (BR); Kimbanza, 19 December 1978, *Pauwels* 6086 (BR, WAG); Kimuenza, May 1901, *Gillet* s.n. (BR), 27 August 1956, *Carlier* 78 (BR), 6 February 1957, *Robyns* 4279 (BR, K), 8 September 1966, *Breyne* 233 (BR, MO); Kinkoko, 10 September 1964, *Pauwels* 4664 (BR); Kinshasa, August 1902, *Gillet* s.n. (BR); Leverville, July 1906, *Sapin* s.n. (BR); Righini, 28 June 1961, *Evrard* 6335 (BR, MO); Sabuka, 6 April 1972, *Evrard* 6937 (BR); Stanley Pool, 23 October 1903, *Laurent, E & M.* s.n. (BR); locality unknown, February 1920, *Pobeguin* 120 (P). **Republic of Congo.** Couloir, 3 August 1902, *Chevalier* 5005 (P); Fort Rousset, 4 June 1961, *Descoings* 7513 (P); Lefini, 12 October 1991, *Thomas & Harris* 8536 (MO); Reneville, 31 July 1912, *Chevalier* 27536 (P); Sedek Forest, 9 June 1919, *Vermoesen* 2438 (BR); Tchekapika, 14 July 1961, *Descoings* 7808 (P); Tonkama, September 1954, *Koechlin* 2822 (P). **Country unknown.** *Staner* 113 (BR).

Habitat. Wet and gallery forests and forest edges, often near rivers.

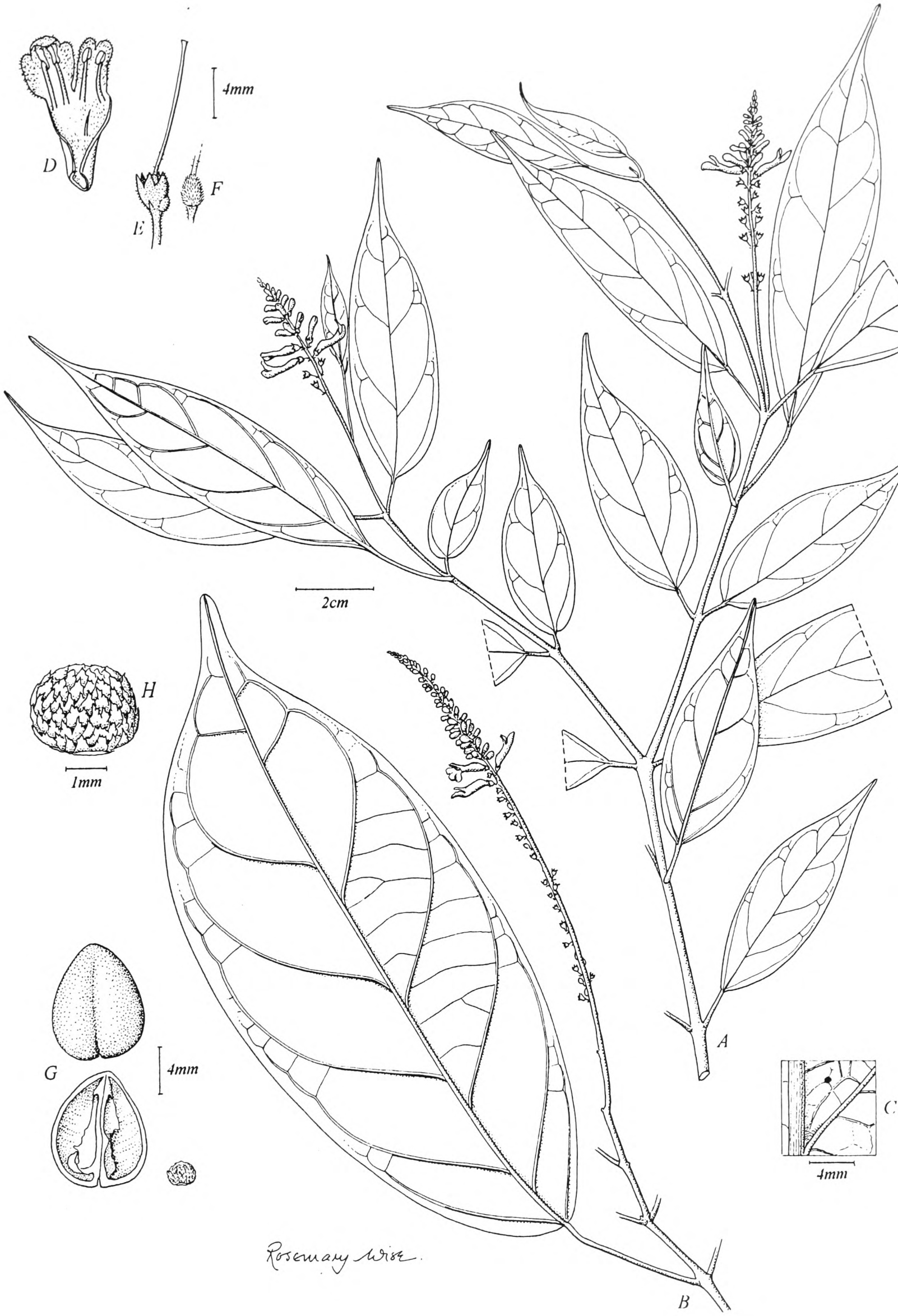


Figure 2.15 *Thomandersia laurentii.* A, B. habit and inflorescence. C. underside of leaf. D. floral dissection. E. calyx. F. gynoecium. G. fruit and seed. H. seed. (A, C – F from *Breyne* 233; B, G, H from *Robyns* 4279).

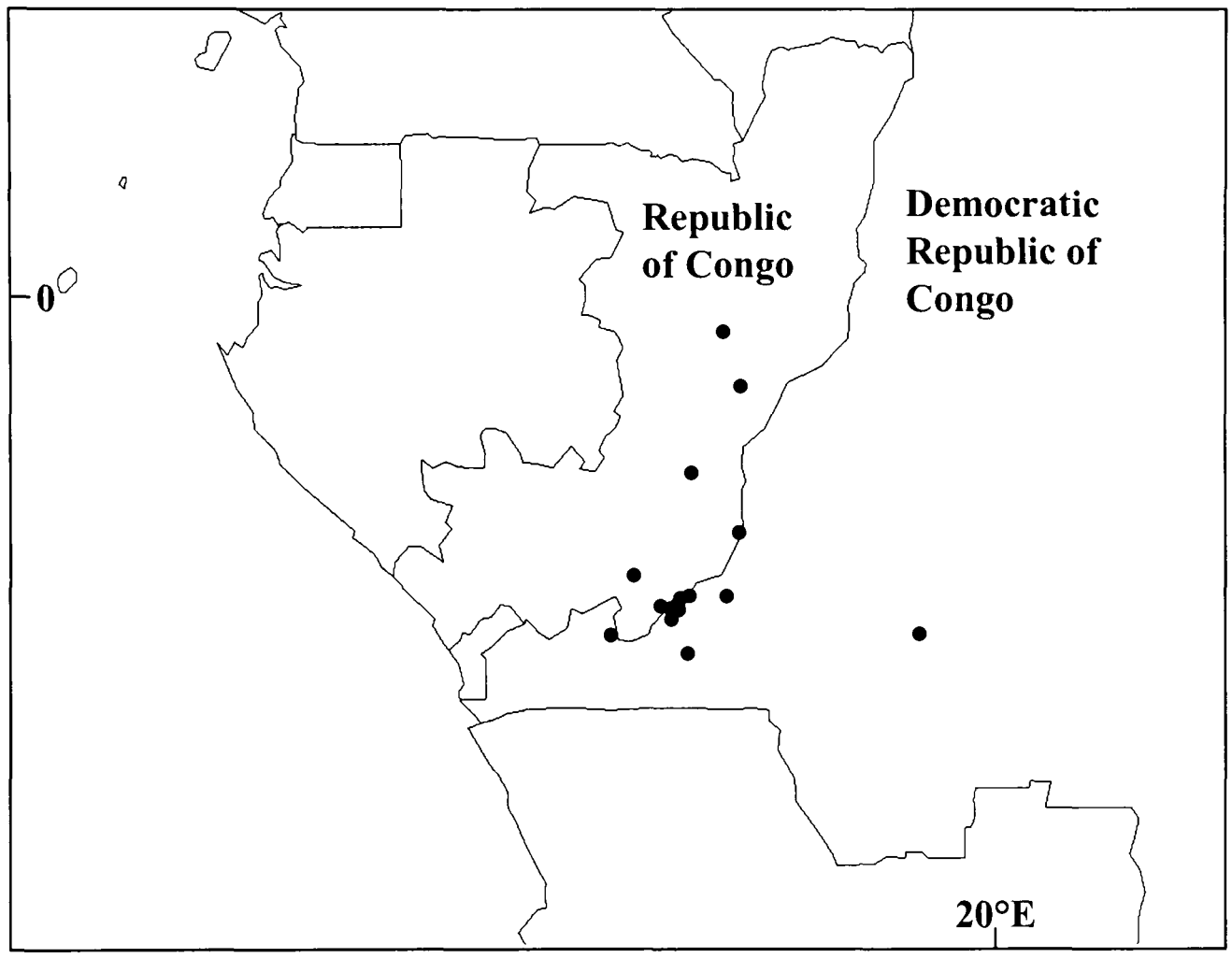


Figure 2.16 Map of western central Africa to show distribution of collections of *Thomandersia laurentii*.

Thomandersia laurifolia (T.Anderson ex Benth.) Baill., Hist. Pl., 10: 456 (1891); Lindau in Engl., Bot. Jahrb. Syst. 13: 55 (1894); Lindau in Engl. & Prantl, Nat. Pflanzenfam. 4(3b): 325 (1895); Burkill & C.B.Clarke in This.-Dyer, Fl. Trop. Afr., 5: 119 (1899); Hutch. & Dalz., Fl. W. Trop. Afr., 2: 255 (1931); Heine in Hutch. & Dalz., Fl. W. Trop. Afr., ed. 2, 2: 413 (1963); Heine in Fl. Gabon., 13: 143-155 (1966); Heine, Bull. Jard. Bot. Etat, 36: 207-248 (1966). Type: Cameroon, Estuaire du Cameroun, January 1863, *Mann* 2207 (lectotype K, designated Heine, Bull. Jard. Bot. Etat, 36: 207-248, 1966).

Scytanthus laurifolius T.Anderson ex Benth. in Benth. & Hook.f., Gen. Pl. 2: 1093 (1876); Benth., in Hooker's Icon. Pl., 13: 8 (1877). Basionym. Type not designated. [In the original species description no specimens were cited and no type chosen (Bentham, 1876), but this did not invalidate publication before 1958 (IBC et al., 2000). The following year, in *Hooker's Icones Plantarum*, Bentham (1877: 8) cited the collections "Old Calabar, Gaboon and Cameroon Rivers, *G. Mann*" which include some specimens now determined as *T. hensii* and others determined as *T. laurifolia*. None of these specimens were designated as lectotypes (Brummitt, pers. comm.). In his revision Heine (1966a) therefore chose a lectotype, *Mann* 2207, from those collections cited by Bentham. *Mann* 2207 was selected because it was of the same species as most material determined as *T. laurifolia* at the time, despite the fact that Bentham (1877) illustrated *Mann* 2321 (now determined as *T. hensii*) in *Hooker's Icones* (Heine, 1966a).]

Straggling shrub or small tree, 1.5-9 m in height, leaves sometimes clumped towards the ends of branches (Fig. 2.17A). *Stems* slender, straight, terete, flattened at nodes,

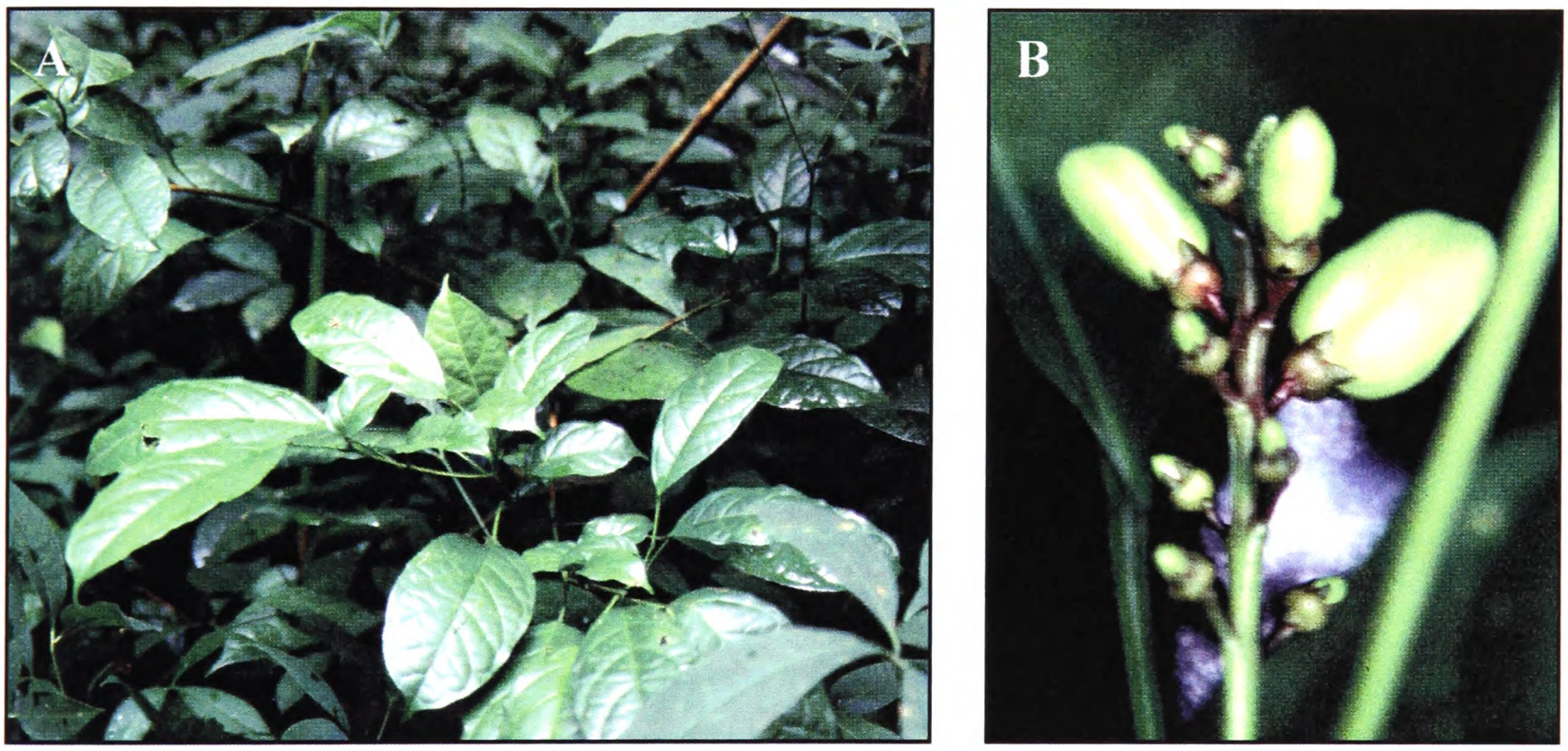


Figure 2.17 *Thomandersia laurifolia.* A. foliage. B. immature fruits.

purplish-green, more or less glabrous; bark pale brown, flaky. *Petioles* green, thickened and darker at both ends, the dark coloration often extending to the base of the midrib, channelled above, especially towards the base of the leaf, glabrous or with very short, sparse, pale hairs, that of larger leaves 10-50 mm long, of smaller leaves 3-8 mm. *Leaves* anisophyllous; lamina, of the larger of each pair 70-240 x 30-110 mm, oblong-elliptic, ovate or obovate, apex shortly acuminate, often cuspidate, base cuneate-rounded, with 3-6 pairs of lateral veins, of the smaller of each pair 25-100 x 15-50 mm, oblong-elliptic, ovate or cordiform, apex shortly acuminate, cuspidate, base rounded-subcordate, with 2-4 pairs of lateral veins, leathery, with many large (up to 1 mm), round glands, especially towards the midrib at base, and smaller gland dots visible with a lens, bright green above, paler below, more or less glabrous above and below, veins glabrous or with short, sparse hairs below; margin entire, occasionally undulate or toothed towards the tip; midrib pale green, drying reddish, embedded or flat above, prominent beneath, lateral veins prominent beneath, tertiary venation lax reticulate-scalariform, prominent below and obscure above; tuft or pit domatia sometimes present in the axils between the lateral veins and midrib, towards the base of the leaf. *Inflorescence* terminal or axillary, 120-250(-450) mm long, straight or curved, the flowers well-spaced, lax; rachis smooth, without irregular ridges, greenish-violet or brown, more or less glabrous or with a few short, erect, dark hairs. *Bracts* c.1 mm long, triangular, slightly hairy, borne at a right-angle to the rachis. *Bracteoles* tiny, triangular-lanceolate. *Flowers* faintly sweet-scented, opposite, occasionally in whorls of three, pedicellate; pedicels 2-5 mm long, slender and curvy, becoming stout when accrescent; buds purplish. *Calyx* 2-3 mm long, campanulate-rotate, violet-green, fleshy, glandular, bearing a single, gently-rounded glandular bump; lobes 1-1.5 mm long, triangular-ovate, spreading (not overlapping), the adaxial

lobe larger, erect and pointed, slightly downy, with palely ciliate margin. *Corolla* tough and leathery, bilabiate, greenish-yellow or white; tube 7-10 mm long, purplish and more or less glabrous outside, with many short glandular-capitate hairs inside; lobes often tinged or striped purple, 4-5 mm long, rounded, more or less spreading, with small glandular hairs inside and out, especially in bud, margin slightly undulate. *Staminode* small (<1 mm long), inserted c.2 mm from base of corolla, white, curly, with a few long hairs. *Stamen* filaments inserted 3-4 mm from the base of the tube, the shorter filaments c.3 mm long, the longer c.4 mm, white, with a few small glandular hairs, especially towards the base; anther locules rounded, one slightly larger than the other, 1-1.2 mm long, pale yellow. *Style* 8-9 mm long, 0.3-0.4 mm diameter, white, glabrous, slightly glandular at base. *Stigma* slightly wider than style (<0.5 mm diameter), cupular, asymmetric but not lobed, papillate. *Gynoecium* 2-3 mm long, flattened ovoid-conical, glabrous, fleshy at base. *Capsule* 15-20 x 10-15 x 4-8 mm, rounded, oblong-ovoid, bright, shiny pale green (Fig. 2.17B), violet-tinged at apex, becoming brown when ripe, glabrous, sometimes dehiscing slightly at top and bottom in the septicidal plane as well as loculicidally, with wall usually much thinner than in *T. hensii* (0.5-1.2 mm); subtended by the accrescent calyx, c.5 mm diameter, rotate, very open, the lobes sometimes even bent backwards. *Seeds* 2-3 per valve, 2-5 mm diameter, spheroidal, black or very dark reddish-brown when mature, with a distinct, pale hilum, covered with spiral rings of scales or roundish warts (Illustration: Fig. 2.18).

Distribution. Cameroon, Democratic Republic of Congo, Equatorial Guinea, Gabon, western Republic of Congo (Fig. 2.19).

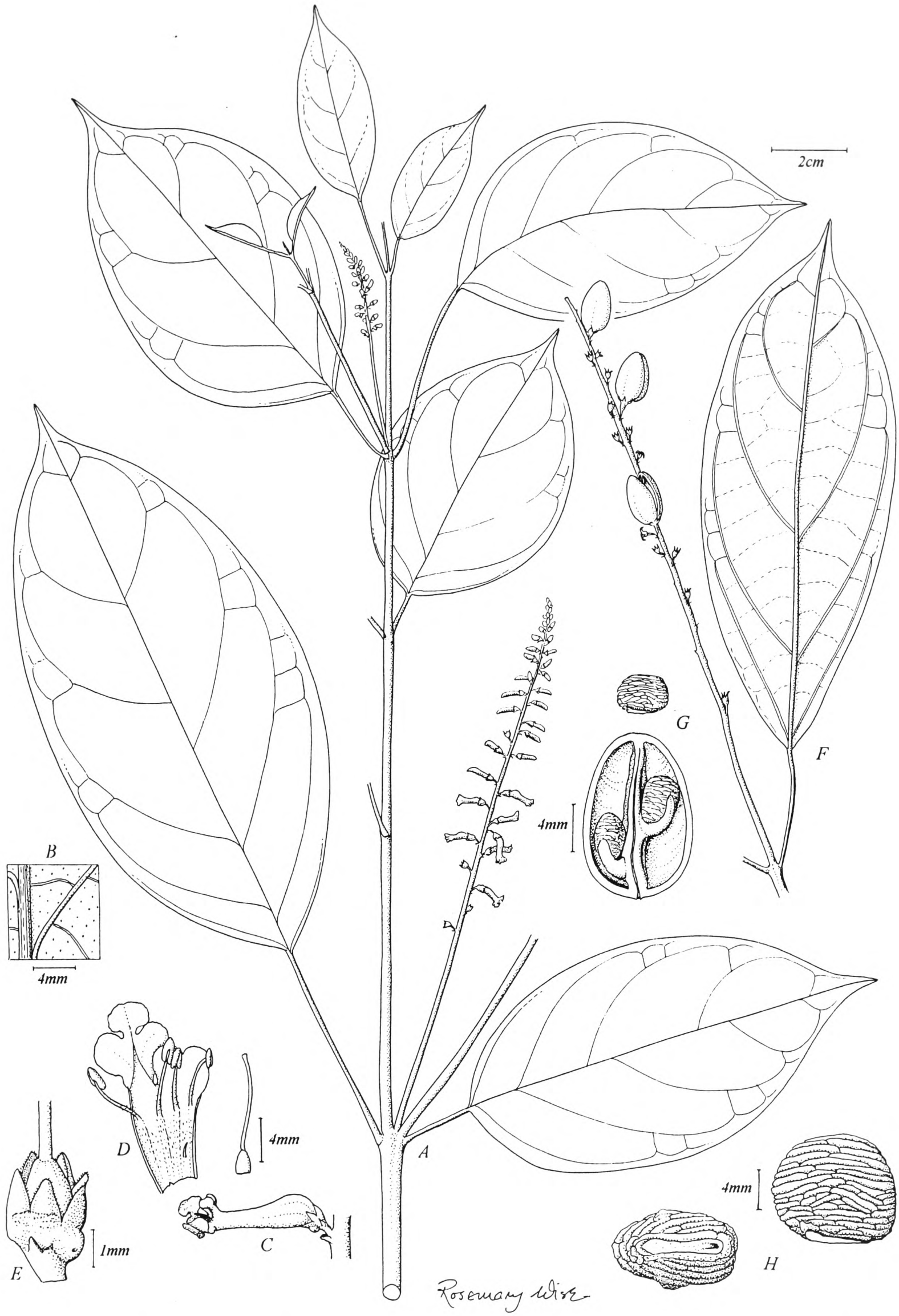


Figure 2.18 *Thomandersia laurifolia.* A. habit and inflorescence. B. underside of leaf. C. flower. D. floral dissection. E. calyx. F. infructescence. G. fruit and seed. H. seed. (A from *Hladik* 2619; B – H from *Louis* 1206).

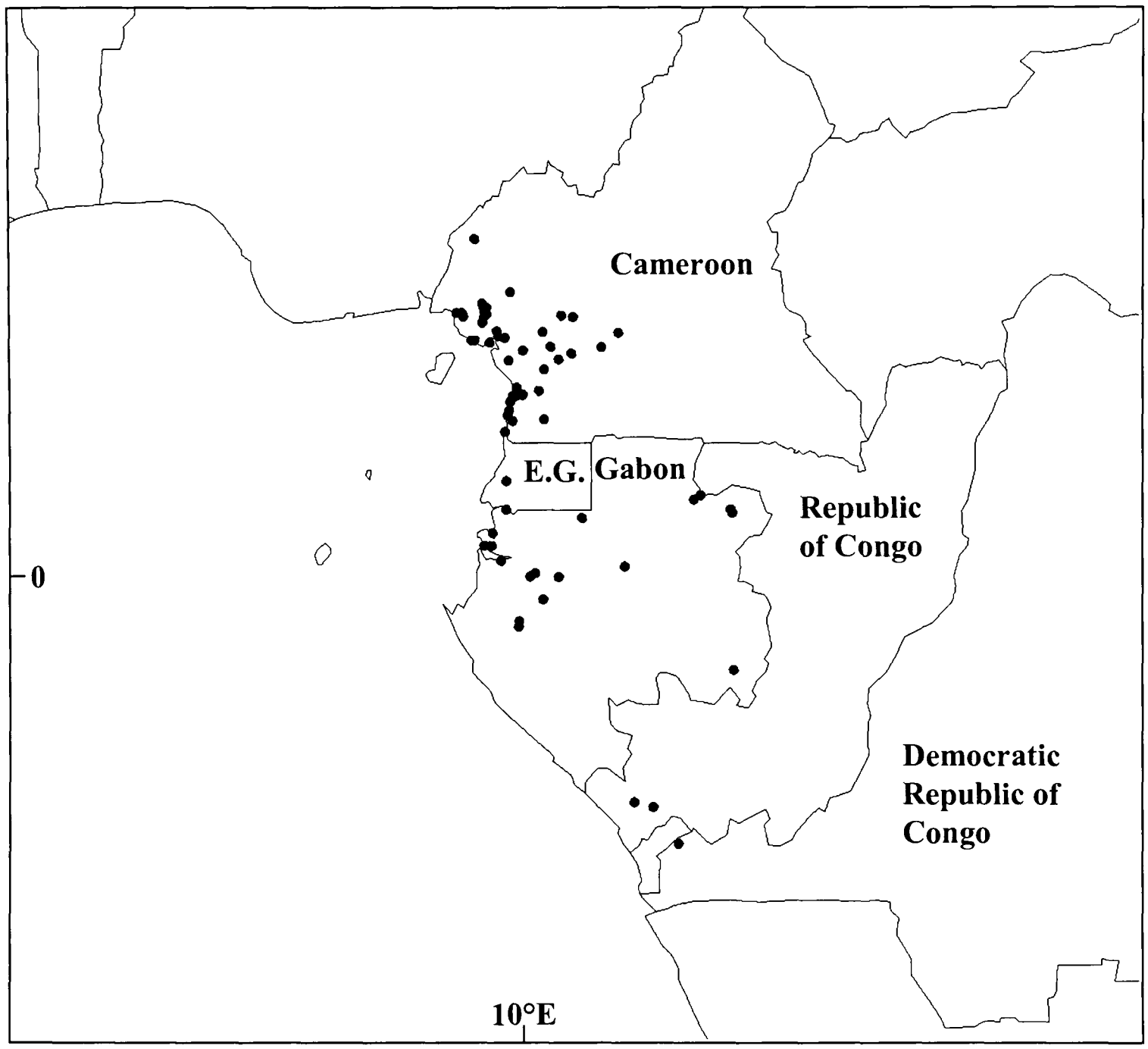


Figure 2.19 Map of western central Africa to show distribution of collections of *Thomandersia laurifolia.* E.G., Equatorial Guinea.

All other collections seen. **Cameroon.** Agborkem-Tabo, 4 June 1975, *Letouzey* 13733 (K, P, YA); Banga, 9 January 1956, *Binuyo & Daramola* 35167, 35168 (FHO, P); Batanga, 29 July 1895, *Bates* 324 (K); Bidou I, 9 April 1969, *Bos* 4330 (BR, MO, P, YA), 6 January 1970, *Bos* 6080 (BR, K, P, YA); Bipaga II, 28 December 1982, *De Kruif* 976 (WAG, YA); Bipindi, *Zenker* 989, 1851 (P), 2739 (BR, K), 3543, 4027, 4392, 4702 (BR, MO, P); Bombe, 21 April 1959, *Daramola* 29808 (BR, K), 30 January 1987, *Manning* 1490 (MO), 25 October 2002, *Wortley* 4 (K, SCA, YA); Campo, 26 March 1968, *Letouzey* 9207 (K, P, YA), 4 January 1983, *Van der Zon* 1996 (WAG, YA); Carbonnier, 1 August 1955, *De Wit* 253/6036 (WAG); Dikolo, 14 October 1997, *Tchouto* 1691 (SCA); Dikulu, 13 December 1993, *Cable, Cheek, Cawe, Gawshaw & Norris* 519 (K, MO, SCA); Douala, 6 February 1927, *Dalziel* 8193 (K), 10 February 1962, *Breteler, De Wilde & Leeuwenberg* 2588 (BR, P, YA), 2 April 1965, *Leeuwenberg* 5337 (BR, MO, P, YA); Douala-Edea, *Rose* 4 (P); Ebone-Yabassi, 27 December 1967, *Bamps* 1638 (BR, P, YA); Ediki, 12 December 1986, *Lisowski* B3374 (BR); Ediki-Mbonge, 12 December 1986, *Lisowski* B3423 (BR), *Lejoly* 86/896 (BR, YA); Ekombe-Mokoko, 21 April 1994, *Tchouto* 1150 (SCA), 24 April 1994, *Akogo* 297 (K, SCA), 25 April 1994, *Mbani* 344 (SCA); Eseka, 8 June 1953, *Mbarga* SRFK171896 (P, YA), 21 November 1963, *De Wilde & De Wilde-Duyfjes* 1239 (BR, K, MO, P, YA), 22 March 1964, *De Wilde & De Wilde-Duyfjes* 2199 (WAG), 9 November 1964, *De Wilde & De Wilde-Duyfjes* 3884 (BR, P); Grand Batanga Road, 24 October 1968, *Bos* 3123 (P, WAG, YA), 31 October 1968, *Bos* 3183 (P, WAG, YA); Ipengedje, 13 March 1975, *De Wilde* 8054 (BR, MO, YA); Japoma, 21 January 1933, *Exell* 744 (BR, K); Kendongi, 17 December 1959, *Latilo* 43814 (FHO, K, YA); Littoral, 18 February 1988, *Nemba, Thomas, Strohmeier & Mambo* 821 (MO); Lolabe, 22 December 1980, *Beentje* 1536 (WAG, YA); Longji, 29

April 1965, *Leeuwenberg* 5654 (BR, MO, P, YA), 11 March 1978, *Lowe* 3596 (K, YA); Mabeta, 19 October 1997, *Tchouto* 1758 (SCA); Makak, October 1938, *Jacques-Felix* 2280 (MO, P), 27 January 1944, *Letouzey* SRFK1149 (P); Makgom, 20 January 1956, *Npom* 178 (YA); Mangombe Forest, 17 April 1952, *Letouzey* SRFK1571 (P, YA); Marienberg, 5 April 1984, *Polhill, Kirkup & Thomas* 5213 (K, YA); Masok, 27 March 1965, *Leeuwenberg* 5209 (BR, MO, P, YA); Massif des Mammelles, 23 April 2001, *Tchouto* 3246 (YA); Mbode Bay, 7 July 1970, *Bos* 7028 (BR, P, YA); Mbongo, June 1973, *Guillon* s.n. (YA); Mongo, June 1917, *Fleury* 33299b (P); Munyenge, 21 March 1993, *Tchouto* 581, (K, SCA); Muyuka, 10 October 1927, *Hedin* 1832a (P); Ndom, 18 March 1984, *Nkongmenech* 698 (YA); Ngong, 12 December 1973, *Letouzey* 12342 (BR, P, YA); Razel, 1955, *Elias* 2066/2 (BR, K, P, YA), 18 January 1958, *Letouzey* 593 (YA), 1897/1 (K, P); Songbong, 11 March 1965, *Leeuwenberg* 5107 (BR, P, YA); Yelfoume, June 1917, *Fleury* 33345 (P); locality unknown, *s.c.* 490 (FHO). **Democratic Republic of Congo.** Ganda-Sundi, *De Briey* s.n. (BR). **Equatorial Guinea.** Cogo, 11 July 1902, *Debeaux* 388 (P); Como River, July 1896, *Bates* 498 (BM, P); Gaboon River, September 1861, *Mann* s.n. (K). **Gabon.** Belimba, 12 November 1964, *Hallé* 3135 (K, P); Belinga, 30 December 1964, *Hallé* 3619 (P), 8 June 1966, *Hallé* 3768 (BR, P), 17 June 1966, *Hallé* 3939 (P); Bellevue, 10 January 1987, *Dibata* 30 (MO, WAG); Bikele, 7 December 1983, *Louis, Breteler & De Bruijn* 1206 (BR, K, MO, WAG); Ekakou, *Griffon du Bellay* 138 (P); Etouk, 28 September 1983, *Floret & Louis* 1515 (WAG), 2 November 1983, *Louis, Breteler & De Bruijn* 293 (WAG); Lac Onangue, 28 September 1953, *Guillery* 1129SRF (P), *Pobeguin* 189 (P); Libange, 24 February 1881, *Soyaux* 227 (K, P); Libreville, April 1871, *Jolly* 150 (P), 1 May 1917, *Fleury* 33681 (P), 15 May 1917, *Fleury* 33663 (P); Mbela, 4 January 2001, *Wieringa, Jongkind, Schoonhoven &*

Mbombet 3883 (WAG); Ndjole, 20 April 1963, *Hallé* 1743 (BR, P); Moyen-Ogooue, April 1883, *Thollon* 243 (P); Mpassa, 11 March 1975, *Hladik* 2619 (P), 30 March 1977, *Florence* 135 (P); Nkogo, 14 August 1912, *Fleury* 26337 (P), 17 August 1912, *Fleury* 26368 (P); Nouna, 30 October 1964, *Hallé* 2914 (P); Ogooue River, *Leroy* s.n. (P); Oguemoue, 21 November 1953, *Guillery* 1208SRF (P); Oveng, 7 May 1985, *Reitsma, J.M. & B. Breteler & Louis* 879 (WAG); Plantation Stephane, 9 October 1912, *Fleury* in *Chevalier* 26988 (P); Remboue, 20 October 1951, *Sebire* 465 (P); locality unknown, 12 April 1963, *Hallé* 1628 (K), *Leroy* s.n. (P). **Republic of Congo.** Dimonika, 24 November 1978, *Cusset* 516 (P), 25 November 1978, *Cusset* 572 (P), 27 April 1986, *De Foresta* 949 (P); Kakamoeka, 22 June 1977, *Makany* s.n. (P); Siko, 8 February 1966, *Farron* 5008 (P). **Country unknown.** 27 September 1896, *Klaine* 161 (P), April 1899, *Klaine* 1479 (P), 21 November 1900, *Klaine* 2063 (P), 16 October 1901, *Klaine* 2397 (P), 22 September 1902, *Klaine* 3071 (P), 13 January 1968, *Hallé & Villiers* 4386 (P), *Duparquet* s.n. (P), *Klaine* 357 (P).

Habitat. Disturbed primary and secondary forest, frequently along paths, roads, coasts, on mountains, in clearings or ravines, on damp lateritic, clayish, or sandy soils.

Vernacular names. Cameroon: Enkoga (Bagielli), Evenzegne (Pahoun), Evezegen, Liabat (Bassa), Lingoga (Bassa), Nditik (Yaounde), Nkitik (Yaounde), Ofes-afan (Boulou, Yaounde), Ofes-afane; Gabon: Ekakou, Ekeze (Pahouin), Kan, Ke (Fang, Pahoun), Ken (Fang), Kess (Fang), Kezek (Fang), Nke (Fang).

Incertae sedis The following collections were sterile or in poor condition so that the species identity could not be conclusively determined. **Cameroon.** Mont d'Elephant, 19 October 2002, *Wortley, Nana, Mackinder, Kiam & Mbamba* 3 (K, YA).

Democratic Republic of Congo. Mayombe, *Gossweiler* 7850 (MO); Yangambi, 14 July 1955, *Devred* 2201 (MO), *Gillet* 3795 (BR). **Gabon.** Medonou, 3 February 1986, *Reitsma, J.M. & B. & Louis* 1819 (MO, WAG). **Republic of Congo.** Komono, 19 January 1965, *Bouquet* 1006 (P); Mandili, 11 October 1965, *Bouquet* 1784 (P); Mayoko, 24 May 1965, *Bouquet* 1422 (P).

Possible hybrid specimens Several collections were difficult to determine to species level, appearing to display a combination of characters intermediate between species, and were collected in regions where the distributions of two or more species overlap (Fig. 2.2). **Democratic Republic of Congo.** Mayombe, 28 November 1990, *Dowsett-Lemaire* 1371 (BR), 1 December 1990, *LaCroix* 4984 (BR, K, MO). **Gabon.** Bakouamba, 2 December 1993, *Breteler, F.J. & B.J.M - Klein Breteler* 12492 (WAG); Bongo, 16 March 1988, *De Wilde, Jongkind & Dibata* 9400 (BR, K, MO, WAG); Haut-Ogooue, 12 October 1970, *Breteler* 6851 (BR, MO, WAG), *Thollon & Savorgnan de Brazza* 212 (P); Massif du Chaillu, 4 November 1983, *De Wilde, Arends, Louis, Bouman & Karper* 380 (BR, MO, P, WAG); Mayumba, 3 November 1907, *Le Testu* 1213 (BR); Moanda, 2 October 1970, *Breteler* 6735 (BR, MO, P, WAG). **Republic of Congo.** Dimonika, 21 November 1986, *Lejoly* 86/331 (BR).

Chapter 3. Floral and wood anatomy of *Thomandersia*

3.1 Introduction

Classifications should be based on monophyletic groups that can be diagnosed by unique combinations of characters (Hennig, 1966; Backlund & Bremer, 1998; Carine & Scotland, 2002). Morphological characters clearly establish the monophyly and diagnosability of *Thomandersia*, but have proven more problematic for its higher-level classification in Lamiales, because subjectively-chosen characters may suggest competing taxonomic arrangements. In classifying *Thomandersia,* emphasis has most often been placed on retinacula, and thus it has been considered a member of Acanthaceae (Bentham, 1876; Lindau, 1894; 1897; Hutchinson & Dalziel, 1931; Heine, 1963; 1966a). The preliminary molecular sequence-based analyses in Chapter 1 imply that this arrangement does not reflect phylogenetic relationships, and therefore that the retinacula of Acanthaceae and *Thomandersia* may not be homologous structures.

In this chapter, a range of morphological characters of *Thomandersia* is re-examined, through detailed anatomical investigation in the context of recent phylogenetic hypotheses for Lamiales (Olmstead et al., 2001; Bremer et al., 2002). Objective anatomical study should make it possible to determine whether retinacula are a primary homology of Acanthaceae and *Thomandersia*, and to identify additional characters of *Thomandersia* that show primary homology, and potential secondary homology, to those of other groups in Lamiales.

The chapter first outlines previous classifications of *Thomandersia*, and the morphological characters on which they were based. New observations of wood, inflorescence, flower, pollen, fruit and seed morphology and anatomy in *Thomandersia* are presented. A detailed investigation into the nature of retinacula is conducted, and the anatomy of another potentially useful character, extrafloral nectaries, is described here for the first time. The results are discussed in the context of the morphology, anatomy and systematics of Lamiales. The characters of *Thomandersia* are then interpreted as plesiomorphies, autapomorphies or potential synapomorphies at the level of Lamiales. In particular, comparisons are drawn between *Thomandersia* and suggested candidates for its sister group.

3.2 Review of previous work

3.2.1 Anatomical investigations of Lamiales. Lamiales presently contains 22,000 species, approximately 12% of all eudicots (Stevens, 2001; Olmstead, in progress), encompassing a wide range of morphological characters. Lamiales has been the subject of many comparative anatomical studies (Van Tieghem, 1908; Bremekamp, 1926; Rao, 1953; Bremekamp, 1965; Rudall, 1981; Armstrong, 1985; Scotland et al., 1994; Kampny, 1995; Donoghue et al., 1998; Schonenberger & Endress, 1998; Schonenberger, 1999; Manktelow, 2000; Scotland & Vollesen, 2000; Bremer et al., 2001; Endress, 2001). Pollen morphology has been studied extensively for its potential systematic applications (Erdtmann, 1945; 1952; Raj, 1961; Straka & Ihlenfeldt, 1965; Goldblatt & Keating, 1976; Raj, 1983a; 1983b; 1985; 1987; Bretting & Nilsson, 1988; Mathew & Shah, 1988; Minkin & Eshbaugh, 1989; Chadwell et al., 1992; Abu & Cantino, 1993; Bove, 1993), and wood anatomy provides useful diagnostic characters for families within the order (e.g. Den Outer & Van Veenendaal,

1983; Carlquist, 1986; 1987; Carlquist & Zona, 1988; Gasson & Dobbins, 1991; Carlquist, 1992; 1996; Dos Santos & Miller, 1997; Baas et al., 1998). For example, Calceolariaceae are distinctively rayless, lack axial parenchyma and have vessels in radial multiples, while Stilbaceae are distinguished by scalariform perforation plates, thick-walled fibres and scarce vasicentric axial parenchyma (Carlquist, 1992).

Like many asterids, Lamiales are characterised by vessel elements with simple perforations, pentamerous flowers, a single style, anatropous ovules and cellular endosperm. Additional plesiomorphic morphological and anatomical characters of Lamiales include a distinct, sympetalous calyx and corolla, dorsifixed anthers, a vascularised disk nectary, unitegmic ovules with a hypostase, and both micropylar and chalazal endosperm haustoria (Yamazaki, 1974; Cantino, 1982; Dahlgren, 1983; Thorne, 1992; Wagenitz, 1992; Judd et al., 1994; Stevens, 2001). These characters are considered to unite the order, although they may be reversed in derived groups. With the exception of the relatively basal branches Oleaceae and Tetrachondraceae, and some Carlemanniaceae, many Lamiales are also distinguished by bilabiate flowers with a bilobed upper labium and trilobed lower one, two or four stamens and three or one staminodes respectively (Dahlgren, 1975; Takhtajan, 1980; Judd et al., 1999; Thorne, 2000; Stevens, 2001).

Five families of Lamiales have been suggested as potential sister groups to *Thomandersia* in morphological classifications and preliminary molecular phylogenetic analyses: Acanthaceae or Pedaliaceae based on morphological classifications (Bentham, 1876; Baillon, 1891; Lindau, 1894; Hutchinson & Dalziel, 1931; Bremekamp, 1942; Heine, 1966a); Bignoniaceae, Schlegeliaceae or

Verbenaceae on preliminary molecular phylogenetic analysis (Chapter 1). The morphological and anatomical characters of these families are discussed briefly below.

Acanthaceae are distinguished by wood with included phloem, flowers often subtended by large bracts and bracteoles, calyx persistent, staminodes often absent, anthers often asymmetric, a bilobed, non-papillate stigma, axile placentation with two to ten ovules in two rows in each locule, endosperm absent (except Nelsonioideae), flat seeds, explosively dehisced, and retinacula. Retinacula are, however, absent from the basal branches Mendoncioideae, Nelsonioideae and Thunbergioideae (De Jussieu, 1789; Lindau, 1894; Van Tieghem, 1908; Bremekamp, 1965; Long, 1970; Metcalfe & Chalk, 1979; 1983; Watson & Dallwitz, 1992; Scotland & Vollesen, 2000).

Pedaliaceae have flowers with axillary nectaries, didynamous stamens, confluent anther thecae, 5-10-colpate pollen, a style that is indurated at the base, stigma with two, broad, wet lobes, thin endosperm and fruit usually with hooks or prickles (Stapf, 1895; Manning, 1991).

Bignoniaceae are always woody, and characterised by scalariform perforation plates, a calyx often open in bud, sagittate anther thecae with an amoeboid tapetum, vascular bundles in the septum as well as the wall of the gynoecium, placentas divided into two per locule, ascending, orthotropous or hemianatropous ovules, endosperm absent, large and persistent cotyledons, and berries or elongate capsules containing multiple, flat seeds, edged with wings or hairs (Gentry, 1974a; 1974b; 1977; 1980; 1982;

Armstrong, 1985; Gentry, 1992a; Watson & Dallwitz, 1992; Gentry, 1997; Judd et al., 1999; Manning, 2000; Stevens, 2001).

Schlegeliaceae are distinguished by the absence of a disk nectary, persistent endosperm, berry-like fruits and seeds without wings, although vestigial seed wings have been noted in *Exarata* (Armstrong, 1985; Gentry, 1992b; Burger & Barringer, 2000; Stevens, 2001; Stevens et al., 2001). Members of Schlegeliaceae have previously been classified in Bignoniaceae or Scrophulariaceae (e.g. Bureau & Schumann, 1896-1897; Williams, 1970; Gentry, 1971; 1975; 1976; Gentry, 1982; Barringer & Gentry, 1995; Gentry, 1997).

Verbenaceae are characterised by square stems, a persistent calyx, imbricate corolla, didynamous stamens, pollen exine thickened towards the apertures, an annular disk nectary, gynoecium subdivided into four locules by false septa, two ovules per carpel, attached at the margins of the locules, a bilobed, wet-type stigma with conspicuous receptive tissue, endosperm absent, and drupaceous or schizocarpic fruits (Cantino, 1982; 1990; Watson & Dallwitz, 1992; Judd et al., 1999; Sanders, 2001; Stevens, 2001).

3.2.2 Treatments of Thomandersia. *Thomandersia* has been classified in a number of families (e.g. Bentham, 1876; Lindau, 1897; Bremekamp, 1942; Heine, 1966a; Sreemadhaven, 1976). The initial description incorporated many widespread features of Lamiales, such as a pentamerous calyx and corolla, superior ovary and adnate stamens, as well as more specialized characters such as racemes with very small bracts, a corolla tube "bent downwards and swollen at rear," a small, club-shaped

staminode, the absence of a disk nectary, ovoid and strongly wrinkled seeds and long, incurved retinacula. Bentham placed *Thomandersia* in Acanthaceae (Bentham, 1876), based largely on floral and fruit characters, particularly the presence of retinacula. Retinacula are hook-like outgrowths of the funicle (Corner, 1976), considered a general earmark of Acanthaceae (Lindau, 1894; Van Tieghem, 1908; Leonard, 1951; Scotland & Vollesen, 2000), although they are absent from three subfamilies. Within Acanthaceae, Lindau (1897) placed *Thomandersia* in Asystasieae, but noted that it differed from the rest of the tribe on pollen morphology and the presence of a staminode.

Bremekamp (1942) first disputed the classification of *Thomandersia* in Acanthaceae, transferring it to Pedaliaceae. He considered the following morphological features important in the systematics of *Thomandersia*: scattered bracts, minute bracteoles, a fleshy corolla, distinctive corolla aestivation, four stamens evenly-spaced on the tube, globose pollen with five or six colpi but no germ pores, capsules rounded, indehiscent or tardily dehiscent, with a leathery or woody pericarp, seeds ovoid, scaly or rugose and, in disagreement with previous authors, "excrescences of the placenta" but no true retinacula. Along with Heine, Bremekamp made the additional significant and diagnostic observation that the abaxial surface of the calyx bears one or two glandular swellings (Bremekamp, 1942; Heine, 1966a).

Heine (1966a) cited inflorescence structure, scaly seeds, dehiscent fruits (in disagreement with Bremekamp) and the presence of true, funicular retinacula *sensu* Corner (1976) as evidence that *Thomandersia* is more similar to Acanthaceae than to Pedaliaceae or any other family. Sreemadhaven (1976; 1977) proposed the erection of

a monogeneric Thomandersiaceae, mainly on the basis of leaf architecture; he interpreted the retinacula of Thomandersiaceae and Acanthaceae as similar but homoplastic. *Thomandersia* was excluded from the most recent classification of Acanthaceae, but no alternative classification was proposed (Scotland & Vollesen, 2000). The genus remains at present unplaced in Lamiales.

3.3 Methods

Taxon sampling. Anatomical investigations were conducted on all species of *Thomandersia*, and representatives of other Lamiales to which the genus may be related: Bignoniaceae, Pedaliaceae, Schlegeliaceae, Verbenaceae and a range of Acanthaceae including those with retinacula (Acanthoideae, in which *Thomandersia* has been classified) and without (represented by Thunbergioideae). Acanthoideae comprises two tribes; *Thomandersia* has previously been classified with members of Ruellieae (Bentham, 1876; Lindau, 1897), but shares some morphological characters, such as colpate pollen and a lack of cystoliths, with Acantheae (Scotland & Vollesen, 2000), so representatives of both Acantheae and Ruellieae were studied.

Material of *Thomandersia* was collected in formalin acetic acid alcohol (FAA) in the Central African Republic. Material of other Lamiales was obtained, for comparison, from the living collections at the Royal Botanic Gardens, Kew (HK) and cultivated collections elsewhere in the UK. Pollen acetolysis was carried out on anthers from herbarium collections. Acetolysed pollen slides made by R. Scotland at the Natural History Museum, London (BM) were also examined. Observations were made on dried collections and spirit material from the herbaria of BM, BR, FHO, K, MO, P and WAG. A list of material studied is given in Appendix 11. The majority of

observations for *Thomandersia* were made on *T. hensii*, for which most material was available.

Anatomical sectioning. Fresh plant material was fixed in FAA for three days, then transferred, with three washings, to 70% ethanol$_{(aq)}$. Dried material was softened in boiling water and taken through a series of aqueous ethanol solutions (30%, 50% and 70%), with at least one hour in each concentration.

Wax embedding was accomplished by passing the specimens through a further series of ethanol$_{(aq)}$ solutions. This comprised two hours in each of 70%, 90% and 95% ethanol$_{(aq)}$ solutions, and two hours in each of two baths of 100% ethanol. The material was then taken through an ethanol: histoclear series. This comprised nine parts absolute alcohol: one part histoclear, seven parts alcohol: three parts histoclear, one part alcohol: one part histoclear, three parts alcohol: seven parts histoclear, one part alcohol: nine parts histoclear and finally 100% histoclear. The material was then covered with 100% histoclear, the containers filled with molten paraffin wax, and kept in an embedding oven at 60°C until all the histoclear had evaporated (after about two weeks). During this time the wax was changed every 48 hours. The specimens were embedded in molten paraplast in a foil mould, solidified in an ice-water bath and allowed to harden at room temperature.

Transverse and longitudinal sections of wax-embedded material, 12 µm thick, were cut using a rotary microtome. The sections were floated onto slides that had been coated in Haupt's adhesive, and left to dry overnight. They were stained with safranin and Alcian blue by passing through the following series of solutions: 100% histoclear

(three minutes), a second period in 100% histoclear (three minutes), 100% ethanol (two minutes), a second period in 100% ethanol (two minutes), 90% $ethanol_{(aq)}$ (two minutes), 70% $ethanol_{(aq)}$ (two minutes), 50% $ethanol_{(aq)}$ (two minutes), safranin in 50% $ethanol_{(aq)}$ (eight minutes), distilled water (two minutes), Alcian $blue_{(aq)}$ (eight minutes), 50% $ethanol_{(aq)}$ (two minutes), 70% $ethanol_{(aq)}$ (two minutes), 90% $ethanol_{(aq)}$ (two minutes), 100% ethanol (five minutes), 100% ethanol (five minutes), 100% histoclear (three minutes) and 100% histoclear (at least three minutes). The stained sections were mounted in DPX and left to harden at room temperature in a fume hood for at least three days.

Pollen acetolysis. Buds were taken from herbarium collections and dissected in distilled water to remove the anthers, which were punctured to release the pollen. The pollen grains were rinsed with distilled water through a 120 μm mesh sieve into a centrifuge tube. The larger pieces of tissue left on the sieve were ground with a glass rod to release any remaining pollen, which was rinsed into the centrifuge tube. The pollen samples were centrifuged for five minutes at 3,500 rpm and the aqueous supernatant decanted, leaving the pollen pellet intact. Approximately 2 cm^3 of glacial acetic acid was added to each tube in a fume hood and stirred to thoroughly dehydrate the pollen grains. The suspension was then centrifuged at 3,500 rpm for five minutes and the glacial acetic acid supernatant decanted. Approximately 2 cm^3 acetolysis mixture (nine parts acetic acid: one part sulphuric acid; prepared by gradual addition with stirring in a fume hood) was added to the pollen pellet in each tube. These were incubated at 110°C for four minutes, stirring intermittently with a glass rod. The suspensions were centrifuged for five minutes at 3,500 rpm and the acetolysis mixture decanted. Each tube was filled with distilled water and stirred to wash the grains, then

centrifuged for a further five minutes at 3,500 rpm. The water was decanted and approximately 2 cm^3 50% glycerol added, stirred and centrifuged again. The surplus glycerol was poured off and the tubes inverted onto a clean tissue for a few minutes to allow the pollen to coat the inside of the tubes.

A clean needle was used to dip small pieces of glycerine jelly into the pollen in each tube. The jelly was placed onto a pre-heated microscope slide on a hot plate at 60°C to melt. Once melted it was spread evenly over a small area of the slide and covered with a cover-slip. Molten paraffin wax was used to seal the edges of the cover-slip.

Microscopy. Light microscope observations were made using Swift Phasemaster and Leitz Laborlux 5 microscopes. Anatomical drawings were taken from the latter, fitted with a Leitz Wetzler drawing tube. Photographs were taken using a Leica DMLB microscope fitted with a Leica MPS60 camera and a Leitz Dialux 20EB microscope fitted with a Fuji S1 digital camera. Further observations were made on dried and spirit material, and photographs taken using a Nikon Coolpix 990 digital camera.

Scanning electron microscopy (SEM). Plant material was fixed in FAA and stored in 70% $ethanol_{(aq)}$. It was then taken through an ethanol series of increasing concentrations: at least one hour in each of 70% and 90% $ethanol_{(aq)}$ and two baths of 100% ethanol. The specimens were transferred to a critical point dryer specimen holder filled with 100% ethanol. They were then dried in a Balzers 030 critical point dryer, using carbon dioxide (CO_2) as the transition liquid. $CO_{2(l)}$ was introduced at 2°C and changed eight times, allowing seven minutes equilibration after each change. The material was heated to 40°C and the pressure released over at least 15 minutes.

The dried specimens were stored in a desiccator or mounted immediately onto adhesive carbon-coated stubs. They were sputter-coated with gold for two minutes at $1x10^{-1}$ mBar pressure and 10 mA. Scanning electron micrographs were taken on a Hitachi S-4700 dual detector field emission microscope, at an accelerating voltage of 2 kV and 10 μA, taking a 50:50 mix of signals from the upper and lower detectors (these were found to be the best conditions for resolution with this material).

3.4 Results

3.4.1 Wood anatomy. Thomandersia has diffuse porous wood with very narrow heterocellular (uniseriate or biseriate) rays. The vessels are small and, as in most dicotyledons (Dickison, 2000), arranged in radial multiples of 2-3 or in isolation (Fig. 3.1A). The fibres are septate, with minute intervascular pitting and simple perforation plates (Fig. 3.1B). Axial parenchyma is scanty vasicentric and included phloem is absent. There are no cystoliths or acicular crystals in any part of the stem (e.g. Fig. 3.1C).

3.4.2 Inflorescence morphology. Thomandersia inflorescences are terminal, subterminal or axillary, indeterminate and superficially racemose, with subsessile or pedicellate flowers. Each flower is subtended by two bracteoles and a small abaxial bract. This arrangement implies that the inflorescence may have evolved by reduction from a compound inflorescence with bracteate flowers borne in threes, and can be interpreted as a reduced indeterminate thyrse (Weberling, 1965; 1992).

3.4.3 Floral anatomy. Vasculature. The inflorescence rachis of *Thomandersia* contains a single ring of vascular traces from which a subsidiary ring branches into

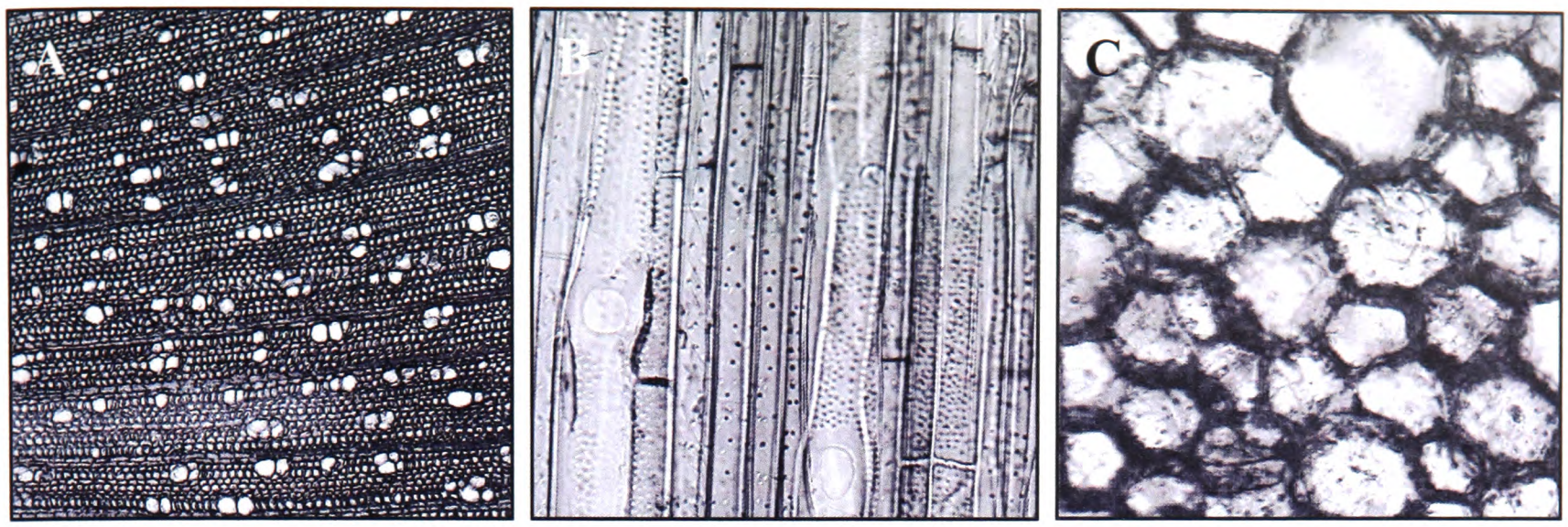


Figure 3.1 *Thomandersia hensii* stem sections. A. T.S. secondary xylem, showing both solitary and grouped vessel elements. B. R.L.S. secondary xylem, showing minute intervascular pitting. C. T.S. pith, showing parenchyma with some cell contents but no acicular crystals.

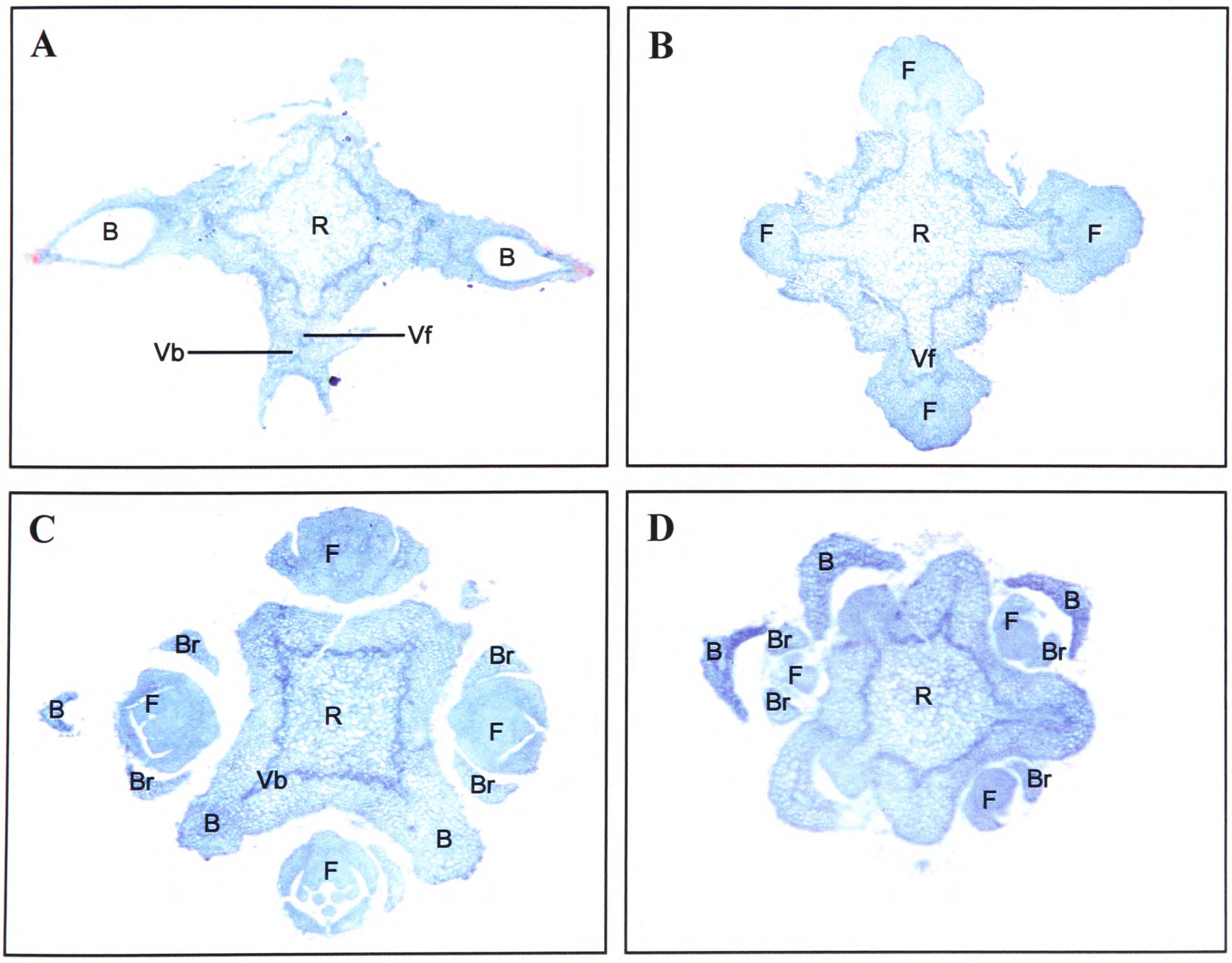


Figure 3.2 *Thomandersia hensii* inflorescence, serial sections (A-D, proximal-distal) through inflorescence axis. **B**, bract; **Br**, bracteole; **F**, flower (of proximal whorl); **R**, rachis; **Vb**, vascular trace to bract; **Vf**, vascular trace to flower.

each flower (Fig. 3.2A). The subsidiary branch diverges from the main axis above each floral bract (Fig. 3.2B). In the pedicel, four separate traces diverge from the floral ring: two lateral traces supply the bracteoles and two supply the extrafloral nectary. The bracts and their vascular traces diverge from the main axis at an angle of 45° to those of the previous whorl (Fig. 3.2C). Each whorl of flowers develops in exactly the same way as, but at right-angles to, the more proximal one, with vascular bundles diverging from the axis to each flower, its bracteoles and extrafloral nectary. Towards the tip of the inflorescence the bracts and bracteoles become increasingly prominent relative to the size of the floral buds themselves (Fig. 3.2D), and superposed floral buds may be found in their axils (Fig. 3.3B).

Calyx. The calyces of all *Thomandersia* species are thick, leathery and approximately campanulate, with five more or less equal lobes. They are open in bud and accrescent in fruit and, when mature, the epidermis stains red, suggesting the lignification of, or accumulation of secondary chemicals by, epidermal cells (Fig. 3.3A).

Corolla. The corolla is zygomorphic, pentamerous and bilabiate. The upper labium consists of two fused petals and the lower one three (Fig. 3.4D,I). The corolla is supplied by a ring of 15 vascular traces, three to each lobe, in addition to those leading to the androecium (Fig. 3.3C). In bud, the large abaxial corolla lobe entirely surrounds the two lateral lobes and overlaps the adaxial lobes (Fig. 3.4A,F). Aestivation is complex: it does not correspond to either cochlear (one lobe wholly outside, one wholly inside, three half-overlapped) or quincuncial (two lobes wholly outside, two wholly inside, one half-overlapped) patterns (Weberling, 1992; Scotland et al., 1994). A quincuncial pattern is seen in sections just proximal to the corolla tip

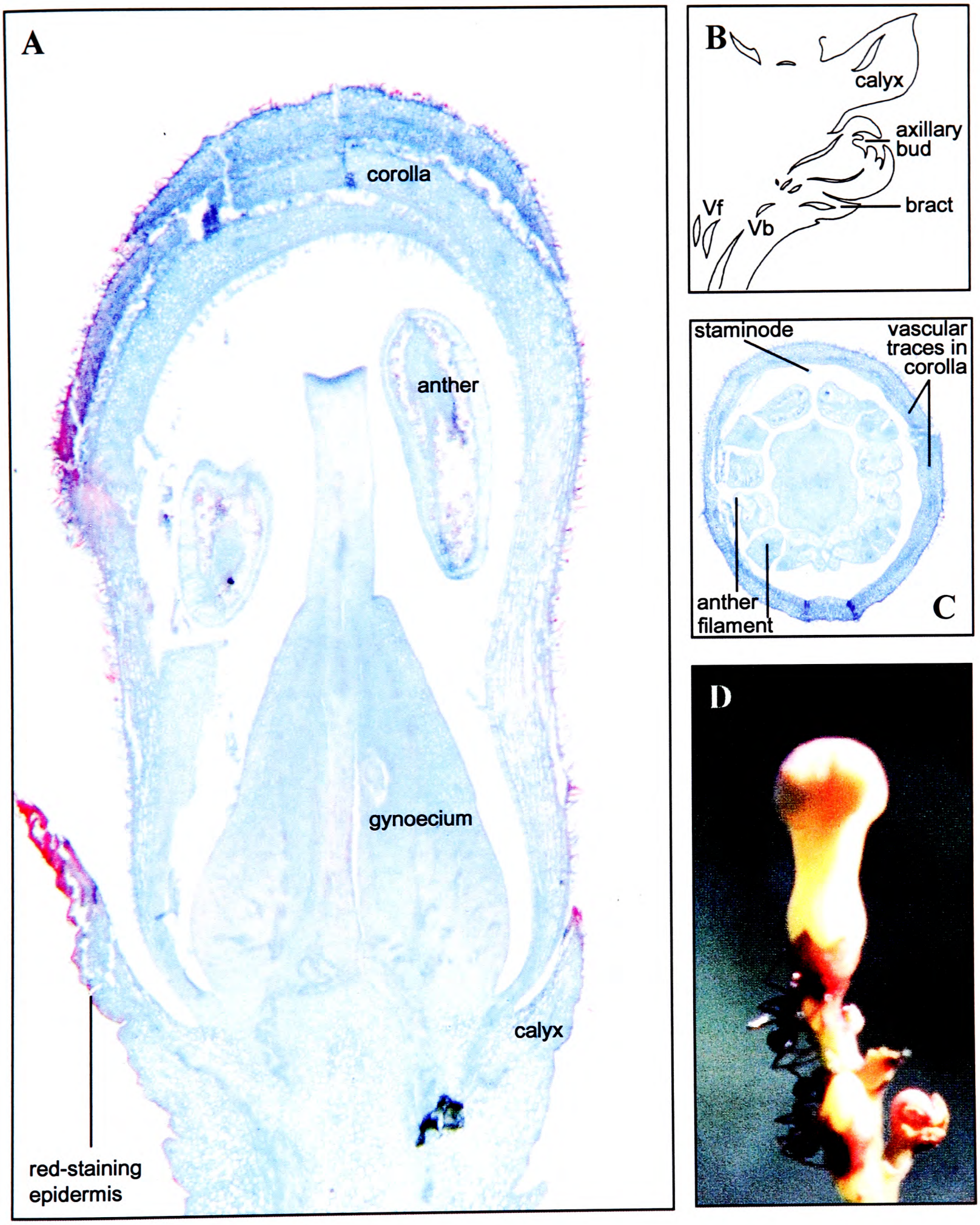


Figure 3.3 *Thomandersia hensii* floral anatomy and morphology. A. L.S. flower, sectioned along abaxial-adaxial axis. B. L.S. pedicel and axillary bud, drawn from anatomical section. C. T.S. flower. D. photograph of bud, Cameroon. **Vb**, vascular trace to bract; **Vf**, vascular trace to flower.

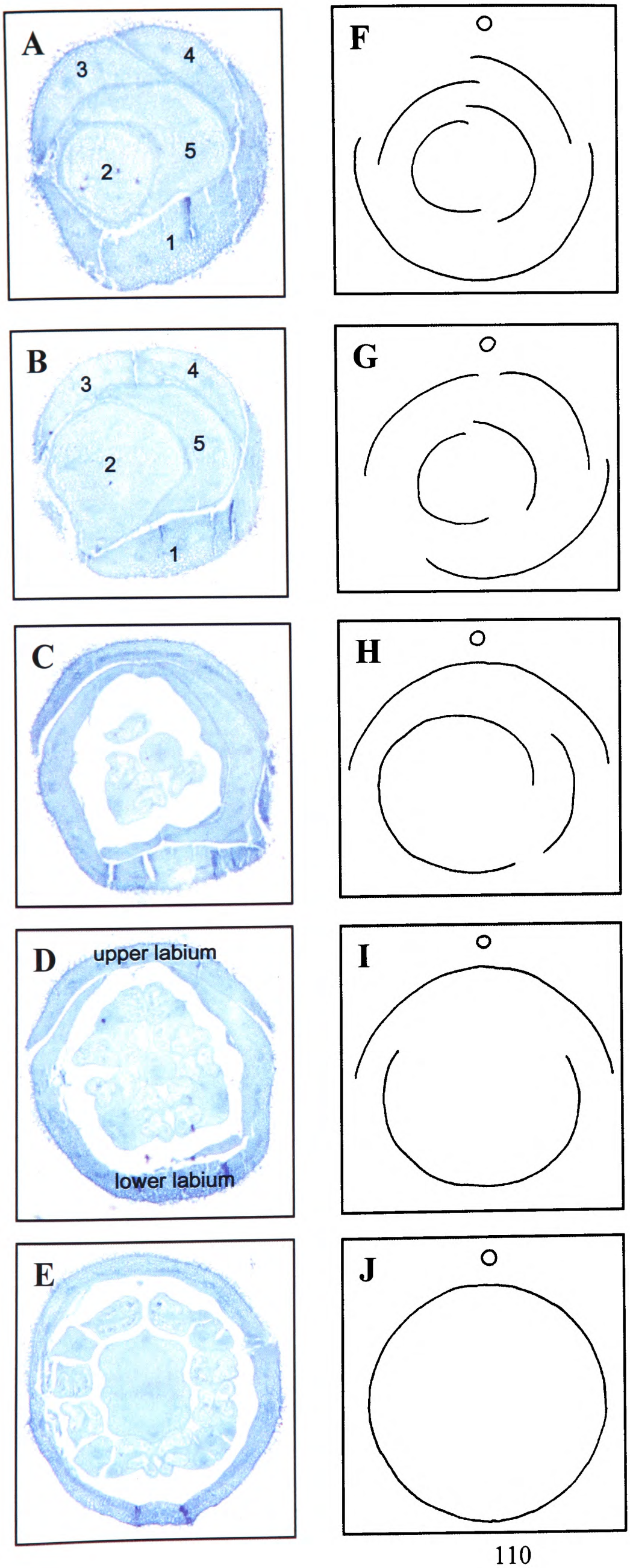


Figure 3.4 *Thomandersia hensii* corolla aestivation. A-E. serial sections (distal to proximal) through bud. Petals numbered clockwise, viewed from above, starting at abaxial petal (*sensu* Scotland et al., 1994). F-J. schematic representation of petals shown in A-E.

(Fig. 3.4B,G), but more proximally (Fig. 3.4C,D,H,I) the two adaxial lobes of the upper labium are fused together and the abaxial lobes are fused with the two lateral lobes in turn. These form the lower labium, which lies inside the upper labium at this level. At the base of the flower (Fig. 3.4E,J), the two labia are united in a single tube. This complex aestivation pattern, with changing relative lobe and labia positions along the corolla, results in a characteristically-shaped bud with protruding folds of tissue (Fig. 3.3D), as the lobes switch from overlapped to overlapping and *vice versa*.

Androecium. Thomandersia has a pentamerous androecium (Fig. 3.3C). The androecial members alternate with the corolla lobes. There are four didynamous fertile stamens and a single adaxial staminode. All androecial members are fused to the corolla tube for part of their length. The stamens each contain a single vascular bundle. The staminode is minute, appears to lack vascular tissue from the free part, and is flanked (in *T. hensii*) by a row of hairs on either side (Figs. 3.3C, 3.5A). The anthers (Fig. 3.5B) are bithecate.

Gynoecium. The gynoecium is superior. At the base it is surrounded by, and continuous with, a dark-staining (secretory) mass of nectary tissue. This is well supplied with phloem-rich vasculature originating from the dorsal carpellary traces (Fig. 3.5E). The pistil is bicarpellary and eusyncarpous. There are two rings of vascular bundles, meeting in the central (ventral) septum, each supplying one side of the dorsal ovary wall (Fig. 3.5E). The ovary wall is thick, extremely so in *T. hensii* (Fig. 3.5F). The epidermis of mature carpels stains reddish, suggesting lignification. The dorsal vascular bundles of both carpels pass into the style. The style is solid with

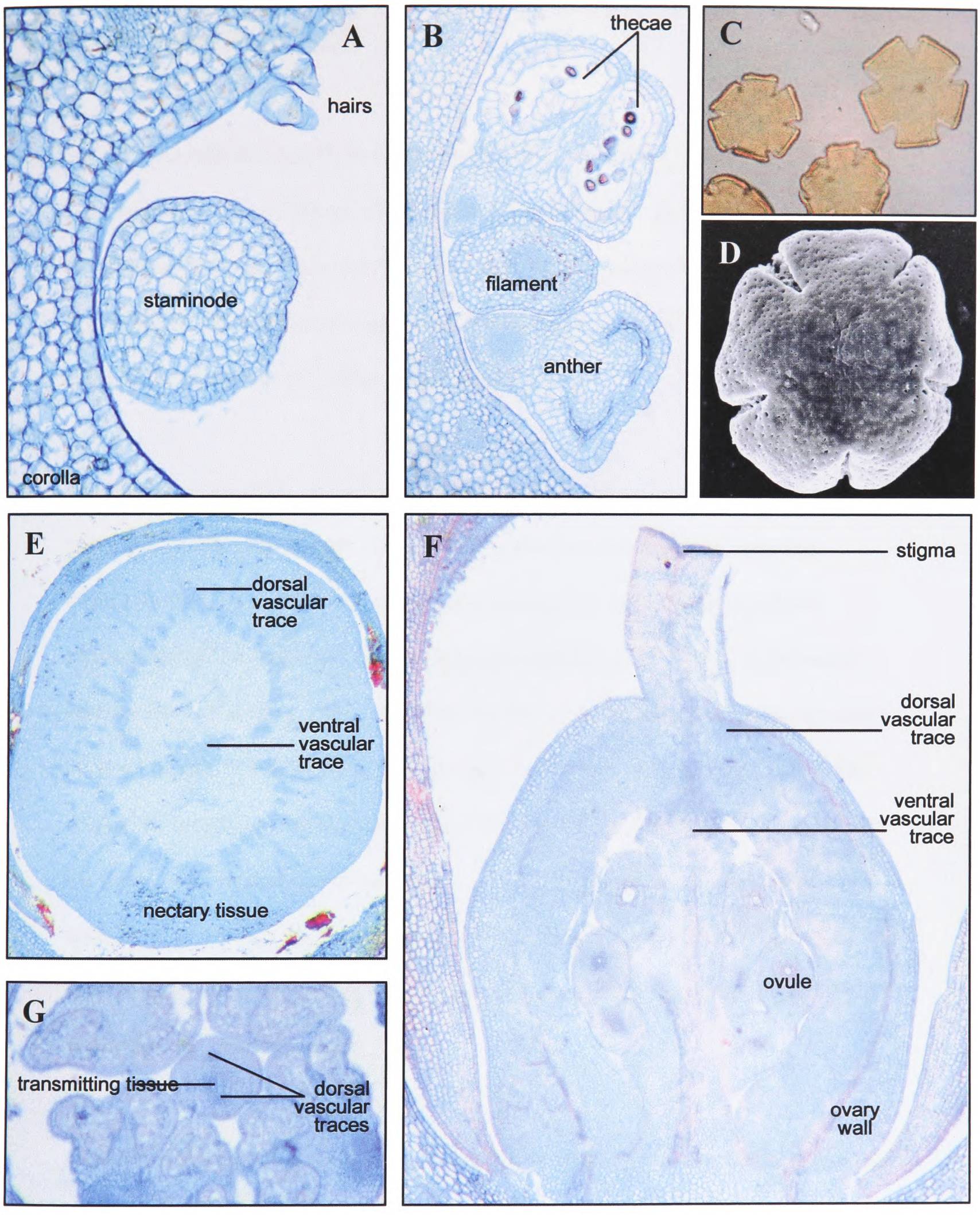


Figure 3.5 *Thomandersia hensii* androecium and gynoecium. A. T.S. staminode. B. T.S. anther. C. acetolysed pollen grains, showing five or six colpi. D. scanning electron micrograph of pollen grain (image supplied by R. Scotland). E. T.S. gynoecium and nectary at base. F. L.S. gynoecium. G. T.S. style.

two vascular traces and a central core of loosely-packed transmitting tissue continuous with the locules (Fig. 3.5F,G).

3.4.4 Pollen morphology. Pollen grain morphology is similar in all species of *Thomandersia*. The grains are 60-80 μm in diameter, oblate, globose and slightly flattened at the poles. There are 5-6 equatorial colpate ectoapertures (e.g. *T. hensii*, Fig. 3.5C,D). The number of colpi varies within species: grains with both five and six colpi were found in every anther examined (Fig. 3.5C).

3.4.5 Embryology. Two or three ovules are borne, one above the other, in each locule of the ovary. They are usually attached at alternate sides and held in opposing directions (Fig. 3.6A). Placentation is axile and the placentas are enlarged and crescent-shaped in transverse section, spreading inside the ovary wall in the spaces between the ovules (Fig. 3.6B). The funicles are short and broad and the demarcation between funicle and placenta is indistinct, the two forming a continuous tissue. The ovules are approximately hemianatropous: the nucellus is oriented at right-angles to the funicle and the embryo sac held horizontally at all stages from young buds to anthesis (Fig. 3.6D,E). At the chalazal end, the nucellus forms a distinct hypostase of suberinised cells, in which the ventral vascular supply of the carpel terminates. The ovules have a single integument.

3.4.6 Fruit and seed morphology. After fertilisation, the gynoecium of *Thomandersia* matures into a woody, bilocular capsule, which dehisces loculicidally, each valve containing 1-3 seeds. The seeds are roughly spherical, with a large hilum (approximately 2 mm long) left by the broad funicular attachment. As previously

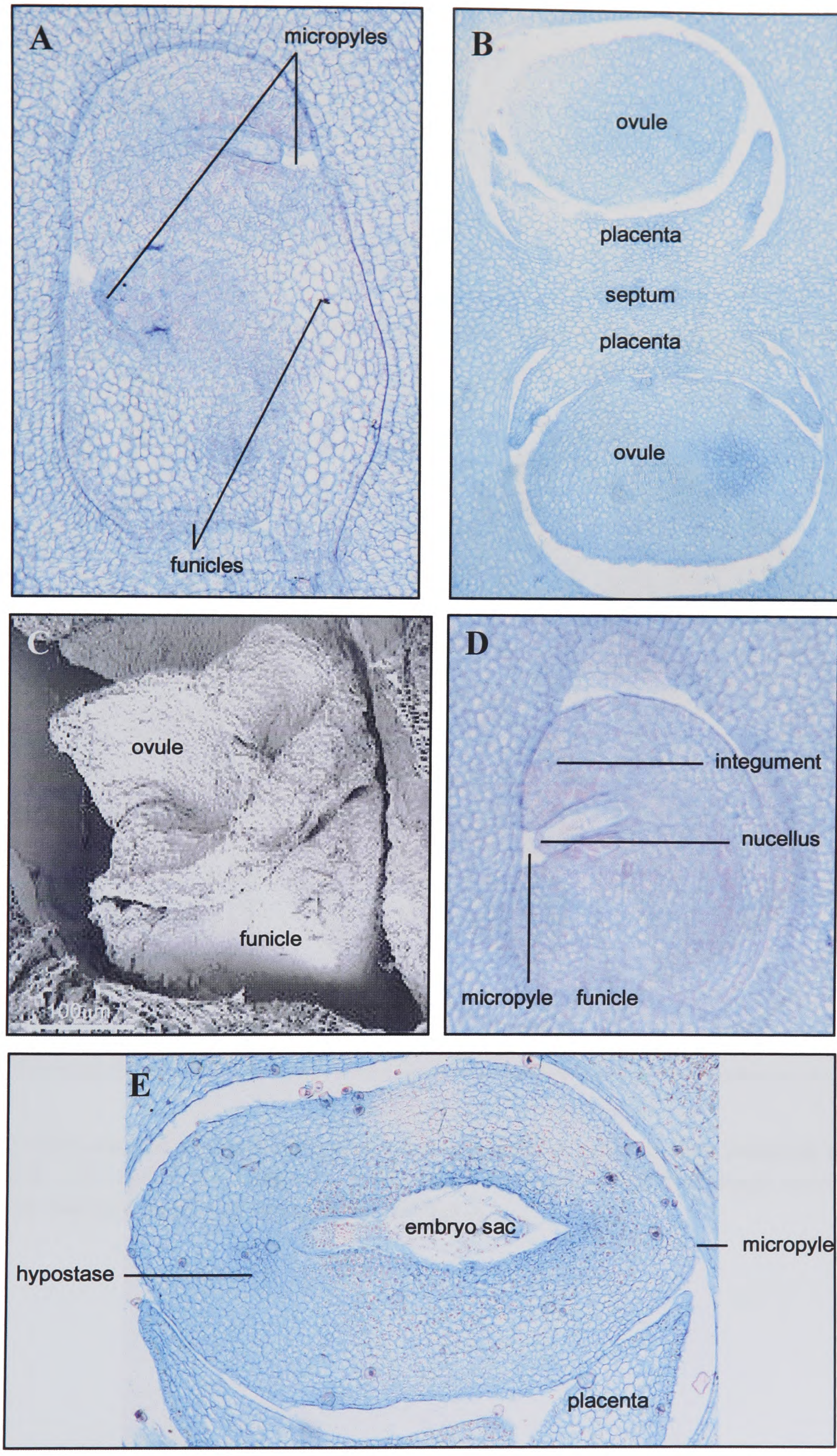


Figure 3.6 *Thomandersia hensii* ovule structure. A. L.S. carpel in bud, sectioned parallel to septum. B. T.S. carpels in bud. C. scanning electron micrograph of ovule in young bud (x130). D. L.S. ovule in young bud, sectioned parallel to septum. E. T.S. ovule, just prior to anthesis.

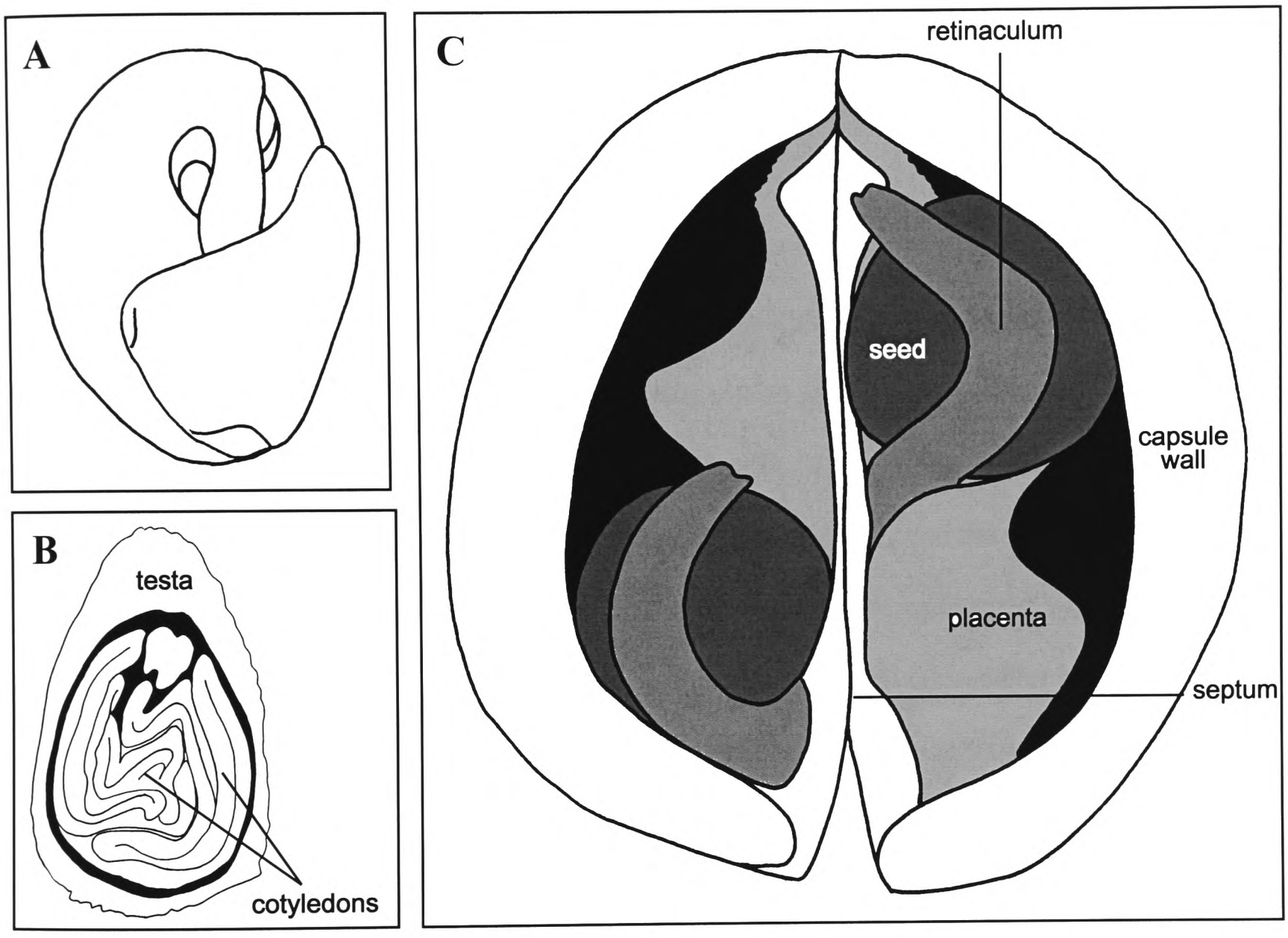
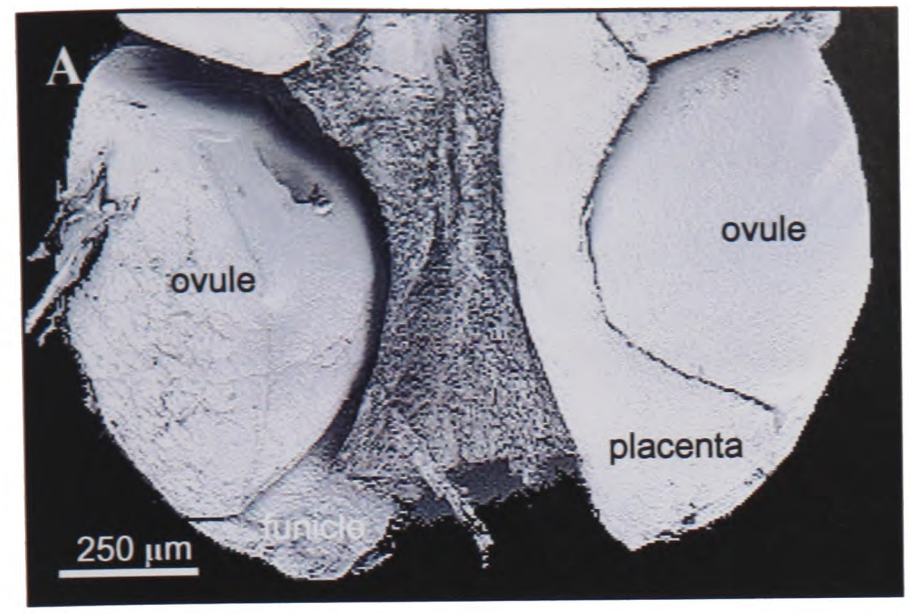


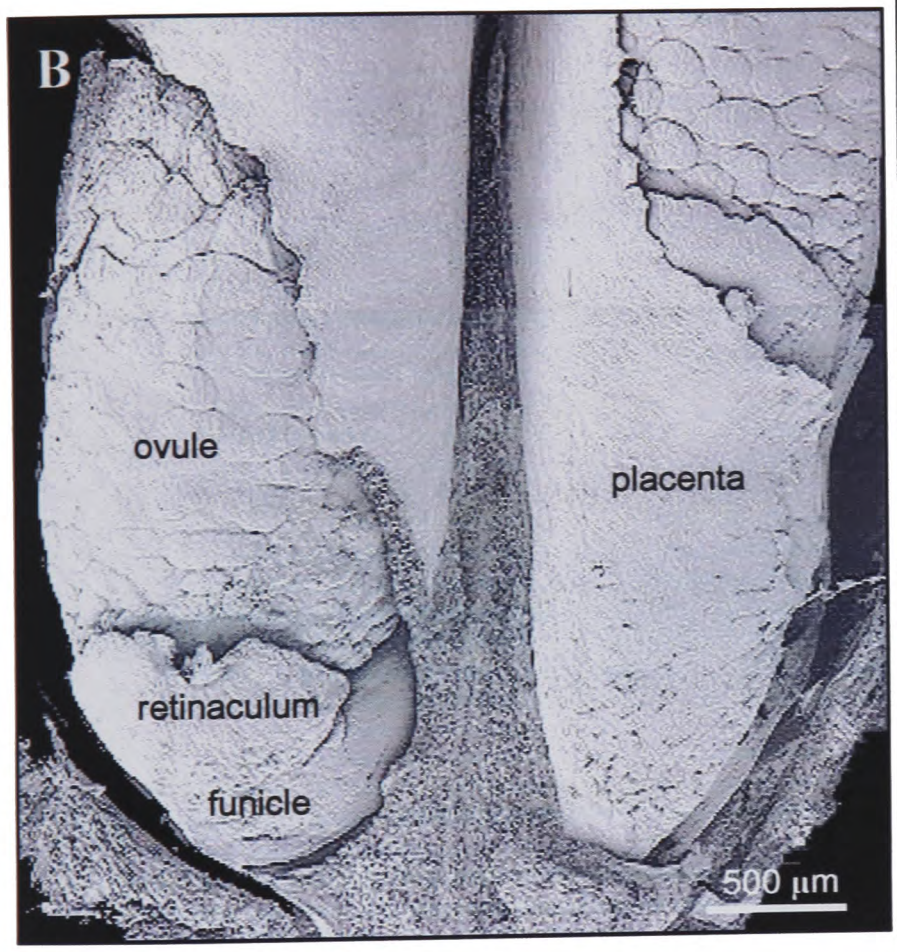
Figure 3.7 Drawings of *Thomandersia hensii* fruit and seed. A. embryo, testa removed, drawn from life. B. L.S. seed, drawn from life. C. schematic representation of fruit, single valve just after dehiscence, showing seeds and associated structures.

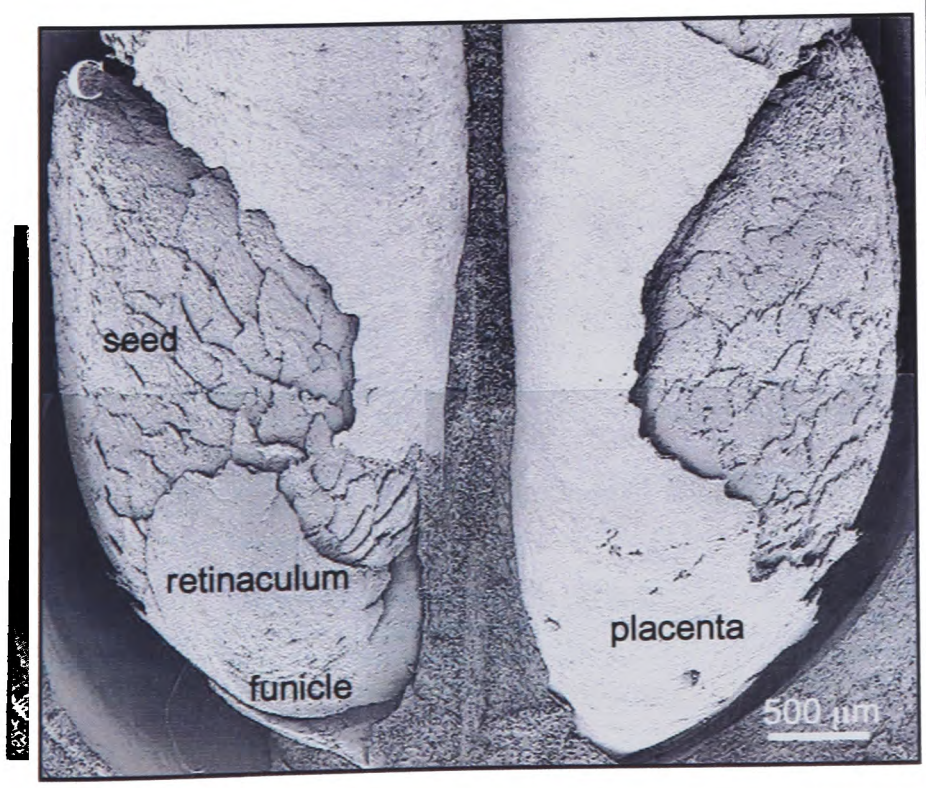
observed (Sreemadhaven, 1976), no endosperm is present in mature seeds. The cotyledons are distinctively folded (Fig. 3.7A,B).

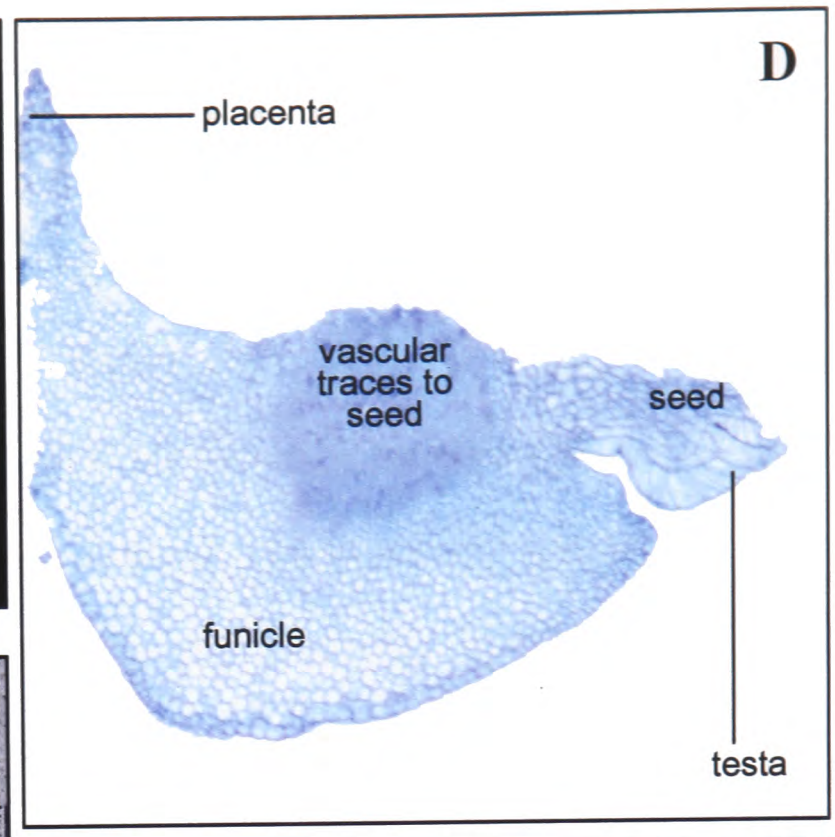
3.4.7 Retinacula. A conspicuous character of all *Thomandersia* species is the hook-shaped retinaculum subtending each seed. The retinaculum develops rapidly, between the placenta and ovule, late in the development of the carpel. It remains attached to the capsule after dehiscence (Fig. 3.7C). Retinacula are absent from flower buds (Fig. 3.8A) and are only found subtending seeds that develop to maturity (frequently one or more are aborted), although the placentas of abortive ovules develop normally. This indicates that the retinaculum develops from the funicle, rather than from the placenta. Transverse sections through a developing retinaculum (Fig. 3.8D,E) show that it diverges from the funicle distally to the hilum: a mass of vascular bundles passes into the seed, which can be identified by the large cells of the testa, and the retinaculum emerges distally to this (Fig. 3.8D,E). Thus it is unlikely to be a placentally-derived structure. Towards the tip (Fig. 3.8E) the retinaculum contains no vascular bundles and consists of uniform parenchyma cells. As the fruit matures, the retinaculum differentiates into two distinct tissue-layers (Fig. 3.8F): a blue-staining outer layer and a red-staining one adjacent to the seed, suggesting cell wall lignification. In mature capsules of *T. hensii* the retinaculum is approximately 5 mm long and 1.5 mm wide, narrowing to 1 mm at the tip.

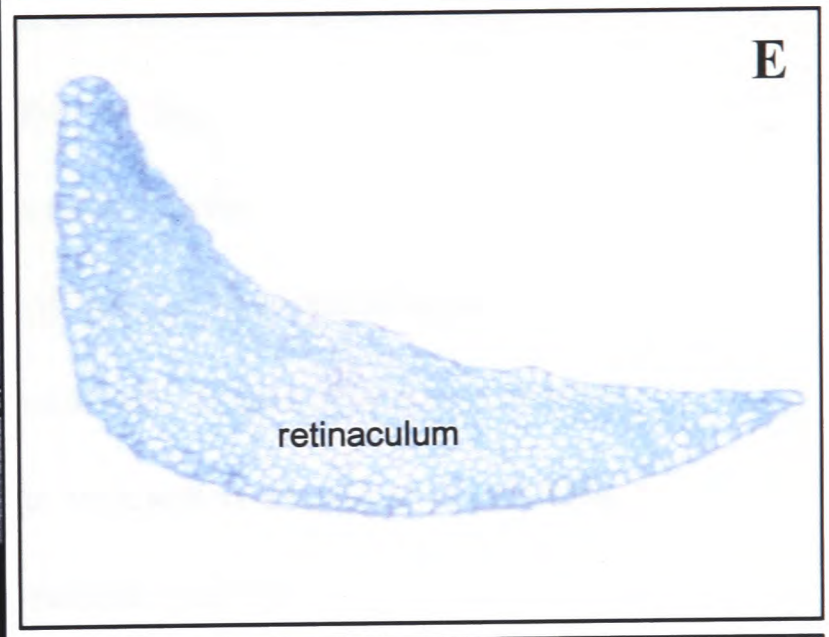
3.4.8 Extrafloral nectary. The base of the calyx bears 1-2 conspicuous swellings, particularly obvious in bud, 1-2 mm in diameter and depth, between the two abaxial lobes. The tissue is richly vascularised, supplied by a branch of the main vascular ring in the pedicel, and contains a multicellular, sometimes branched gland, approximately











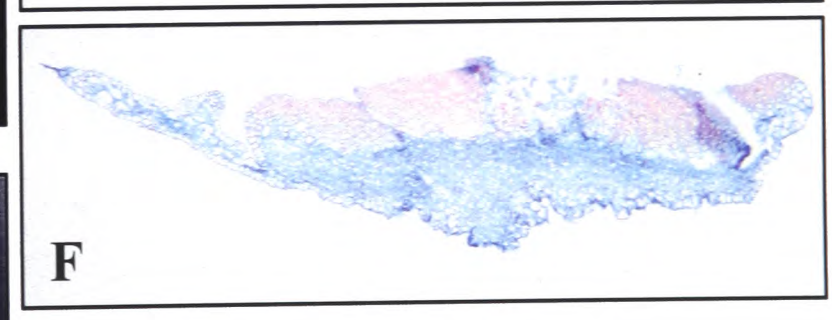


Figure 3.8 *Thomandersia hensii* retinacula. A-C. scanning electron micrographs of developing ovary (placenta removed from left carpel in each case; A, x70; B, x35; C, x30). D,E. T.S. developing retinaculum, serial sections from base to tip. F. T.S. tip of mature retinaculum.

1 mm long (Fig. 3.9A). In *T. hensii, T. butayei, T. congolana, T. laurentii* and *T. laurifolia* there is always a single swelling, although in some specimens of *T. hensii* the swelling contains two distinct glands with separate vascular supplies (Fig. 3.9B), and in *T. congolana* two ostioles are sometimes observed (D. Champluvier, pers. comm.). In *T. anachoreta* there are always two swellings, each containing a gland (Fig. 3.9C-E); each gland has a separate ostiole. In *T. hensii* the opening takes the form of a narrow channel (Fig. 3.9A); in *T. anachoreta* it is broader and cup-shaped (Fig. 3.9C). The glands are composed of clearly defined cell layers (Fig. 3.9F). The central cavity is surrounded by dark-staining columnar epithelial cells, which are probably secretory, as are similar cells in other taxa (Subramanian & Inamdar, 1989). The secretory cells are surrounded by a single layer of large, colourless cells, possibly a barrier layer as in many species' extrafloral nectaries (Schnepf, 1969; Subramanian & Inamdar, 1989). Surrounding this is a region of smaller, dark-staining cells containing starch grains, around which the vascular trace passes. The gland channel frequently contains secretions (Fig. 3.9F) and the structure is attractive to ants (Fig. 3.3D), confirming that it may function as an extrafloral nectary.

3.5 Discussion and systematic implications

The characters of *Thomandersia* can be interpreted in three ways in the context of Lamiales: plesiomorphies of Lamiales; autapomorphies of *Thomandersia*; or primary homologies shared with potential sister groups.

3.5.1 Wood anatomy. The wood anatomy of *Thomandersia* is typical of Lamiales (Carlquist & Zona, 1988; Carlquist, 1992; Dickison, 2000). It differs from more derived families of the order, and lacks any unique characters that might indicate

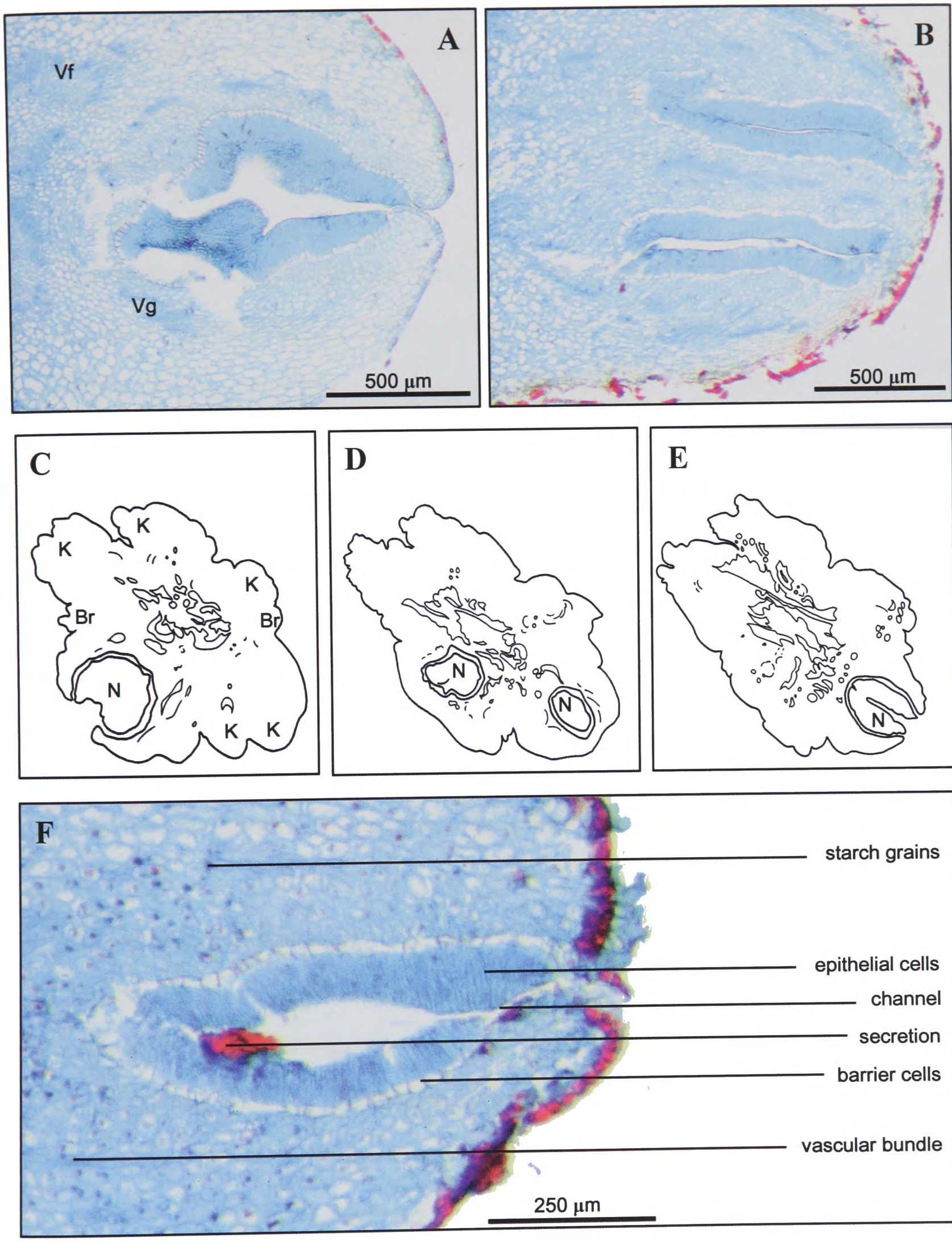


Figure 3.9 *Thomandersia* extrafloral nectary. A,B. *T. hensii,* T.S. calyx. C-E. *T. anachoreta,* T.S. calyx, drawn from serial anatomical sections. F. *T. hensii*, L.S. nectary gland. **Br**, bracteole; **K**, calyx lobe; **N**, nectary; **Vf**, vascular trace to flower; **Vg**, vascular trace to gland.

phylogenetic relationships with other families. Plesiomorphic wood characters shared by *Thomandersia* and a wide range of Lamiales include vessel arrangement (in pairs, threes or solitary), simple perforation plates, scanty vasicentric axial parenchyma, septate fibres and heterocellular (uniseriate or biseriate) rays (Fig. 3.1).

One family of Lamiales notable for its distinctive wood characteristics is Acanthaceae (Carlquist & Zona, 1988). Acanthaceae are distinguished by included phloem (Fig. 3.10A), cystoliths, and acicular crystals (Fig. 3.10B), all of which *Thomandersia* lacks (Fig. 3.1). If *Thomandersia* were a member of Acanthaceae, and particularly if it were in Acanthoideae (Bentham, 1876; Baillon, 1891; Lindau, 1894; 1897; Burkill & Clarke, 1899; Thiselton-Dyer, 1906; Heine, 1966a), it might be expected to share some of these derived characters. In addition, the intervascular pitting of *Thomandersia* (Fig. 3.1B) is characteristically minute, smaller than that of Acanthaceae examined. Thus the wood anatomical characters of *Thomandersia* are congruent with its placement in Lamiales, but do not imply membership of any one family and in particular are incongruent with its inclusion in Acanthaceae.

3.5.2 Inflorescence morphology. Thomandersia inflorescences display characters that are typically plesiomorphic, or at least widespread within Lamiales. Racemose inflorescences with bracts and bracteoles are found in all potential sister groups of *Thomandersia*, including all subfamilies of Acanthaceae. Likewise, a pentamerous, sympetalous calyx and corolla are almost ubiquitous in Lamiales (as in many other asterids), excepting some members of the basal groups Carlemanniaceae, Oleaceae and Tetrachondraceae (Wilson & Wood, 1959; Watson & Dallwitz, 1992; Wagstaff & Olmstead, 1997; Judd et al., 1999; Stevens, 2001).

3.5.3 Floral anatomy. Calyx. The calyx of *Thomandersia* shares characters with various Lamiales taxa. It is thick, leathery, red-staining and accrescent to the fruit, like that of *Schlegelia*, some Verbenaceae and some Bignoniaceae. *Schlegelia*, in particular, has a thick, leathery, and richly vascularised calyx. Its epidermis stains red, suggesting the accumulation of secondary chemicals. It is also dotted with small, stalked, eight-celled oil glands which have not been found in *Thomandersia* but are common throughout Lamiales (Uphof & Hummel, 1962).

Corolla. The corolla of *Thomandersia* is typical of Lamiales: zygomorphic and bilabiate. It is adaxially swollen (gibbous) at the base, a state also seen in some Bignoniaceae (e.g. *Eccremocarpus scaber*) and Pedaliaceae (e.g. *Sesamothamnus gurichii*). Corolla aestivation in *Thomandersia* is partially quincuncial (Fig. 3.4), but does not exactly parallel that of any Acanthaceae (Scotland et al., 1994). Aestivation in most other Lamiales has not been studied in detail.

Androecium. The androecium of *Thomandersia* is also typical of Lamiales (Fig. 3.3C). It comprises four didynamous, bithecate anthers, fused to the corolla, and an adaxial staminode. Staminodes are thought to evolve through reduction of one side of the androecium during transitions to zygomorphy (Donoghue et al., 1998), although they may reappear in a lineage once lost (Walker-Larsen & Harder, 2000). It is therefore likely that the staminode of *Thomandersia* evolved by reduction of the fifth, adaxial stamen. The free part of the staminode lacks vascular bundles, as does that of *Petrea* (Verbenaceae; Fig. 3.11A), whereas those of *Schlegelia* (Fig. 3.11B) and *Eccremocarpus* (Bignoniaceae; Fig. 3.11C) are vascularised. Staminodes are often absent from the flowers of Acanthaceae (Endress, 2001). The didynamous androecial

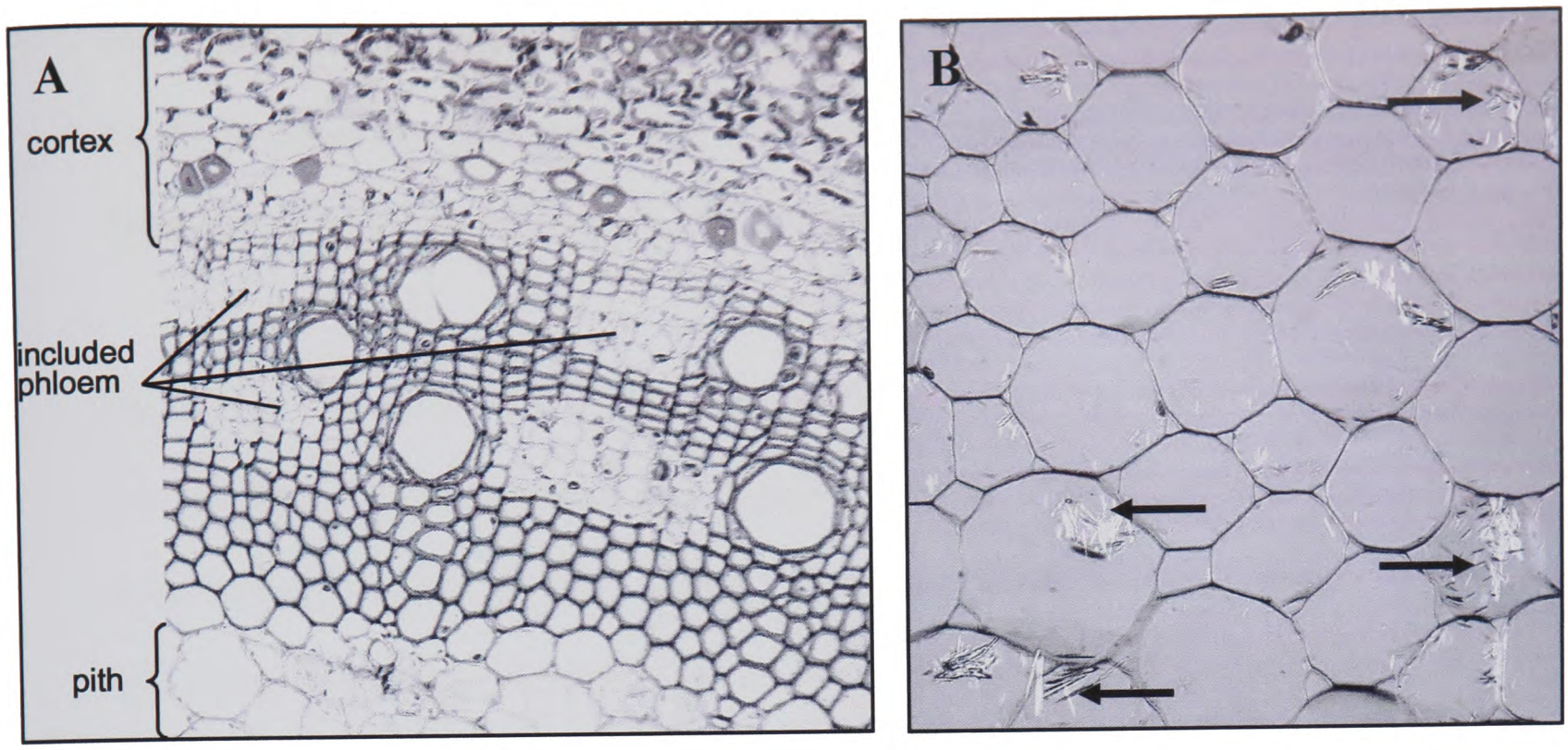


Figure 3.10 *Thunbergia grandiflora* (Acanthaceae) stem sections. A. T.S. cortex, showing included phloem. B. T.S. pith (under phase contrast), showing acicular crystals in parenchyma cells.

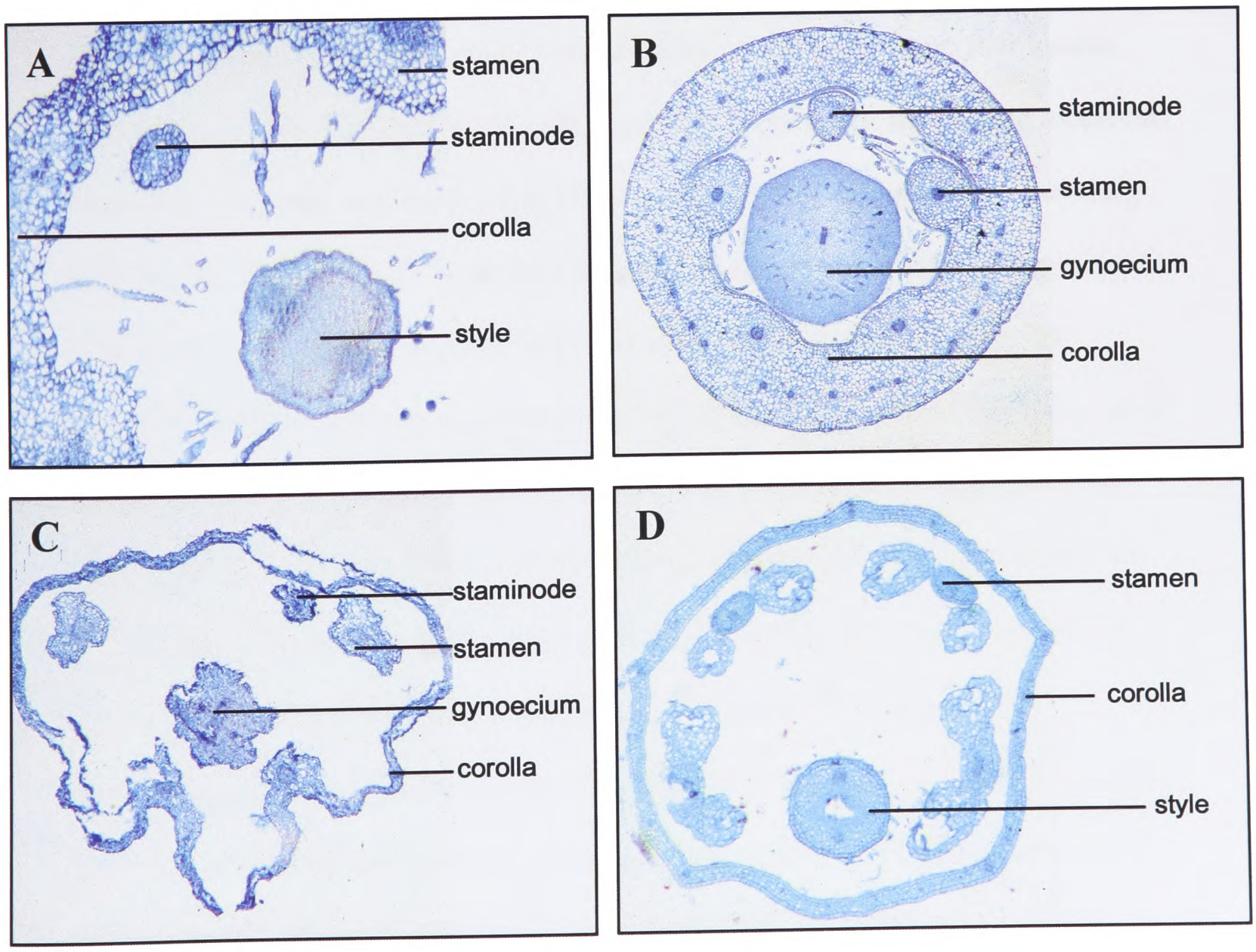


Figure 3.11 Androecia of Lamiales. A. *Petra volubilis* (Verbenaceae), T.S. flower. B. *Schlegelia violacea* (Schlegeliaceae), T.S. flower. C. *Eccremocarpus scaber* (Bignoniaceae), T.S. flower. D. *Ruellia tuberosa* (Acanthaceae), T.S. flower.

arrangement of four fertile stamens in unequal-sized pairs is common throughout Lamiales, including Acanthaceae (Fig. 3.11D), Bignoniaceae (Fig. 3.11C), Pedaliaceae, Schlegeliaceae (Fig. 3.11B) and Verbenaceae.

Gynoecium. The gynoecium of *Thomandersia* is of a typical Lamiales type: eusyncarpous with a bilocular ovary containing two rings of vascular bundles. In Acanthaceae the gynoecium is also bilocular, and (sometimes only proximally) syncarpous, containing a single ring of discrete vascular bundles (Fig. 3.12A). In Bignoniaceae the gynoecium may be bilocular or secondarily unilocular; in species where it is bilocular it contains vascular bundles in the septum (i.e. there are two rings of vascular bundles in the gynoecium, as in *Thomandersia*). In Verbenaceae the ovary is usually bilocular, syncarpous and subdivided by a false septum into four locules. Sometimes (e.g. in *Petrea*) it is bilocular (probably secondarily: the aborted locule can be seen in transverse section as a small cavity; Fig. 3.12B). In Pedaliaceae the ovary may also be 2-4-loculed. In Schlegeliaceae the gynoecium is bilocular and syncarpous (Fig. 3.12C) and contains derivatives of two carpellary rings (Fig. 3.12D). The distinctively thick, well-vascularised ovary wall of *Thomandersia*, which ripens into a woody pericarp with a red-staining epidermis, is similar to those of Schlegeliaceae and Verbenaceae (Rueda, 1994). In Verbenaceae the epidermis also stains red (Fig. 3.12G). In *Schlegelia* the ovary wall is glabrous, thick and richly-vascularised (Fig. 3.12C). In Acanthaceae the ovary wall is thinner than in *Thomandersia* and is frequently glandular or hairy (e.g. *Ruellia*, Fig. 3.12I). *Thomandersia* has a solid style (Fig. 3.5G). In Acanthaceae the style may either be solid (e.g. *Pseuderanthemum*) or hollow, usually where carpel fusion is incomplete such as in *Thunbergia* and *Ruellia* (Fig. 3.11D). It contains loosely-packed transmitting tissue continuous with the

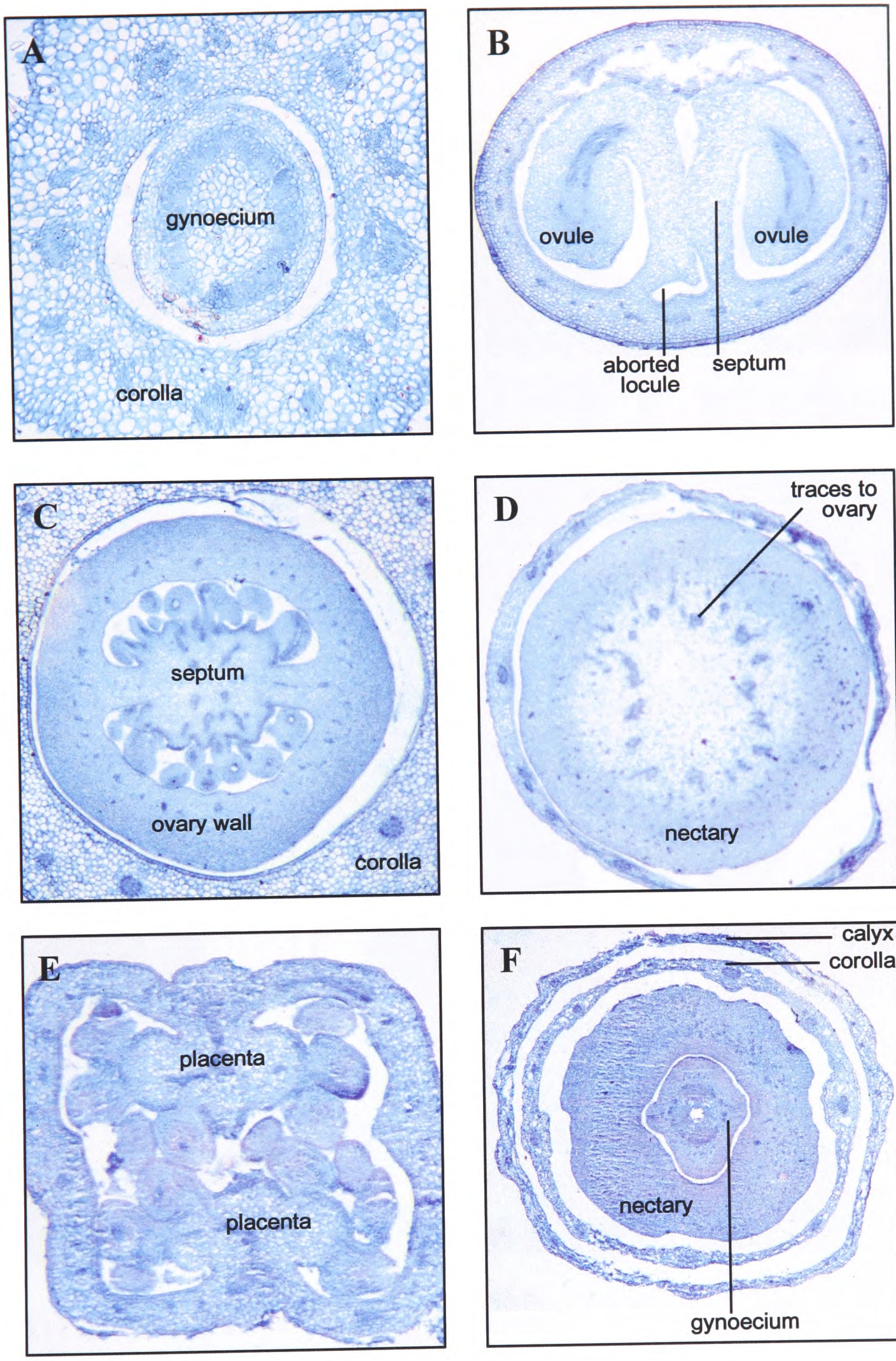


Figure 3.12 Gynoecia of Lamiales. A. *Ruellia tuberosa* (Acanthaceae), T.S. bud, at base of carpels. B. *Petra volubilis* (Verbenaceae), T.S. gynoecium. C,D. *Schlegelia violacea* (Schlegeliaceae), T.S. gynoecium, serial sections (distal to proximal). E,F. *Eccremocarpus scaber* (Bignoniaceae), T.S. gynoecium in bud, T.S. flower at base of carpels **(continued overleaf).**

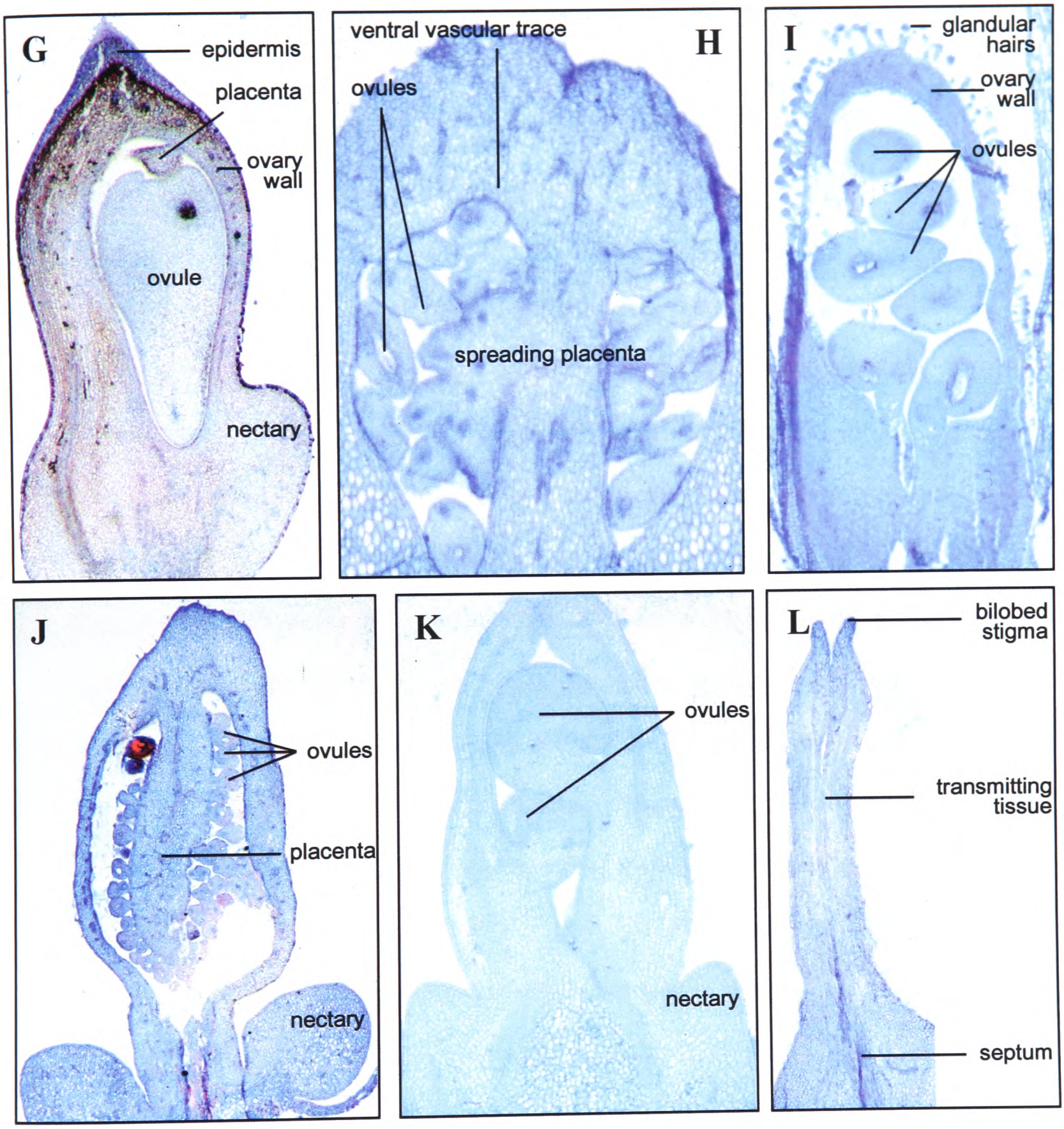


Figure 3.12 (continued) G. *Petra volubilis* (Verbenaceae), L.S. gynoecium. H. *Schlegelia violacea* (Schlegeliaceae), L.S. gynoecium. I. *Ruellia tuberosa* (Acanthaceae), L.S. gynoecium. J. *Eccremocarpus scaber* (Bignoniaceae), L.S. gynoecium, nectary. K. *Thunbergia grandiflora* (Acanthaceae), L.S. gynoecium. L. *Schlegelia violacea* (Schlegeliaceae), L.S. style, stigma.

placenta. In Verbenaceae the style may also be proximally hollow. In Schlegeliaceae the style has a central core of loosely-packed transmitting tissue continuous with the septum (Fig. 3.12L). In *Thomandersia*, by contrast, transmitting tissue is continuous with the locules (Fig. 3.5F). Compared to the cylindrical or slightly bilobed stigma of *Thomandersia* species, the stigmas of Lamiales are usually strongly bilobed, e.g. *Eccremocarpus* (Bignoniaceae), *Petrea* (Verbenaceae), and *Schlegelia* (Fig. 3.12L).

Floral nectary. A well-vascularised floral nectary, continuous with the base of the gynoecium, is a potentially informative character in *Thomandersia* systematics. Similar nectaries are found in Pedaliaceae, Verbenaceae (Fig. 3.12G) and Schlegeliaceae (Fig. 3.12D). Gentry (1980) distinguished Schlegelieae from other Bignoniaceae (in which they were classified) primarily on the basis of this character. In most Bignoniaceae, as well as Pedaliaceae, the floral nectary takes the form of an annular disk, separated from the gynoecium and supplied by its own ring of vascular traces (e.g. *Eccremocarpus scaber*; Fig. 3.12F,J). Floral nectaries are uncommon in Acanthaceae; where there is a nectary it is distinct from the pistil (e.g. *Thunbergia*; Fig. 3.12K). Floral nectaries are widespread in dicotyledons and usually considered plesiomorphic in Lamiales (Lu, 1990; Watson & Dallwitz, 1992; Stevens, 2001). Therefore their absence from Acanthaceae, like wood anatomical characters and the absence of staminodes and vascular bundles in the septum of the gynoecium, may be indicative of the derived position of this family in Lamiales (Carlquist & Zona, 1988; Endress, 2001). Acanthaceae, with Lamiaceae, Orobanchaceae, *Mimulus* and *Paulownia*, is suggested to form group nested within the rest of Lamiales (Endress, 2001).

3.5.4 Pollen morphology. Pollen grains of *Thomandersia* are similar in all species, although the number of colpi varies within species. This contrasts with the description by Heine (1966a), who stated that all species of *Thomandersia* had five-colpate pollen except *T. congolana*, which had six-colpate pollen.

Pollen similar in morphology to that of *Thomandersia* is found in a range of Lamiales. Bignoniaceae display a great variety of pollen grain morphologies (Gentry & Tomb, 1979) including grains with simple colpi, some of which appear similar to those of *Thomandersia*. For example, pollen grains of *Incarvillea emodii* are approximately 50 μm in diameter with seven equatorial colpi (Gentry & Tomb, 1979). Verbenaceae is also highly eurypalynous, and in many genera the pollen is colpate rather than colporate (Raj, 1983a). Pollen of many Pedaliaceae is also colpate (Raj, 1961; Straka & Ihlenfeldt, 1965). Most pollen grains of Verbenaceae and Pedaliaceae are of a similar size and shape to those of *Thomandersia*, and a few Verbenaceae (e.g. *Cornutia*) have five colpi. While pollen of most Pedaliaceae has seven equatorial apertures, some, such as *Pedalium murex*, have 5-6 colpi as in *Thomandersia* (Raj, 1961). Pollen morphology of Schlegeliaceae is also quite variable but in all genera is triaperturate (Gentry & Tomb, 1979). Pollen of Acanthaceae is extremely varied (Raj, 1961; Scotland & Vollesen, 2000). It is usually colporate in Ruellieae, so the colpate pollen of *Thomandersia* is considered anomalous in this tribe (Lindau, 1897; Bremekamp, 1942; Raj, 1961).

The degree of intrafamilial variation in pollen morphology in Lamiales makes it useful for generic-level systematics, but probably of little use at interfamilial level in this order. While uniting the six species of *Thomandersia*, it cannot be used to

indicate the relationships of the genus. Pollen grains similar to those of *Thomandersia* in size, shape, aperture type and number are found in a number of Lamiales families including Bignoniaceae, Pedaliaceae and Verbenaceae. Extensive studies by Raj (1961) found no instances of this type of pollen in Acanthaceae. However, contrary to Bremekamp's (1942) opinion, Acanthaceae should not be ruled out as a sister group to *Thomandersia* on the basis of pollen morphology alone, since there is such a range of morphologies present in the family.

3.5.5 Embryology. Embryological characters may be potentially informative for systematics in Lamiales (Johri & Singh, 1959; Phatak & Ambegaokar, 1961; Makeshwari, 1963; Mohan Ram & Masand, 1963; Davis, 1966; Yamazaki, 1974; Dahlgren, 1991). The number of ovules in each carpel of *Thomandersia* was considered by previous authors to be constant at two per locule (e.g. Bremekamp, 1942; Heine, 1966a). In reality it ranges from one in some specimens (through abortion) to three in others. In Acanthaceae there may be two (e.g. *Thunbergia*, Fig. 3.12K) to multiple (e.g. *Ruellia*; Fig. 3.12I) ovules per carpel. In other Lamiales studied, ovules are usually multiple. *Thomandersia* ovules are hemianatropous (Fig. 3.6D) like those of some Bignoniaceae (Watson & Dallwitz, 1992). Most other families of Lamiales have anatropous ovules. Fully syncarpous, bilocular ovaries are usually characterised by axile placentation, as in *Thomandersia.* In Acanthaceae, placentation is predominantly axile but distally parietal where the carpels are not fully fused (e.g. in *Pseuderanthemum*). In some Bignoniaceae, such as *Eccremocarpus* (Fig. 3.12E), there are two bilobed, parietal placentas. The distinctively expanded, crescent-shaped placentas of *Thomandersia* may be unique to the genus, although there is a placental swelling above the ovules in *Petrea volubilis* (Verbenaceae; Fig.

3.12G), and in *Schlegelia violacea* (Schlegeliaceae) the placenta is large, spreading inside the carpels beyond the point at which it is fused to the septum (Fig. 3.12H).

3.5.6 Fruit and seed morphology. The capsules of *Thomandersia* are loculicidally, but not explosively, dehiscent. In Acanthaceae, by contrast, the capsules are usually explosive. *Thomandersia* seeds are spherical and scaly, compared to those of Acanthaceae, which are usually round, flat and frequently hairy (e.g. *Ruellia*, Fig. 3.13A), and Bignoniaceae, which are usually winged. *Thomandersia* seeds have folded cotyledons and lack endosperm. Endosperm is also lacking from mature seeds of Bignoniaceae, most Verbenaceae, and all subfamilies of Acanthaceae except Nelsonioideae (Stevens, 2001). Endosperm is present in the seeds of Schlegeliaceae and thin in Pedaliaceae (Stevens, 2001). The only families of Lamiales in which folded cotyledons have been reported are Phrymaceae and Acanthaceae (Watson & Dallwitz, 1992; Schwarzbach & McDade, 2002).

3.5.7 Retinacula. The seeds of both *Thomandersia* (Fig. 3.8) and Acanthoideae (Fig. 3.13A) are subtended by a retinaculum. To determine whether these are potentially homologous, or analogous as proposed by Bremekamp (1942), their similarity in structure, development and position was assessed (Jardine, 1970; Patterson, 1982; De Pinna, 1991; Lee, 1998; Rieppel & Kearney, 2002). Topographical position was examined relative to associated structures: the placenta, ovule and funicle. In *Thomandersia* the retinaculum develops, after fertilisation, from the funicle (Fig. 3.8B) and lies between the placenta and ovule. In Acanthaceae it also emerges at the distal end of the funicle rather than the placenta (Fig. 3.13B,C). In both cases the retinaculum remains attached to the fruit following dehiscence and seed expulsion. In

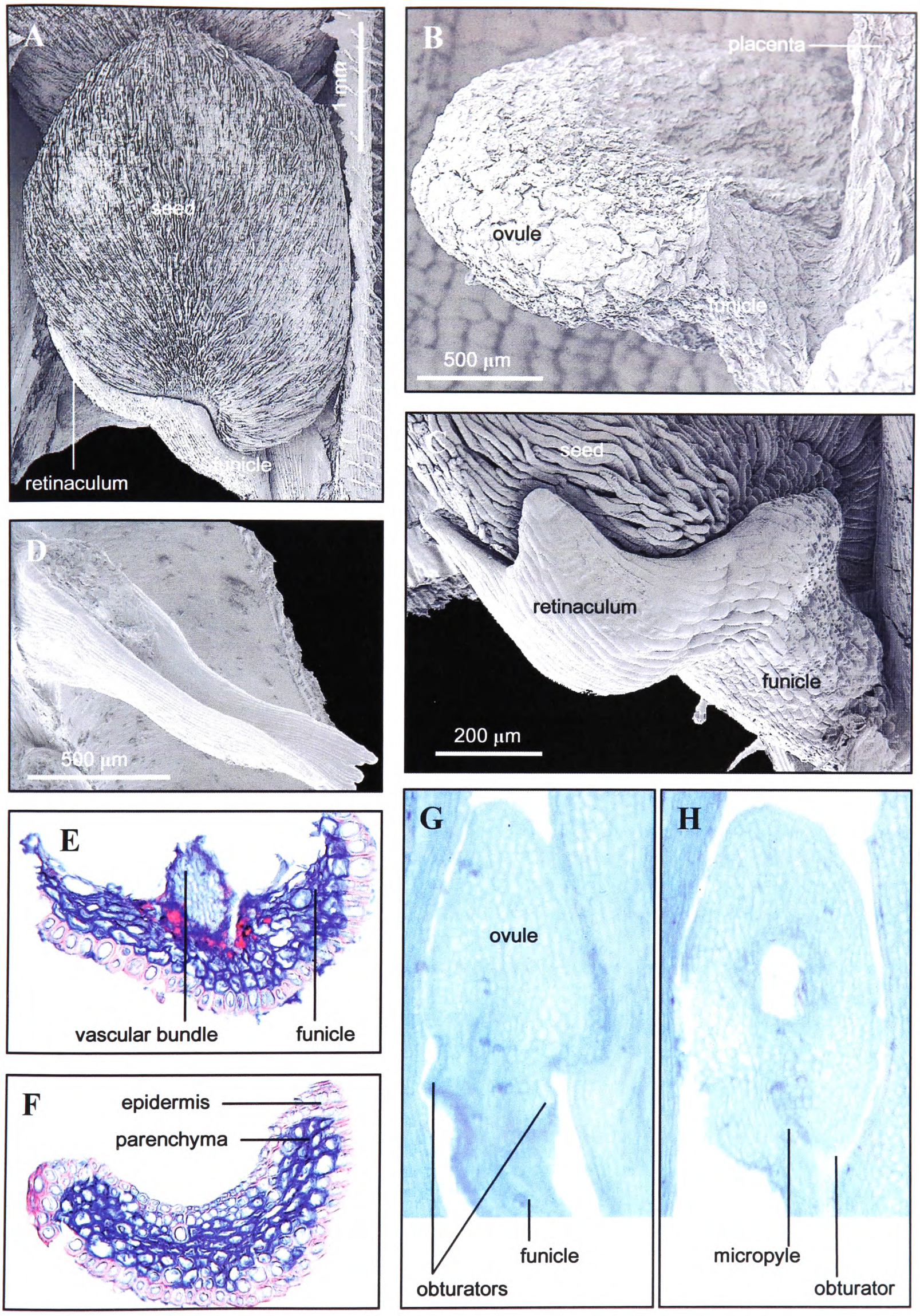


Figure 3.13 Acanthaceae fruit, seed and retinacula. A. *Ruellia tuberosa*, scanning electron micrograph of seed (x35). B,C. *Ruellia tuberosa,* scanning electron micrographs of developing ovule, showing emergence of retinaculum from funicle (B, x500; C, x150). D. *Pseuderanthemum tunicatum,* scanning electron micrograph of retinaculum (seed removed, x90). E,F. *Ruellia tuberosa,* T.S. developing retinaculum, serial sections from base to tip. G,H. *Pseuderanthemum tunicatum,* L.S. ovules (sectioned along adaxial-abaxial axis).

these respects the retinacula of *Thomandersia* and Acanthaceae are topographically correspondent funicular outgrowths.

The retinacula of *Thomandersia* and Acanthaceae initially appear dissimilar in shape. Those of *Thomandersia* are broad and flat (Fig. 3.8F) while in Acanthaceae they are narrower and U-shaped in cross section (Fig. 3.13D). These differences, however, could be attributed to constraints imposed by the shape of the adjacent developing seed. In *Thomandersia* the retinaculum is constrained to lie flat between the surface of the spheroid seed and the carpel wall; in Acanthaceae the seeds are usually disc-shaped and the retinaculum, lying along one edge of the seed, is forced into the shape of a channel (Fig. 3.13D).

The retinacula of *Thomandersia* are larger than those of many Acanthaceae. However, the range of retinacula across Acanthaceae as a whole encompasses a variety of sizes, from rudimentary in *Nelsonia* and *Elytraria* to 3 mm long in *Trichanthera* (Mohan Ram & Masand, 1963; Rosales, 1997). The retinaculum of *Thomandersia* initially appears broader than in those Acanthaceae investigated here, but this also may be a result of its position, flattened against the side of the capsule by the seed.

In cellular structure, the retinacula of *Thomandersia* and Acanthaceae are similar, comprising a mass of small parenchyma cells without vascular tissue (Figs. 3.8E, 3.13F). There is an epidermal layer of larger, red-staining cells (Figs. 3.8F, 3.13F) which may accumulate lignin or other chemicals associated with the stiffening of the structure. In Acanthaceae (at least in *Ruellia*) the uniseriate red layer extends around

the entire perimeter of the retinaculum (Fig. 3.13F), whereas in *Thomandersia* it is confined to the inner epidermis (Fig. 3.8F) and is a number of cells thick.

Retinacula develop late in the maturation of *Thomandersia* fruits, once the associated ovule has been fertilised. It is possible that Bremekamp (1942) was misled by the enlarged placentas of *Thomandersia* when stating that the retinacula were placental structures developing early in the ontogeny of the gynoecium. In Acanthoideae, retinacula also develop after fertilisation, although again this observation is hampered by structures surrounding the developing ovule. The funicle in Acanthoideae may be associated with swollen proliferations of dark-staining epidermal tissues that are apparent very early in floral development (Fig. 3.13G). For instance, in *Pseuderanthemum* these can be seen on both the dorsal and ventral sides of the developing funicle, at the base of the ovule (Fig. 3.13G). There is also an integumentary swelling of elongated cells adjacent to the micropyle (Fig. 3.13H). Such tissues are usually considered to be obturators or strophioles (Tilton & Horner, 1980; Shamrov, 1998; Werker, 1999). Most pertinent to this study, the distinctive retinacula do not emerge until a much later stage of maturity (Fig. 3.13B,C).

Three criteria are conventionally invoked to assess primary homology (Rieppel & Kearney, 2002): similarity in relative position (topographic correspondence), detailed structure, and ontogeny (Jardine, 1970; Patterson, 1982; De Pinna, 1991; Lee, 1998; Rieppel & Kearney, 2002). According to these criteria, the retinacula of *Thomandersia* and Acanthaceae are primary homologues. They differ in shape and size, but this can plausibly be attributed to mechanical constraints on development imposed by adjacent structures. In relative position, both are outgrowths of the

funicle. In composition and anatomy the two outgrowths are also more or less similar. In development, *Thomandersia* retinacula emerge after the ovules are fertilised and Acanthaceae retinacula also become visible at a late stage, perhaps after fertilisation. Previous authors have described the appearance of the retinaculum in Acanthaceae only in association with post-fertilisation ovules, just as observed here for *Thomandersia* (Mohan Ram, 1960; Phatak & Ambegaokar, 1961; Mohan Ram, 1962).

Retinacula are therefore primary homologies (*sensu* Remane, 1952; Patterson, 1982; Rieppel, 1988) of *Thomandersia* and Acanthaceae. However, they are probably not secondary homologies. Homoplasy can be demonstrated by incongruence with other morphological or molecular characters (Wilson, 1960; Patterson, 1982; Brower & Schawaroch, 1996). Preliminary molecular phylogenetic analyses of Lamiales imply that *Thomandersia* is not a member of Acanthaceae (Chapter 1). If this is a correct representation of relationships then retinacula must be a convergent, homoplastic character.

Retinacula formed the basis for most classifications of *Thomandersia* in the past. One possible explanation for this is that they were interpreted as relatively complex structures, stiffened with secondary compounds and shaped for the specific positioning and possible expulsion of the seed (Bremekamp, 1926; Witztum & Schulgasser, 1995). However, as the present study demonstrates, retinacula are in fact a simple tissue with no vascular bundles, comprising only parenchyma cells and an epidermis that stains red in the same way as that of many other parts of the flower of *Thomandersia* and other Lamiales. Their shape can be attributed to the constraints imposed by the adjacent seed, although this hypothesis has yet to be tested. Another

reason is their relative uniqueness: unlike many characters studied here, which are widespread and very homoplastic, retinacula are so far known only in Acanthaceae and *Thomandersia*. The function of the retinacula in *Thomandersia* remains undetermined.

3.5.8 Extrafloral nectary. A conspicuous character, possibly unique to *Thomandersia*, is the single or paired extrafloral nectary at the base of the calyx (Figs. 3.9, 3.14A). The cellular organisation of these nectaries is common to a wide range of plant secretory glands (Fahn, 1988). Multicellular, multi-layered extrafloral nectaries are widespread in Lamiales, and members of a number of families bear nectary-like structures potentially similar to those of *Thomandersia.* However the nectaries of *Thomandersia* are exceptional in terms of both the size of the gland itself and the conspicuous swelling in which it is contained.

Bremekamp (1942) hypothesised that the nectaries of *Thomandersia* were homologous to nectaries in the axils of the bracteoles of Pedaliaceae, which are derived from a much-reduced axillary bud (Singh, 1960; Monod, 1986). This hypothesis is supported by the fact that the nectaries of *Thomandersia* are found at the base of the flowers in a compound raceme and, in *T. anachoreta*, occur in pairs between paired bracteoles. Examination of a number of inflorescences demonstrated the presence of a variety of nectaries and buds in the axils of the bracts of individual flowers, particularly towards the tip of the indeterminate inflorescence (e.g. Fig. 3.3B). On the other hand, in *Thomandersia* the nectaries are found on the calyx rather than at the base of the pedicel and are adjacent to the bracteoles rather than in their axils. There are no derivatives of floral whorls in the structure of the glands of

Thomandersia, as were found in anatomical investigations of Pedaliaceae, proving the floral origin of those structures (Singh, 1960; Monod, 1986). Instead, the extrafloral nectaries of *Thomandersia* consist of distinct layers of specialised secretory cells, with vascular traces passing around the associated secretory tissues but not feeding directly into the gland.

Among other Lamiales, conspicuous extrafloral nectaries are very frequent in Bignoniaceae, on various parts of the plant including the abaxial exterior of the calyx (Seibert, 1948; Fahn, 1979; Stevens, 2001). In most cases these calycine glands differ from *Thomandersia* in that they are multiple, small (less than 100 μm diameter), possess a single large barrier cell, are not vascularised, and are flush with the epidermis rather than in a tissue swelling (e.g. Seibert, 1948; Thomas & Dave, 1992). In species of *Campsis* (Fig. 3.14B) and *Tecomaria* the abaxial calycine nectaries have a cellular structure very similar to those of *Thomandersia* and are subtended by vascular bundles (Elias & Gelband, 1976; Subramanian & Inamdar, 1989). However, these nectaries reach a maximum of 0.3 mm in diameter (compared to 2 mm in *Thomandersia*) and are found in aggregations of c.28 nectaries per flower (Subramanian & Inamdar, 1989). Seibert (1948) described "exceptionally large" calycine nectaries in *Callichlamys*, but these reach a maximum size of 0.5 mm, and are multiple rather than solitary. None of the nectaries described in Bignoniaceae are embedded in the calyx with a channel to the surface; rather they are broad, cup-shaped structures open to the epidermis (Fig. 3.14B). However, these features may be only superficial; even in *Thomandersia* the extent of channelling varies between *T. hensii* and *T. anachoreta.* Similarity is more reliably determined by underlying cellular structure. In summary, species of Bignoniaceae and *Thomandersia* bear extrafloral

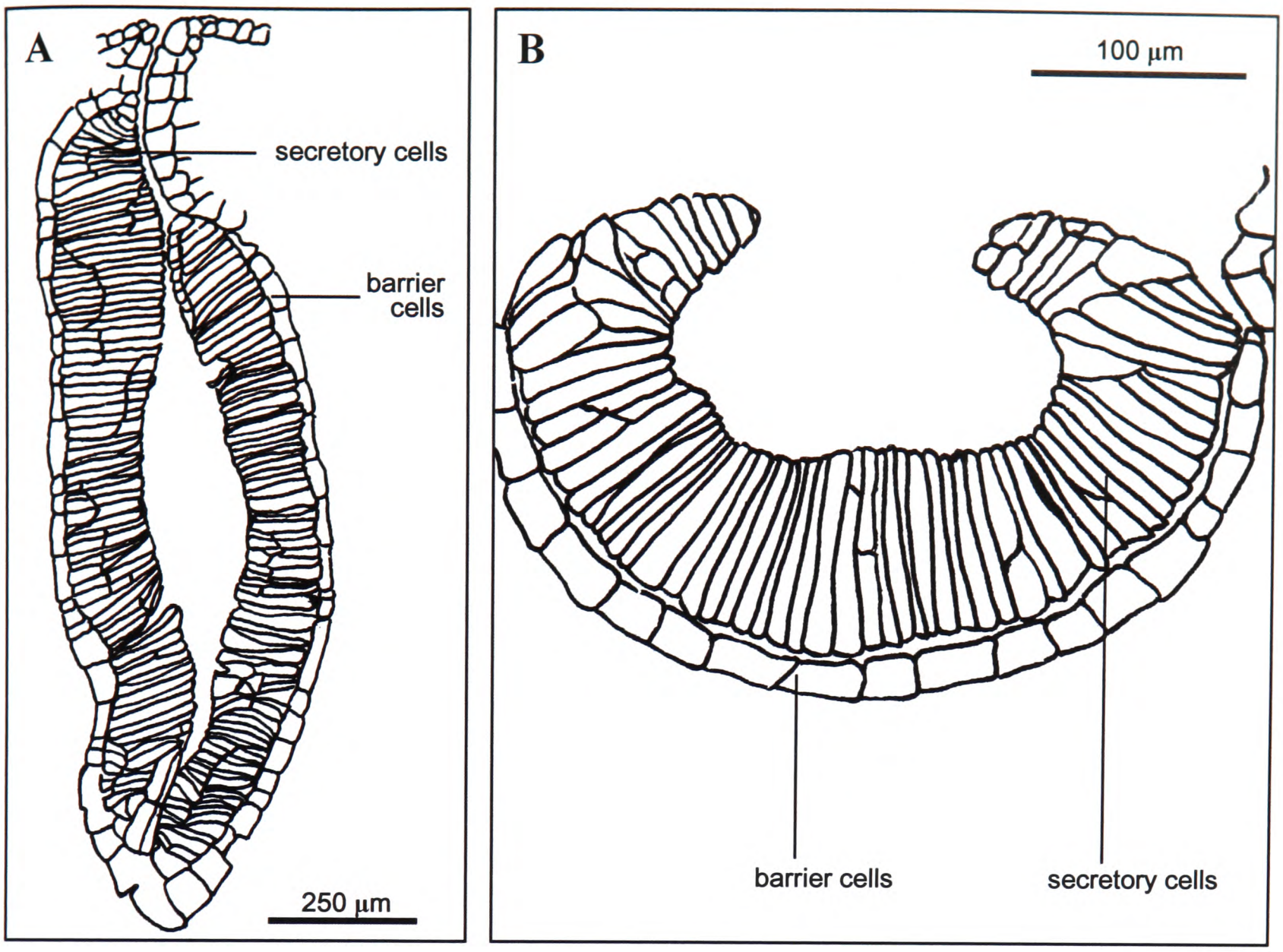


Figure 3.14 Comparison of calycine nectaries in *Thomandersia* and Bignoniaceae (represented by *Campsis*). A. *Thomandersia hensii*, L.S. nectary, drawn from light micrograph. These nectaries are solitary, approximately 260 µm across and 1500 µm long, channelled and embedded in a swelling in the calyx. B. *Campsis radicans*, L.S. nectary, drawn from Elias & Gelband (1976). These nectaries are found in aggregations of up to 20, approximately 260 µm across but only 170 µm deep, cup-shaped and flush with the calyx surface.

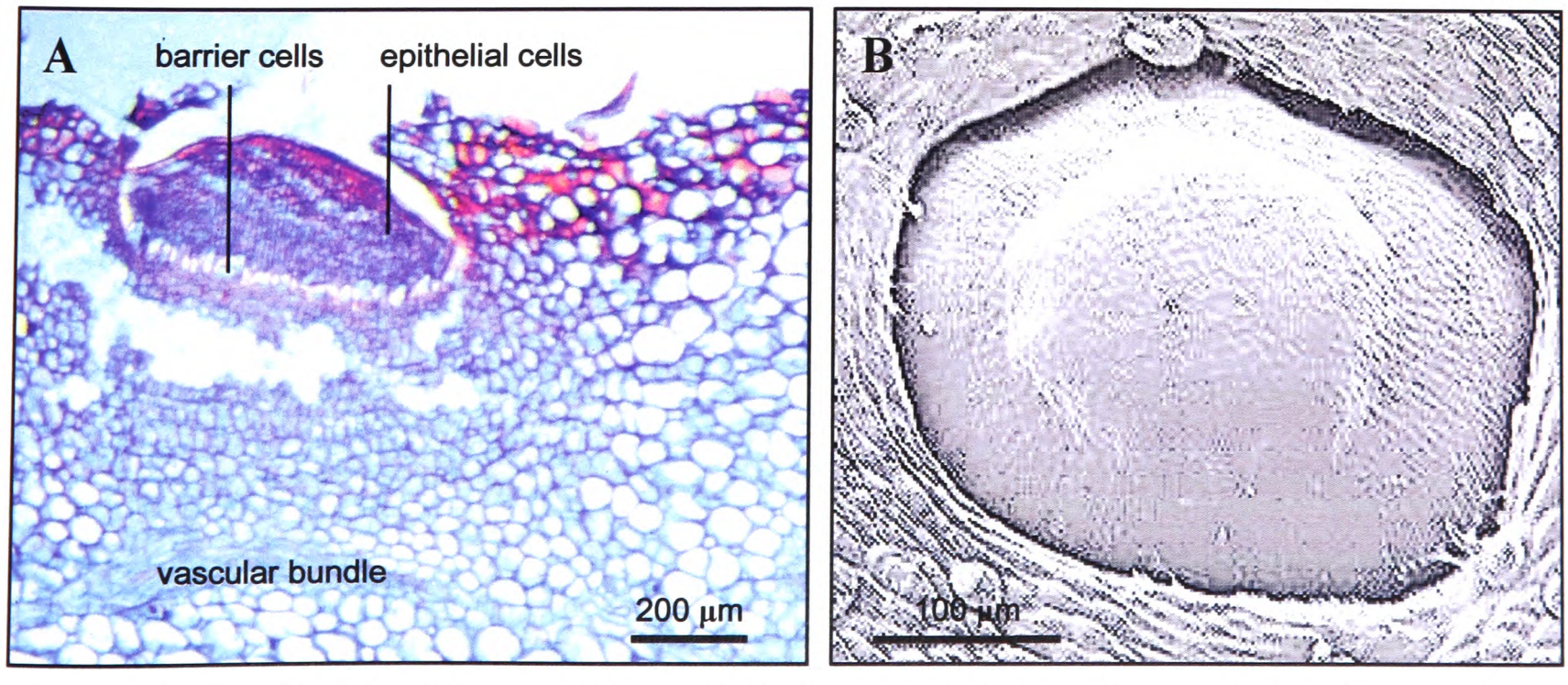


Figure 3.15 *Schlegelia violacea* calycine gland. A. section through tissues of calyx. B. scanning electron micrograph of gland in calyx surface (x200).

nectaries in similar topographic positions, sometimes with a similar anatomical structure, although in Bignoniaceae they are smaller, multiple, with a broad opening and lack a surrounding swelling.

Schlegelia also displays multiple large, multicellular glands on the abaxial side of the calyx, which have not previously been described (e.g. Bureau & Schumann, 1896-1897; Gentry, 1974a; Burger & Barringer, 2000). The glands are larger (c.0.5 mm in diameter) than in Bignoniaceae but smaller than in *Thomandersia*. They are found in groups on the abaxial surface of the calyx, sunken in the epidermis but not in a noticeable swelling. Their cellular organisation (Fig. 3.15A) is similar to that of *Thomandersia*, comprising columnar, apparently secretory cells, opening to the epidermis, subtended by a single layer of large, colourless barrier cells, themselves surrounded by a region of small, dark-staining cells with crystals that are visible under phase contrast. A vascular supply surrounds this layer of cells. The opening of the gland to the epidermis is broader than the narrow channel seen in *Thomandersia hensii* and the gland itself rises to the opening rather than invaginating around a central channel (Fig. 3.15B).

To properly assess the homology of the extrafloral nectaries it is necessary to apply the criteria of similarity outlined above (Jardine, 1970; Patterson, 1982; Lee, 1998; Rieppel & Kearney, 2002). Similarity of position is fulfilled for the nectaries of *Thomandersia,* Schlegeliaceae and some Bignoniaceae, since all are found in the epidermis of the calyx, although in Bignoniaceae they are also common elsewhere. The nectaries of Bignoniaceae and Schlegeliaceae differ in relative size, number, and shape from those of *Thomandersia*, but are anatomically very similar. This implies

that the criteria of similar detailed structure and ontogeny are also fulfilled. The extrafloral nectaries of *Thomandersia* might therefore be primary homologues of those in *Schlegelia* and some Bignoniaceae such as *Campsis* (Elias & Gelband, 1976), although their large size may be autapomorphic for *Thomandersia*. As with retinacula, the secondary homology of the character cannot be determined without testing it against a rigorous phylogenetic hypothesis based on the distribution of many characters.

3.5.9 Systematic implications for Thomandersia *in Lamiales.* Past classifications of *Thomandersia* have been based upon intuitive assessments regarding which morphological characters are significant for systematics. In the majority of treatments, *Thomandersia* has been assigned to Acanthoideae (e.g. Bentham, 1876; Baillon, 1891; Lindau, 1894; 1897; Heine, 1966a). The retinaculum was heavily weighted as a unique character linking *Thomandersia* to Acanthaceae. The anatomical investigation conducted here reveals a variety of additional characters that could link *Thomandersia* with other families in Lamiales, as well as noting characters unique to the genus and those that are plesiomorphies of the order.

Acanthaceae are likely a highly derived family, shown by both wood and floral anatomy (Endress, 2001). *Thomandersia*'s membership of Acanthaceae (e.g. Bentham, 1876; Lindau, 1897; Heine, 1966a) is contradicted by the fact that it lacks many derived features of this family, such as included phloem, cystoliths and acicular crystals, large bracts and bracteoles, the loss of a staminode, and explosively-dehiscent capsules. *Thomandersia* does share with Acanthaceae a persistent calyx and lack of endosperm (both characters also shared with other putative sister groups) as

well as folded cotyledons and retinacula. However, *Thomandersia* also shares characters with each of the other families of Lamiales investigated (Table 3.1).

Table 3.1 Anatomical characters of *Thomandersia* and their presence in other Lamiales studied, excluding those that are plesiomorphic for Lamiales.

Character	Acanthaceae	Pedaliaceae	Bignoniaceae	Schlegeliaceae	Verbenaceae
Calyx open in bud	-	-	+	-	-
Calyx persistent	+	-	-	-	+
Calyx red-staining	-	-	+	+	+
Calycine extrafloral nectary	-	-	+	+	-
Corolla gibbous	-	+	+	-	-
Staminode without vascular bundles	-	-	-	-	+
Gynoecium with continuous nectary	-	+	+	+	+
Gynoecium with two vascular rings	-	-	+	+	-
Ovules hemianatropous	-	-	+	-	-
Endosperm lacking	+	-	+	-	+
Cotyledons folded	+	-	-	-	-
Retinaculum	+	-	-	-	-
Pericarp woody	-	-	-	+	+

A relationship between *Thomandersia* and Pedaliaceae has been suggested by Bremekamp (1942) on the grounds of pollen morphology, extrafloral nectaries and his (incorrect) assessment of *Thomandersia* retinacula as placental excrescences. This hypothesis is not upheld, although *Thomandersia* does share with Pedaliaceae a gibbous corolla and vascularised nectary continuous with the gynoecium.

Three families are associated with *Thomandersia* on preliminary molecular grounds (Chapter 1). Of these, Bignoniaceae shares with *Thomandersia* a thick, red-staining calyx, apparently open in bud, calycine extrafloral nectaries, a frequently gibbous corolla tube, vascularised nectary continuous with the gynoecium, two rings of vascular bundles in the gynoecium, hemianatropous ovules and non-endospermic seeds. With Schlegeliaceae, *Thomandersia* shares a red-staining calyx, calycine extrafloral nectaries, vascularised floral nectary continuous with the gynoecium, two rings of vascular bundles in the ovary wall and septum, and a thick, woody pericarp. With Verbenaceae, *Thomandersia* shares a red-staining, persistent calyx, a staminode lacking vascular bundles, vascularised nectary continuous with the gynoecium, very thick ovary wall and woody pericarp, and non-endospermic seeds.

Pollen morphology, embryology and floral anatomy reveal a number of characters that are potential primary homologies of *Thomandersia* and other families in Lamiales, including all three families to which molecular sequence analyses have suggested a possible relationship. Detailed investigation and application of similarity criteria suggests the extrafloral nectaries of *Thomandersia* may be primary homologues of those of some Bignoniaceae and Schlegeliaceae, while the retinaculum is a primary homologue of that in Acanthoideae. Thus morphological data remain equivocal regarding the systematic relationships of *Thomandersia* and it is inevitable that some primary homologies will be found to be homoplastic.

3.6 Conclusions

This is the first detailed investigation into the anatomy of *Thomandersia,* and provides a basis for assessing which characters of the genus are unique, which shared with a

wide range of Lamiales, and which might be shared with potential sister groups or diagnostic of such relationships. It enables a more informed evaluation of possible classifications of the genus, within the context of aiming at a diagnostic, monophyletic classification for Lamiales.

The majority of anatomical features of *Thomandersia* can be interpreted as plesiomorphies. This is particularly true for wood anatomical characters. Unlike Acanthaceae, *Thomandersia* displays typically Lamialean wood characters. This supports its membership of Lamiales, but does not indicate a relationship to any particular family. Many floral and inflorescence characters of *Thomandersia* can also be interpreted as plesiomorphic, such as racemose inflorescences with bracts and bracteoles, a pentamerous, sympetalous calyx and corolla, four didynamous stamens and a superior, eusyncarpous, bicarpellary pistil.

Unique characters of *Thomandersia* include the particular structure and size of the calycine nectary, form of the expanded placentas, corolla aestivation pattern, cylindrical stigma and spherical, scaly seeds.

Thomandersia shares some features with all other Lamiales investigated (Table 3.1). Two non-plesiomorphic anatomical characters are shared with Pedaliaceae, four with Acanthaceae, five with Schlegeliaceae, six with Verbenaceae and eight with Bignoniaceae. However, most of these characters (Table 3.1) are shared with more than one family and/or variable within families.

This investigation of morphology and anatomy in *Thomandersia* shows that morphological characters are equivocal for determining its phylogenetic relationships in a traditional, intuitive manner (e.g. Bremekamp, 1942; Heine, 1966a; Sreemadhaven, 1976). Many of the characters that do show potential primary homology with other Lamiales taxa are incongruent with one another and are often polymorphic within families. It is therefore unsurprising that previous classifications of *Thomandersia*, based on one or a few morphological characters, have contradicted one another and do not necessarily reflect evolutionary relationships (Bremekamp, 1942; Heine, 1966a; Sreemadhaven, 1976). Rigorous phylogenetic hypotheses based on numerical analysis are needed to determine which of the primary homologies identified here are secondary homologies, and which are homoplasies. To date, neither morphological cladistic analysis (Lu, 1990), nor preliminary molecular-sequence based analyses (Chapter 1) have provided reliable or well supported hypotheses of the phylogeny of Lamiales. Chapters 4 and 5 investigate the best ways to build a more robust phylogeny of Lamiales including *Thomandersia*, which may lead to a more reliable classification based on monophyletic groups.

Chapter 4. The utility of morphological data to resolve the phylogenetic position of *Thomandersia*

4.1 Introduction

Thomandersia is a clearly-defined, diagnosable group and a member of Lamiales, a fact that is supported by both molecular phylogenetic analysis and a combination of morphological characters. The family-level relationships of *Thomandersia* have so far proved more difficult to determine with either type of data. Chapters 4 and 5 investigate in detail the potential of morphological and molecular datasets to resolve the relationships of *Thomandersia* through phylogenetic analysis.

Phylogenetic analyses of molecular sequence data have thus far provided only partial resolution in Lamiales (Albach et al., 2001a; Olmstead et al., 2001; Bremer et al., 2002). The order is characterised by a number of well-supported families and several isolated lineages, often coincident with the rank of genus (Table 4.1). These groups are separated by short, unstable internal branches, and there is low bootstrap support for interfamilial relationships.

Phylogenies similar to that of Lamiales, comprising well-supported clades separated by short, unstable branches, are common throughout the tree of life. At least 25 of the 45 orders recognised in the most recent angiosperm classification display this type of topology when reconstructed using molecular data (Angiosperm Phylogeny Group 2003). Examples include Commelinales, Fabales, Poales and Saxifragales (Givnish et al., 1999; Fishbein et al., 2001; Kajita et al., 2001; Bremer, 2002). The problem is not

Table 4.1 Families and genera of Lamiales in previous studies.

Taxon (Olmstead et al., 2001; APGII, 2003)	**Alternative taxon (Lu, 1990)**	**Genera/species (Mabberley, 1997; Olmstead, in progress)**	**Support (Olmstead et al., 2001)**
Lamiales Bromhead	**Lamiiflorae** Dahlgren		
Acanthaceae Juss. (inc. Avicenniaceae)	Acanthaceae Nelsoniaceae Thunbergiaceae Mendonciaceae	202/3,510	91
Bignoniaceae Juss.	Bignoniaceae	114/725	100
Calceolariaceae Olmstead	-	3/275	100
Carlemanniaceae Airy Shaw	-	1/3	-
Gesneriaceae DC.	Gesneriaceae	140/3,000	94
Lamiaceae Martynov	Lamiaceae/ Verbenaceae	252/6,700	99
Verbenaceae J.St.-Hil.		38/1,020	73
Lentibulariaceae Rich.	Lentibulariaceae	3/245	-
Byblidaceae Domin		2/4	-
Martyniaceae Horan.	Martyniaceae	4/17	-
Oleaceae Hoffman. & Link	excluded	25/600	-
Paulowniaceae Nakai	-	1/6	-
Pedaliaceae R.Br.	Pedaliaceae	13/70	-
Phrymaceae Schauer	Phrymaceae	11/195	-
Plocospermataceae Hutch.	-	1/1	-
Schlegeliaceae Reveal	-	4/20	-
Scrophulariaceae Juss.	Buddlejaceae Myoporaceae Selaginaceae	52/1,681	78
Orobanchaceae Vent. (inc. Cyclocheilaceae, Nesogenaceae)	Scrophulariaceae	65/1,538	99
Stilbaceae Kunth	Retziaceae	11/39	69
Tetrachondraceae Wettst.	-	2/3	-
Veronicaceae Durande	Callitrichaceae Plantaginaceae Globulariaceae Hippuridaceae	97/1,854	48
Unplaced genera			
Craterostigma	-	1/9	-
Cubitanthus	-	1/1	-
Ellisiophyllum	-	1/1	-
Limnophila	-	1/37	-
Lindernia	-	1/80	-
Lithophytum	-	1/1	-
Peltanthera	-	1/1	-
Pierranthus	-	1/1	-
Rehmannia	-	1/9	-
Sanango	-	1/1	-
Thomandersia	-	1/6	-
Torenia	-	1/40	-
Totals	-	**1,052/21,696**	-

confined to orders but is also found, for example, between species within *Gossypium*, orders within core eudicots and phyla in Metazoa (Halanych, 1998; Cronn et al., 2002; Soltis et al., 2003). Developing an approach to phylogeny reconstruction – based on morphological data, molecular data, or both – that improves support and resolution in Lamiales may suggest ways to resolve these similarly problematic phylogenies.

This chapter investigates the potential of morphological data to reconstruct the phylogeny of Lamiales and the position of *Thomandersia*. The utility of morphology in phylogeny reconstruction is seen as increasingly uncertain, as systematics focuses on larger molecular datasets (Hillis, 1987; Patterson, 1988; Donoghue & Sanderson, 1992; Kadereit, 1994; Mishler, 1994; Tehler, 1995; Givnish & Sytsma, 1997; Hillis & Wiens, 2000; Baker & Gatesy, 2002; Endress, 2003; Scotland et al., 2003; Wahlberg & Nylin, 2003). The relative usefulness of datasets can be established according to criteria such as the support, resolution, internal consistency or robustness of the phylogenies inferred from them (Lecointre et al., 1993; Huelsenbeck, 1995; Bremer et al., 1999), or may be examined *a priori* using measures such as the character-taxon ratio (Sanderson & Donoghue, 1989; Sanderson, 1991).

The chapter begins by introducing the theoretical debate concerning morphological and molecular datasets in phylogeny reconstruction. There follows a comparison of morphological, molecular, and combined datasets in phylogenetic analysis in practice. Both *a priori* measures of potential informativeness and *a posteriori* assessments of support and resolution are employed. In this context, the potential utility of morphological characters is then investigated for the specific case of Lamiales

phylogeny. A large morphological dataset is analysed both independently and in simultaneous analysis with molecular sequence data. The processes of selecting morphological characters are discussed and the impact of character selection upon Lamiales phylogeny is investigated.

4.2 Morphological versus molecular data in phylogenetic analysis

The relevance of morphological data to phylogeny reconstruction, a discipline increasingly dominated by molecular sequence data, has been widely debated (e.g. Hillis, 1987; Sytsma, 1990; Patterson et al., 1993; Baker et al., 1998; Baker & Gatesy, 2002; Scotland et al., 2003; Wahlberg & Nylin, 2003). A crucial role for morphology is widely acknowledged in the systematic activities of species description and delimitation, studies of character evolution and evolutionary-developmental ("evo-devo") biology (Doyle & Endress, 2000; Wilson, 2000; Heywood, 2001; Wortley et al., 2002; Endress, 2003; Scotland et al., 2003), but its relevance to computer algorithm-based phylogenetic analysis remains controversial.

Molecular evidence is considered superior to morphological data for accurate phylogeny reconstruction on the grounds that it is less susceptible to convergence homoplasy (Sytsma, 1990; Givnish & Sytsma, 1997; Baker & Gatesy, 2002; Collin, 2003). The genetic basis of convergence is thought to be more completely understood for molecular datasets, where primary homology can be determined more objectively across a larger range of taxa (Mishler, 1994; Raff et al., 1994; Smith & Sytsma, 1994; Scotland et al., 2003).

The most significant advantage of molecular datasets is thought to be the greater number of independent characters they can contain (Felsenstein, 1985; Hillis, 1987; Mishler, 1994; Soltis & Soltis, 2000; Scotland et al., 2003). The ease of collecting large numbers of molecular characters is demonstrated by a recent surge in multi-gene and whole-genome phylogenetic studies (e.g. Graham & Olmstead, 2000; Herniou et al., 2001; Matsuoka et al., 2002; Rokas et al., 2003). Larger datasets contain a greater number of potentially informative characters and therefore increase the statistical power of phylogenetic inference (Hillis, 1987; Donoghue & Sanderson, 1992; Farris, 2000). Large datasets also reduce the effect of any mistakes in character coding or conceptualisation upon the resulting phylogeny (Raff et al., 1994). Thus molecular datasets, due to their size, are often considered superior to morphological matrices for computer-based phylogeny reconstruction (Hillis, 1987; Sanderson & Donoghue, 1989; Lamboy, 1994; Soltis & Soltis, 2000; Baker & Gatesy, 2002; Scotland et al., 2003).

Morphological datasets may have a role in phylogeny reconstruction despite the apparent size advantage of molecular matrices (e.g. Wiens, 2000; Stuessy et al., 2003). For instance in ancient, rapid radiation events, morphological characters may have evolved at the correct rate to "track" relationships without being overwritten by subsequent evolutionary change (Donoghue & Sanderson, 1992; Bateman, 1999). Morphological characters are applicable to a range of taxa (largely fossil species) for which molecular characters are unobtainable (Smith, 1994). Although homoplasy can be prevalent in morphological datasets, homoplastic characters may themselves be an important source of phylogenetic information and should not be rejected *a priori* (Kallersjö et al., 1999; Baker & Gatesy, 2002). Furthermore, molecular data can also

be subject to adaptive convergence or long-branch attraction and incorrect homology assessment through misalignment (Felsenstein, 1978; Raff et al., 1994; Huelsenbeck, 1995; Wheeler, 1995b; Crandall et al., 1999; Lee, 2001).

Perhaps the most widespread application of morphological datasets in phylogenetic analysis today is in simultaneous analysis with molecular sequence data (recent examples include Hauk et al., 2003; Lundberg & Bremer, 2003; Michelangeli et al., 2003; Sauquet et al., 2003; Virtanen, 2003). In principle, "total evidence" simultaneous analysis should maximise statistical and explanatory power (Hillis, 1987; Kluge, 1989; Swofford, 1991; Tehler, 1995; Nixon & Carpenter, 1996; Wahlberg & Nylin, 2003). Combining datasets is expected to provide new and useful information, especially if they contain phylogenetic signal at different hierarchical levels (e.g. Pennington, 1996; Nandi et al., 1998; De Luna et al., 1999). Even a small number of morphological characters may improve resolution where the molecular signal is weak or noisy (Sytsma, 1990; Doyle & Endress, 2000; Wahlberg & Nylin, 2003), and empirical studies suggest that morphological characters can also contribute significantly to support values (e.g. Baker et al., 1998).

Simultaneous analysis is not without controversy (e.g. Barrett et al., 1991; Bull et al., 1993; De Queiroz et al., 1995; Huelsenbeck et al., 1996b; Nixon & Carpenter, 1996). Combining morphological and molecular datasets that contain significantly different evolutionary signals can hinder or mislead phylogenetic inference (Bull et al., 1993; De Queiroz et al., 1995; Reed & Sperling, 1999). Alternatively, partitions that are shown to be statistically incongruent, for instance by the partition homogeneity test (Mickevich & Farris, 1981), can contribute positively to supported, resolved

phylogenies in simultaneous analysis (Baker et al., 1998; Baker & Gatesy, 2002; Collin, 2003). The accuracy of combined phylogenies is therefore open to question: it may be possible for a morphological dataset to lower the resolution of a molecular phylogeny or even generate unopposed, inaccurate relationships in simultaneous analysis (Donoghue & Sanderson, 1992; Givnish & Sytsma, 1997). Combining a large molecular dataset with a smaller morphological one could also risk "swamping" the signal in the morphological data (Miyamoto, 1985; Smith & Sytsma, 1994).

This chapter explores the theoretical perceptions of morphological and molecular datasets in practice. Section 4.3 considers the relationship between dataset size and potential informativeness. Several *a priori* measures are compared across a sample of recent molecular and morphological matrices, to assess their relative potential informativeness for phylogeny reconstruction. Section 4.4 explores the actual usefulness of morphological datasets in combined analysis with molecular sequences. A sample of recent total evidence analyses is examined to determine how the addition of morphological characters to a molecular dataset affects the decisiveness, resolution and support of the resulting phylogeny.

Character selection is an essential step in any phylogenetic analysis, particularly using morphological data. Section 4.5 discusses the principles of character selection in morphological phylogenetics. Section 4.6 applies these concepts to the specific problem of Lamiales: I empirically investigate whether a morphological matrix actually and potentially provides new, useful information for phylogeny reconstruction in Lamiales, how this depends upon character selection, and how it might affect the resulting tree. A large, inclusive morphological matrix is compared

with a smaller, stringent subset selected according to explicit criteria. Both matrices are analysed alone and in combination with a two-gene molecular dataset.

4.3 Measuring the potential informativeness of morphological data *a priori*

4.3.1 Introduction. The usefulness of datasets for phylogenetic inference may be evaluated before or after analysis: *a priori* measurements of potential informativeness can provide an indication of the likely support and resolution that a dataset might contribute to phylogenetic analysis (e.g. Felsenstein, 1985; Sanderson, 1995; Bremer et al., 1999); *a posteriori* assessments of support or resolution can demonstrate the actual effect of each dataset in a given phylogenetic study.

Potential informativeness is related to matrix size. Size, in this context, is usually measured as the number of aligned characters in the matrix (Felsenstein, 1985; Hillis, 1987; Mishler, 1994). However, this may not be the most instructive measure of the informativeness of a matrix for phylogeny reconstruction, particularly when comparing different types of data such as morphological and molecular characters. Several other possible measures of potential informativeness (summarised in Table 4.2) will be compared here, for a sample of empirical datasets.

Table 4.2 Measures of dataset potential informativeness.

Measure of informativeness	Notes
Number of characters	Measured after alignment for molecular datasets; equivalent to the number of columns in a matrix
Number of variable characters	Most relevant measure for maximum likelihood analysis
Number of parsimony-informative characters	Excludes autapomorphies; commonly calculated by phylogeny reconstruction computer programs such as PAUP* (Swofford, 1991)
Minimum number of parsimony-informative character state transformations	Most precise measure of those investigated here; relevant to parsimony analysis

The simplest measure of potential informativeness is the number of characters, i.e. the number of columns in a matrix. This is sometimes reported relative to the number of taxa, as the character-taxon ratio, i.e. the ratio of columns to rows in the matrix (e.g. Sanderson & Donoghue, 1989; Sanderson, 1991; Scotland et al., 2003). By this measure, the maximum numbers of molecular and morphological characters available for phylogeny reconstruction are often an order of magnitude apart: most morphological datasets are restricted to tens or hundreds of characters whereas molecular sequences can potentially be as long as an entire genome and frequently run to thousands of base pairs in length (e.g. Graham & Olmstead, 2000; Herniou et al., 2001; Matsuoka et al., 2002; Rokas et al., 2003).

However, potential informativeness depends upon more than just the number of characters; it is a complex entity related to character state transformations. It may therefore be more accurate to measure the number of *variable* characters in a matrix (although invariable characters may be informative in the sense that they enable correct alignment and therefore homology assessment of the rest). Morphological matrices typically include only variable characters, whereas a large proportion of the characters in molecular sequence datasets are invariant across all taxa studied. Thus the number of variable characters in a molecular matrix can be much fewer than its overall size suggests.

In model-based phylogeny reconstruction methods such as maximum likelihood, all variable sites provide potential information. For parsimony analysis (the method most commonly applied to morphological data) not all variable characters are informative. Only those characters displaying at least two states in at least two taxa can indicate

relationships. Phylogeny reconstruction programs such as PAUP* (Swofford, 1991) often display this as the number of *parsimony-informative characters.*

Potential informativeness can be measured more precisely still, because not all parsimony-informative characters contain the same amount of information. Firstly, multistate characters (for example, DNA sequence characters) tend to imply a greater number of state changes, and thus provide more potential evidence for groups, than binary characters (Baker et al., 1998). Secondly, one state of every character is plesiomorphic and uninformative. For example, a three-state character, implying a minimum of two changes on a tree, is potentially twice as informative as a two-state character requiring only a single change (rather than one-and-a-half times, as the number of states might suggest). A better way to compare the potential informativeness of matrices *a priori,* in a parsimony framework, is therefore by the *minimum number of parsimony-informative character state transformations* they entail. The different measures of potential informativeness are investigated below for a sample of morphological and molecular datasets.

4.3.2 Methods. To empirically investigate the potential informativeness of different types of matrix, *a priori,* for phylogenetic analysis, I compared the 50 molecular sequence (DNA or amino acid) and 50 non-sequence (morphological, anatomical, secondary chemical and behavioural) studies most recently contributed (at 1st November 2003) to TreeBASE (http://www.treebase.org/treebase; see Table 4.3). Only the largest (in terms of number of characters, whether single gene or concatenated) molecular or morphological matrix submitted in each study was used, to avoid including non-independent data points from multiple matrices. In some cases

both a morphological and molecular dataset were drawn from a single study. Where more than one matrix had been submitted for the same characters in the same study, the one covering the greatest number of taxa was used, thus maximising the potential number of informative character state transformations. The sample of analyses covered a variety of organisms, hierarchical levels of analysis, and analytical methods. Using PAUP* (Swofford, 2002) and manual counting, the number of aligned characters, variable characters, parsimony-informative characters, parsimony-informative states, and minimum number of parsimony-informative character state transformations was calculated for each matrix (Table 4.3). The matrices were then ranked according to each of these parameters.

4.3.3 Results. The simplest measure of potential informativeness, number of characters, showed a continuous, hollow distribution (Fig. 4.1) comprising a few very large matrices and a large number of much smaller ones. There was no distinct boundary between the sizes of molecular and morphological matrices in the studies examined – there was a slight overlap – but almost all molecular sequence datasets contained a greater number of characters than almost all morphological ones, a difference which was statistically significant ($p<0.0001$, two-tailed t-test).

A similar distribution was found when matrices were ranked according to their number of variable characters (Fig. 4.2), parsimony-informative characters (Fig. 4.3), or minimum parsimony-informative character state changes (Fig. 4.4). In each case the potential informativeness of the molecular sequence matrices was significantly greater than that of non-molecular ones (all p-values <0.0001). However, the distinction between molecular and morphological studies became less clear as more

Table 4.3 Structure of matrices compared from TreeBASE. PI, parsimony-informative. (continued overleaf).

	Taxa	Aligned characters	Variable characters	PI characters	PI states	Minimum PI changes	Character-taxon ratio
Non-molecular studies							
S635 (Bailey et al., 2001)	33	17	17	17	36	19	0.52
S407 (Baker et al., in press)	38	66	63	59	125	66	1.74
S662 (Berbee, 2001)	297	6	6	6	12	6	0.02
S756 (Beyra-Matos & Lavin, 1999)	42	50	50	46	123	77	1.19
S513 (Bininda-Emonds et al., 2001)	16	431	427	290	580	290	26.94
S611 (Bradford & Barnes, 2001)	39	48	48	46	105	59	1.23
S743 (Budd, 2002)	21	33	33	33	71	38	1.57
S465 (Coddington, 1991)	19	61	61	59	128	69	3.21
S723 (Cotton, 2001)	49	97	97	97	236	139	1.98
S410 (Davies, 1999)	22	36	36	28	62	34	1.64
S471 (De Jong et al., 1996)	75	103	103	100	258	158	1.37
S835 (Farmer & Schilling, 2002)	70	110	88	83	294	211	1.57
S589 (Geisler, 2001)	68	186	186	185	450	265	2.74
S953 (Gernandt et al., 2003)	19	20	20	19	61	42	1.05
S677 (Góes-Neto et al., 2001)	14	17	17	12	27	15	1.21
S411 (Griswold et al., 1999)	43	137	137	129	274	145	3.19
S560 (Hedin, 2001)	12	13	13	10	24	14	1.08
S945 (Hughes et al., 2004)	95	55	55	53	125	72	0.58
S540 (Hugot, 2002)	21	22	22	20	57	37	1.05
S435 (Hugot, 1999)	50	45	45	44	142	98	0.90
S432 (Jackman et al., 1999)	55	16	16	16	44	28	0.29
S372 (Johnson, 1999)	38	38	38	38	99	61	1.00
S718 (Kearney, 2003)	28	176	176	163	344	181	6.29
S438 (Marcus & McCune, 1999)	28	86	82	76	161	85	3.07
S739 (Marshall, 2002)	45	147	147	134	305	171	3.27
S970 (Nylander et al., 2004)	32	166	166	162	362	200	5.19
S387 (O'Leary & Geisler, 1999)	40	123	123	122	279	157	3.08
S399 (Page et al., 1995)	122	58	58	58	179	121	0.48
S877 (Pedersen & Hedenäs, 2003)	37	30	30	29	63	34	0.81
S638 (Platnick et al., 1991)	43	67	67	67	144	77	1.56
S486 (Prendini, 2000)	71	115	115	110	245	135	1.62
S543 (Pryer et al., 2001)	35	136	135	128	299	171	3.89
S437 (Quicke & Belshaw, 1999)	33	118	118	118	261	143	3.58
S445 (Ramírez, 1999)	23	44	44	41	86	45	1.91
S503 (Rodman et al., 1996)	24	97	96	84	186	102	4.04
S512 (Rouse, 2000)	91	140	138	133	266	133	1.54
S886 (Schulman & Hyvönen, 2003)	65	117	117	91	255	164	1.80
S935 (Simpson et al., 2004)	18	20	23	18	46	28	1.11
S914 (Smith et al., 2003)	44	77	63	47	96	49	1.75
S535 (Smith, 2000b)	61	138	138	131	343	212	2.26
S555 (Stock et al., 2001)	22	22	22	20	55	35	1.00
S576 (Taylor & Purvis, 2002)	234	357	357	349	746	397	1.53
S575 (Thayer, 1997)	10	40	40	40	89	49	4.00
S577 (Thayer, 2000)	29	132	132	132	282	150	4.55
S828 (Thewissen & Madar, 1999)	28	9	9	9	20	11	0.32
S697 (Waters et al., 2002)	17	112	112	110	245	135	6.59
S370 (Wheeler & Hayashi, 1998)	17	93	93	79	172	93	5.47
S423 (Whitfield, 1992)	17	36	36	31	64	33	2.12
S797 (Wilson & Edgecombe, 2003)	61	165	165	162	390	228	2.70
S368 (Zehethofer & Sturmbauer, 1998)	29	16	12	12	24	12	0.55

Molecular sequence studies	Taxa	Aligned characters	Variable characters	PI characters	PI states	Minimum PI changes	Character-taxon ratio
S949 (Alves et al., 2004)	39	549	211	158	341	183	14.08
S926 (Bailey et al., 2003)	15	1242	172	88	183	95	82.80
S927 (Behnke et al., 2003)	28	1698	415	250	591	341	60.64
S940 (Cox et al., 2004)	34	7668	2369	1388	2978	1590	225.53
S925 (Den Bakker et al., 2003)	11	1736	245	94	191	97	157.82
S950 (Dettman et al., 2003)	147	2141	731	494	1049	555	14.56
S976 (Fliegerová et al., 2004)	17	430	301	237	580	343	25.29
S938 (Garnica et al., 2003)	32	1320	307	133	294	161	41.25
S953 (Gernandt et al., 2003)	56	4169	433	169	342	173	74.45
S958 (Göker et al., 2003)	51	1893	873	428	798	370	37.12
S967 (Gurgel et al., 2004)	21	1466	80	33	66	33	69.81
S931 (Hao et al., 2004)	58	1719	610	456	1087	631	29.64
S945 (Hughes et al., 2004)	95	2967	1663	1264	2774	1510	31.23
S947 (Huhndorf et al., 2004)	85	1342	801	501	1310	809	15.79
S944 (Johnston et al., 2003)	37	561	156	131	320	189	15.16
S960 (Kasuga et al., 2003)	80	1585	300	182	374	74	19.81
S943 (Koontz et al., 2004)	32	1788	313	161	334	173	55.88
S916 (Koster et al., 2003)	13	2252	110	101	203	102	173.23
S917 (Kropp & Matheny, 2004)	16	2649	621	362	764	402	165.56
S941 (Levin et al., 2004)	93	1884	1001	516	1124	608	20.26
S937 (Liu et al., 2004)	25	2243	322	127	260	133	89.72
S921 (Lopez-Vaamonde et al., 2003)	94	602	411	300	798	498	6.40
S955 (Lutz et al., 2004)	40	550	350	268	670	402	13.75
S942 (Matheny & Ammirati, 2003)	25	1341	594	434	957	523	53.64
S974 (Matheny & Watling, 2004)	18	730	284	242	537	295	40.56
S933 (Melnik et al., 2004)	36	1043	236	133	286	153	28.97
S956 (Meve & Liede, 2004a)	18	2510	426	153	305	152	139.44
S924 (Meve & Liede, 2004b)	66	2227	589	268	558	290	33.74
S922 (Miller et al., 2003)	63	4726	1543	642	1337	695	75.02
S961 (Mostert et al., 2004)	21	571	302	176	393	217	27.19
S918 (Niessen et al., 2004)	13	622	146	107	221	114	47.85
S929 (Nuytinck et al., 2004)	18	728	218	72	151	79	40.44
S970 (Nylander et al., 2004)	32	1154	326	198	423	225	36.06
S971 (Oh & Potter, 2003)	19	2694	57	46	92	46	141.79
S928 (Pennington et al., 2004)	75	745	377	297	711	414	9.93
S975 (Percy et al., 2004)	53	981	494	378	789	411	18.51
S969 (Plana, 2003)	83	630	230	126	273	147	7.59
S954 (Pryor & Bigelow, 2003)	40	769	90	55	116	61	19.23
S915 (Roalson et al., 2003)	32	907	36	19	38	19	28.34
S919 (Saar et al., 2003)	34	1593	577	390	813	423	46.85
S952 (Santos et al., 2004)	8	277	27	10	20	10	34.63
S935 (Simpson et al., 2004)	20	822	373	240	528	288	41.10
S920 (Steppan et al., 2004)	46	4483	1597	974	2116	1142	97.46
S957 (Tuthill & Frisvad, 2004)	40	1185	326	186	425	239	29.63
S959 (Tuthill, 2004)	18	1161	148	101	212	111	64.50
S948 (Voglmayr, 2003)	101	1694	1352	407	1010	603	16.77
S965 (Wang et al., 2004)	28	3183	1109	575	1227	652	113.68
S962 (Waters & Roy, 2004)	28	1603	429	199	408	209	57.25
S966 (Weeks et al., 2004)	87	49	48	48	96	48	0.56
S939 (Zhang et al., 2004)	63	684	322	256	431	175	10.86
Totals	4644	88010	29443	18619	41244	22507	28.33

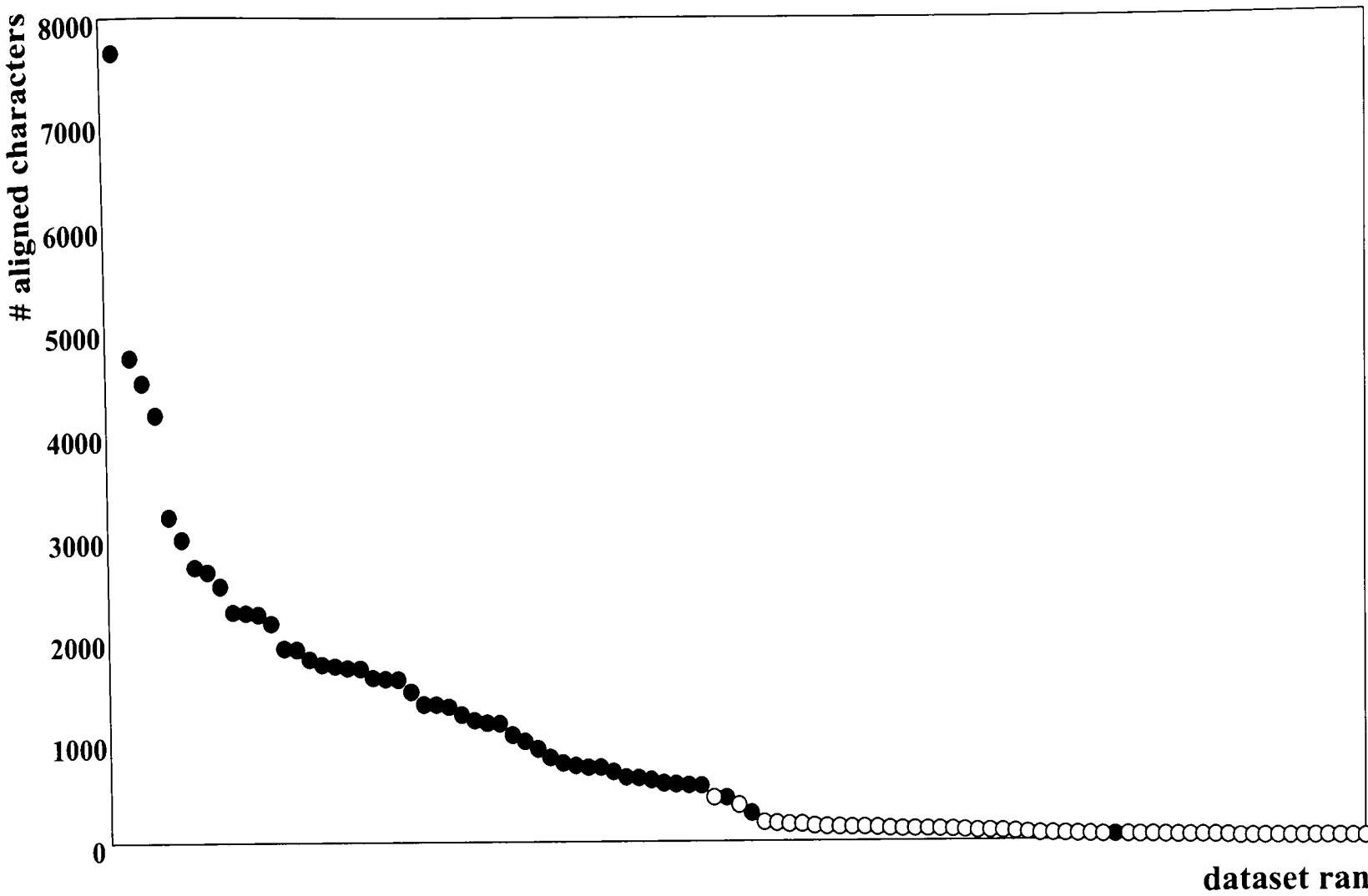


Figure 4.1 Distribution of 100 matrices ranked according to number of characters (after alignment, in the case of molecular data). Filled circles indicate molecular matrices; empty circles indicate non-molecular matrices.

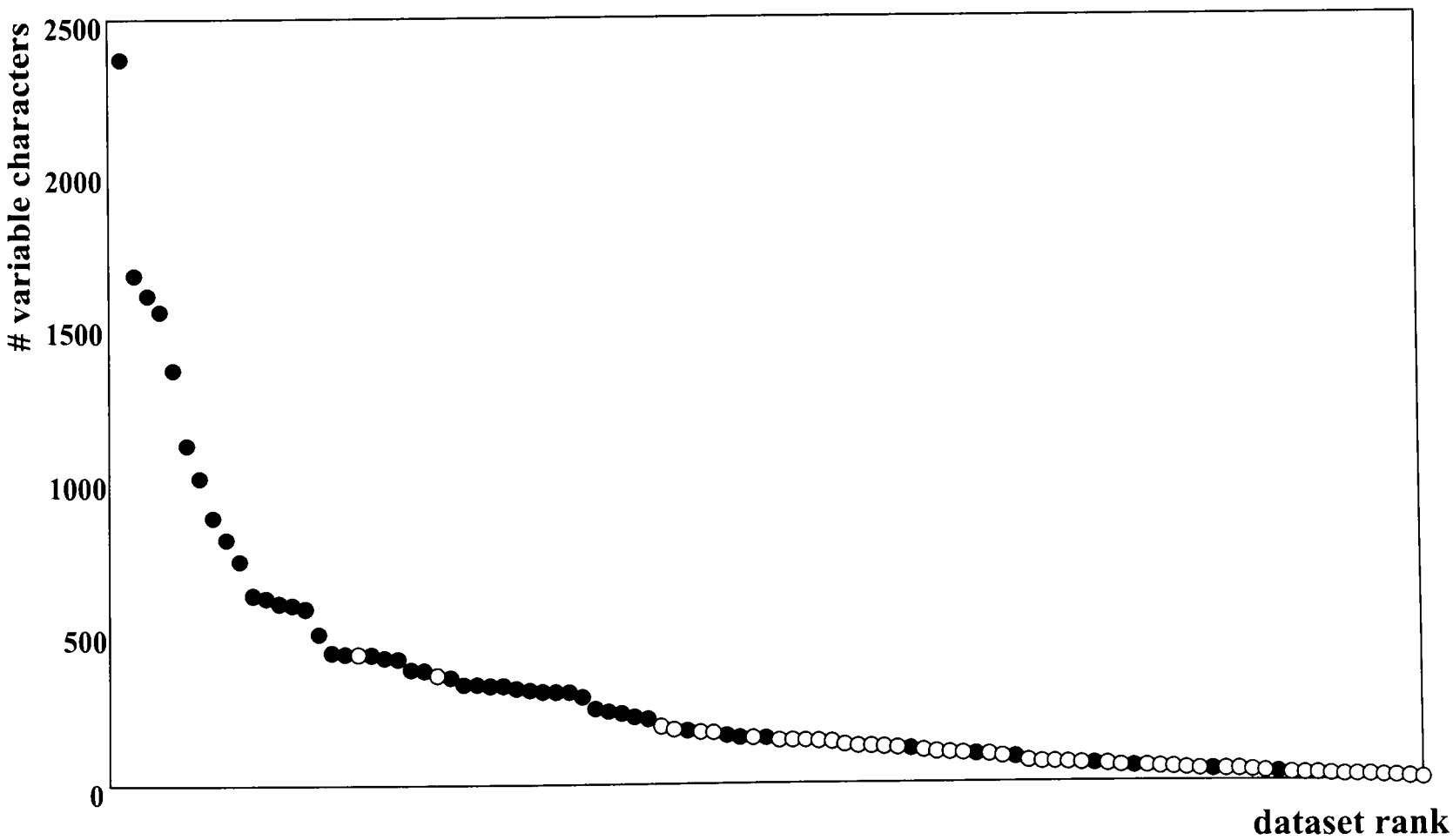


Figure 4.2 Distribution of 100 matrices ranked according to number of variable characters. Filled circles indicate molecular matrices; empty circles indicate non-molecular matrices.

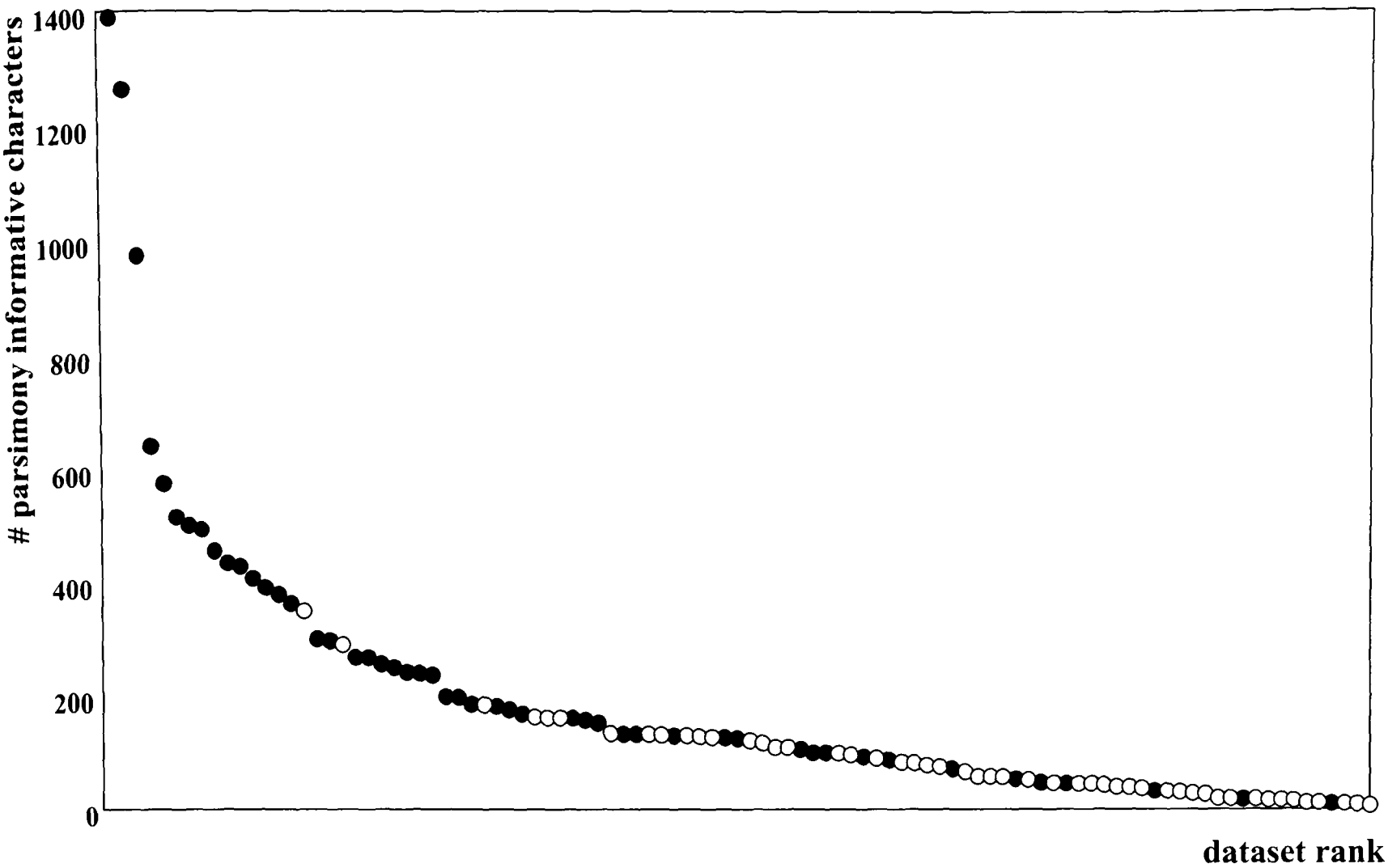


Figure 4.3 Distribution of 100 matrices ranked according to number of parsimony-informative characters. Filled circles indicate molecular matrices; empty circles indicate non-molecular matrices.

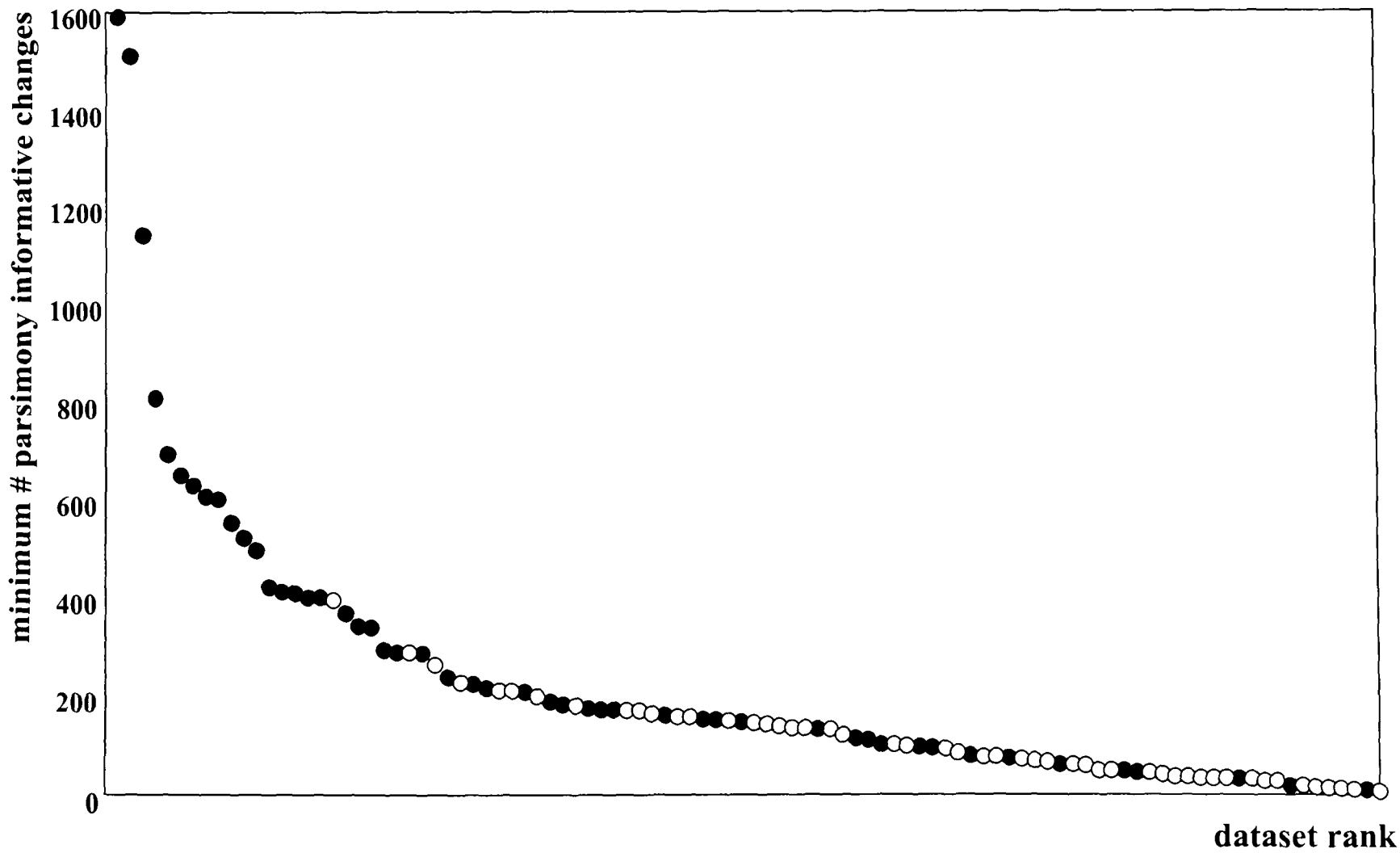


Figure 4.4 Distribution of 100 matrices ranked according to minimum number of parsimony-informative character state changes. Filled circles indicate molecular matrices; empty circles indicate non-molecular matrices.

sophisticated measures of potential informativeness were applied. For example, in terms of parsimony-informative character state changes, most molecular matrices contained significantly more than morphological ones but seven molecular matrices were potentially less informative than the majority of morphological studies. Similarly, four morphological matrices contained more parsimony-informative character state changes than the majority of the molecular matrices.

The most sophisticated measure of informativeness tested here was the minimum number of parsimony-informative character state changes. This is rarely reported in phylogenetic studies. For morphological datasets, the minimum number of parsimony-informative changes was strongly correlated with the more commonly-reported measure, number of characters (squared Pearson product moment correlation coefficient, representing the proportion of the variance in y attributable to the variance in x, r^2=0.837). For molecular datasets, it was not correlated with number of characters (r^2=0.423; Fig. 4.5), but was more closely correlated with the number of variable characters (r^2=0.859; Fig. 4.6). The conventionally-reported measure correlating most closely with potential informativeness, for both morphological (r^2=0.910) and molecular (r^2=0.968) datasets, was the number of parsimony-informative characters (Fig. 4.7).

4.3.4 Discussion. There is a widely-held assumption that molecular matrices provide significantly more potential information for phylogeny reconstruction than morphological ones (e.g. Hillis, 1987; Scotland et al., 2003). This is perpetuated by a comparison of the number of characters they contain (Fig. 4.1), and mirrored closely by the commonly-reported character-taxon ratio ($p<0.0001$, two-tailed t-test; Fig. 4.8).

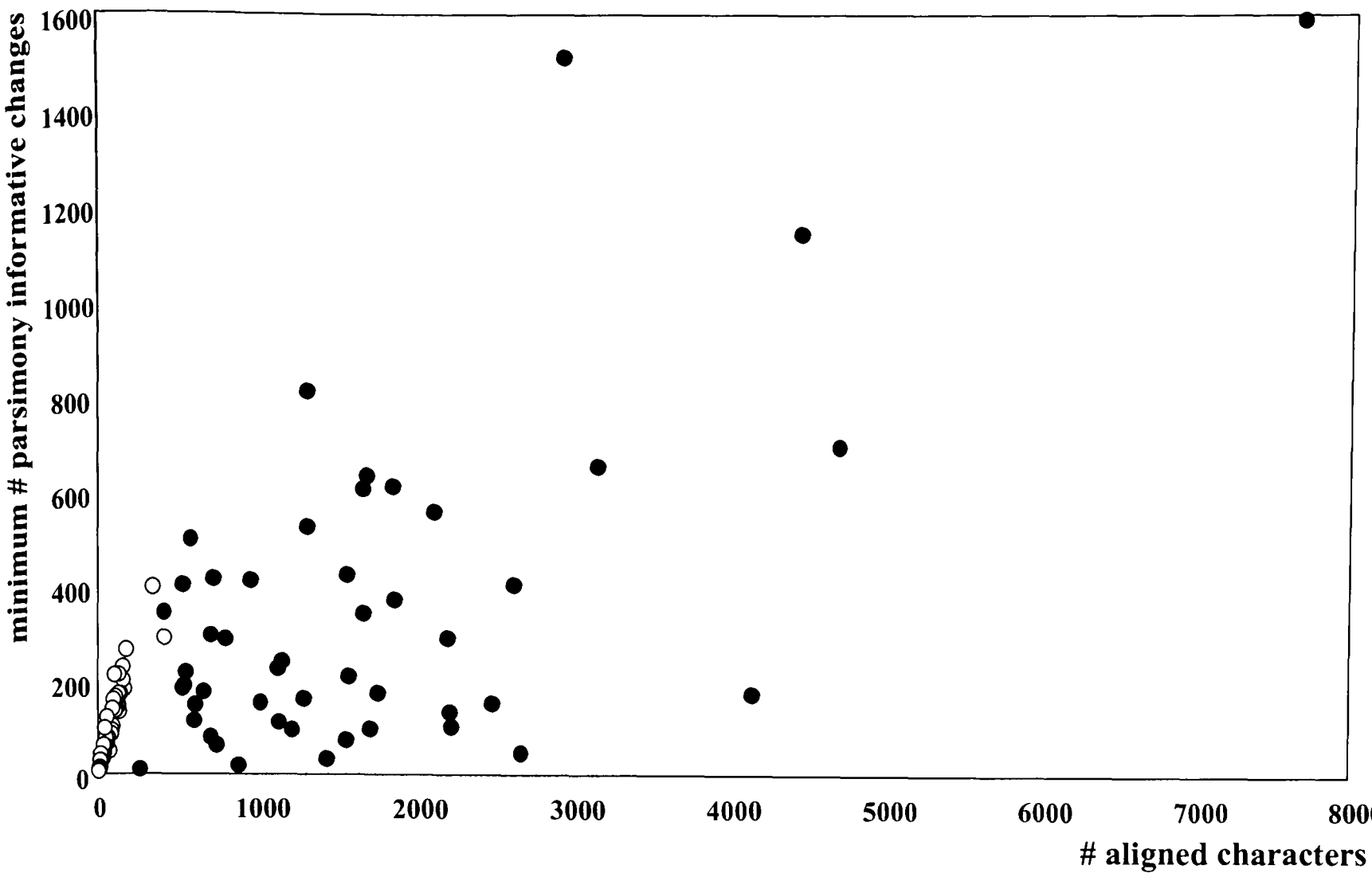


Figure 4.5 Relationship between minimum number of parsimony-informative character state changes and number of characters (after alignment, in the case of molecular data) for 100 matrices. Filled circles indicate molecular matrices; empty circles indicate non-molecular matrices.

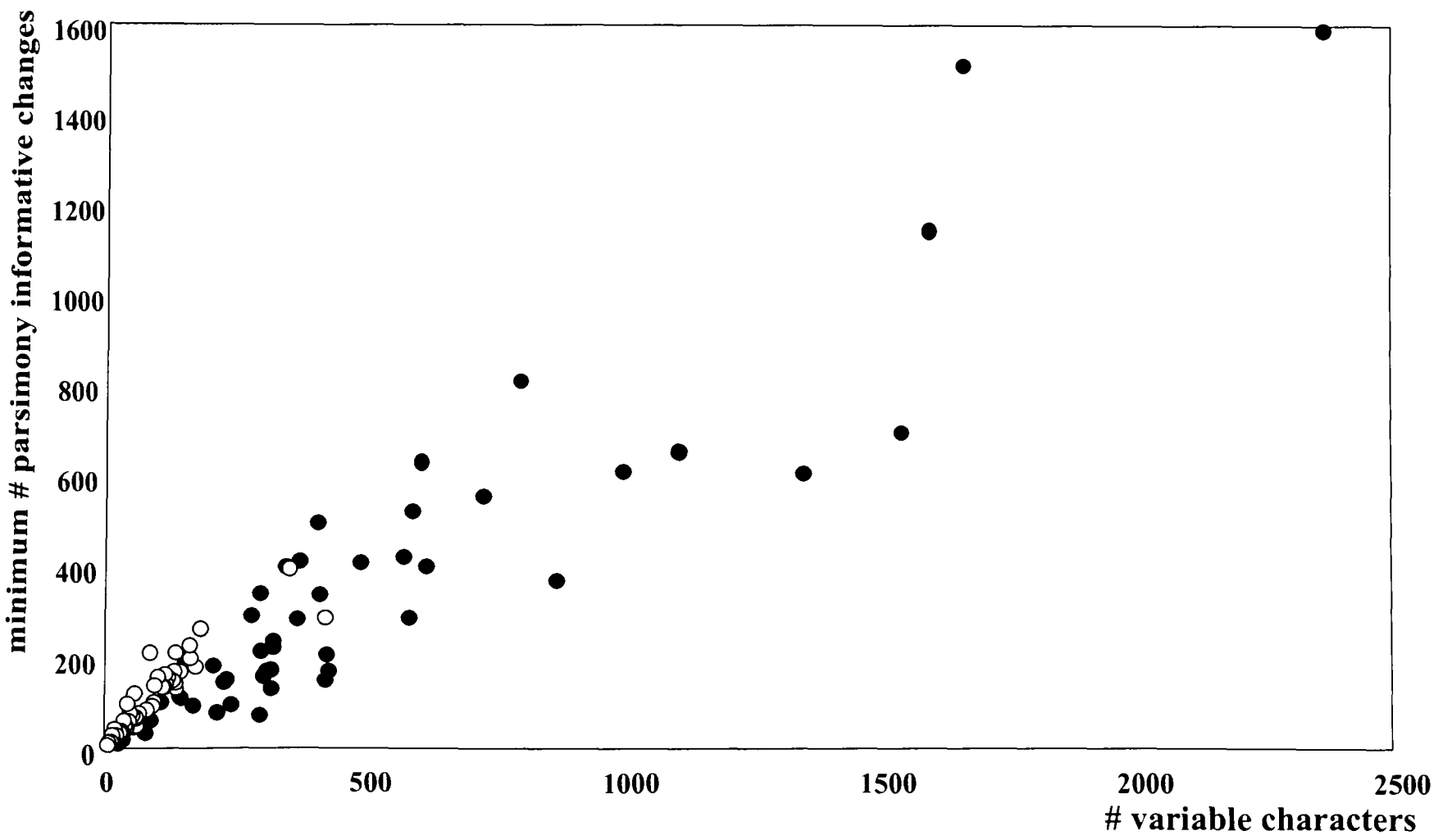


Figure 4.6 Relationship between minimum number of parsimony-informative character state changes and number of variable characters for 100 matrices. Filled circles indicate molecular matrices; empty circles indicate non-molecular matrices.

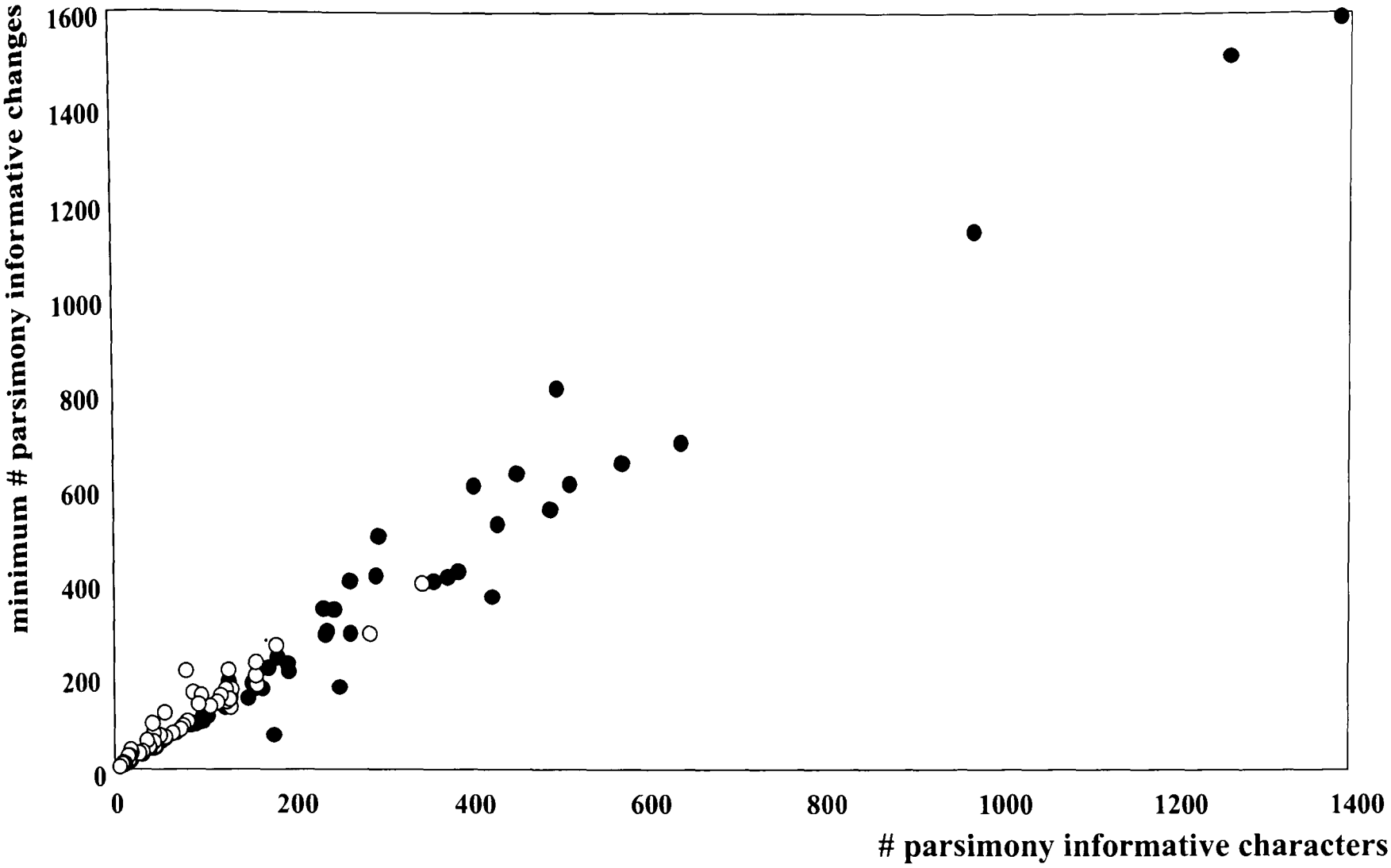


Figure 4.7 Relationship between minimum number of parsimony-informative character state changes and number of parsimony-informative characters for 100 matrices. Filled circles indicate molecular matrices; empty circles indicate non-molecular matrices.

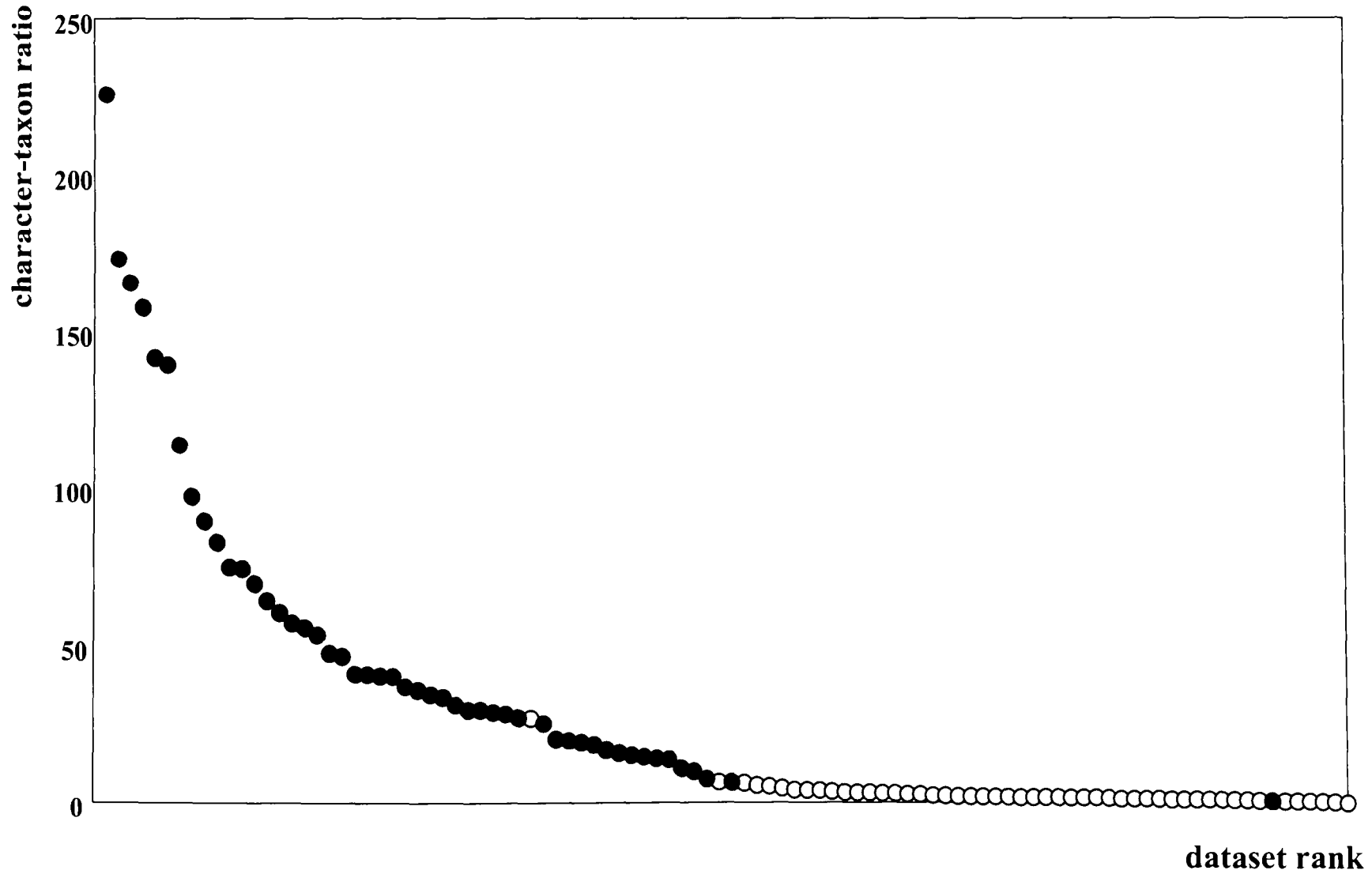


Figure 4.8 Distribution of 100 matrices ranked according to character-taxon ratio. Filled circles indicate molecular matrices; empty circles indicate non-molecular matrices.

However, when datasets are compared in terms of parameters more closely related to the amount of informative data they provide for phylogenetic inference, the distinction between molecular and morphological datasets becomes less clear-cut (Figs. 4.2-4.4). This result can be attributed to the large number of invariant characters in molecular matrices. For morphological data, where invariant characters are usually excluded *a priori*, there is an approximate 1:1 correspondence between the number of variable characters and the total number of characters (Fig. 4.9; r^2=0.999); whereas for molecular data there is little correlation (r^2=0.614). Morphological datasets are usually smaller than molecular datasets, but their information content *per aligned character* is almost always greater than for molecular datasets, because molecular datasets contain fewer variable characters than aligned characters (below the 1:1 line indicated on Fig. 4.9).

There is great variation in the potential informativeness of molecular datasets, making it hard to predict their potential informativeness and therefore difficult to choose the most useful molecular marker for an analysis *a priori.* The variation in potential informativeness is prevalent even among very large datasets. For example, two recent very large studies of coding sequences from whole genomes (Herniou et al., 2001; Rokas et al., 2003), comprising 127,026 and 25,688 aligned nucleotide sites respectively, also contain large numbers of parsimony-informative characters (a good proxy for potential informativeness, as shown above), even though the genes were not selected to contain large numbers of variable characters. By contrast, a dataset of 10,988 aligned molecular characters, selected for their potential usefulness in solving a particular phylogenetic problem (the resolution of a rapid radiation in *Gossypium*),

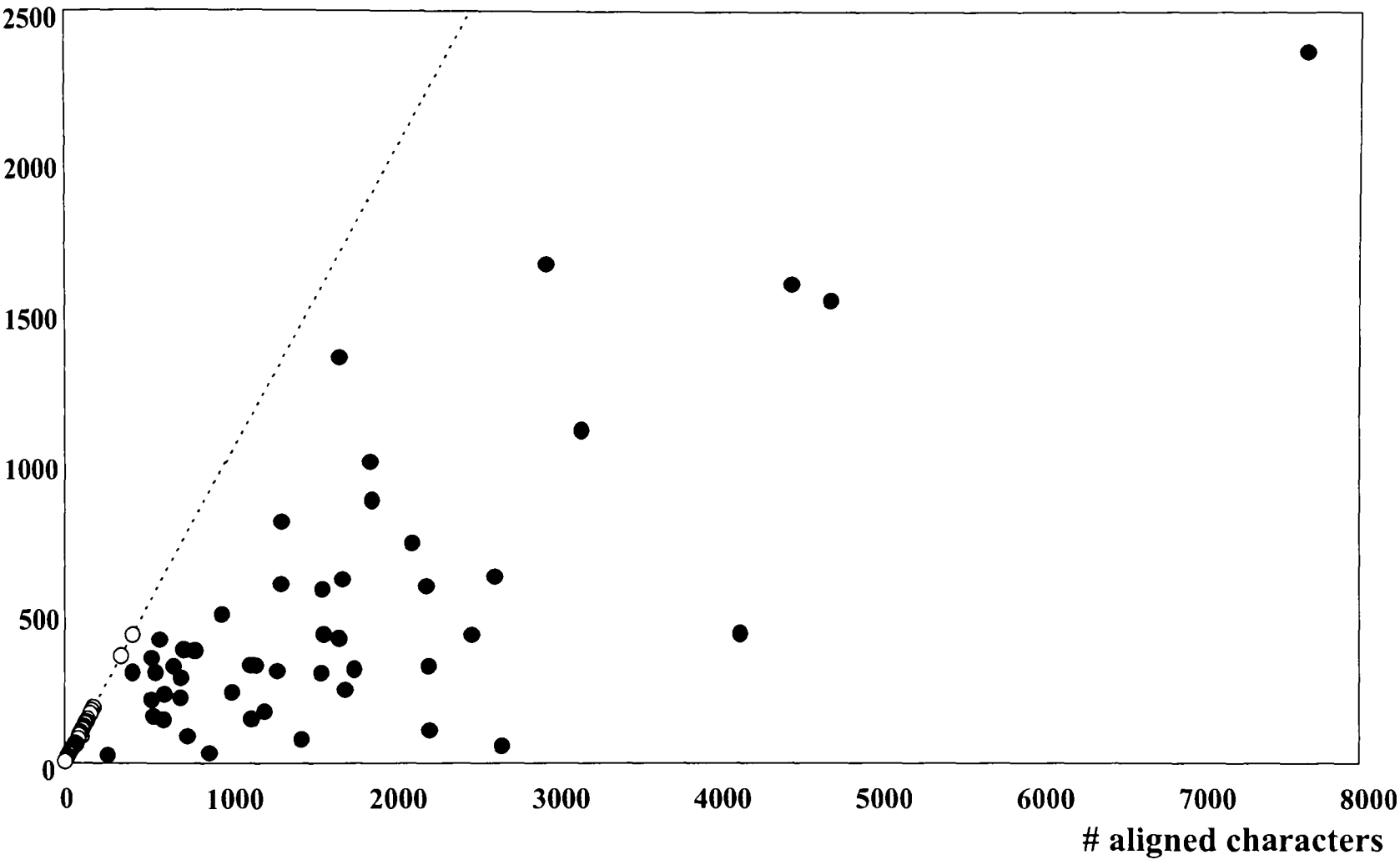


Figure 4.9 Relationship between number of variable characters and total number of characters for 100 matrices. Filled circles indicate molecular matrices; empty circles indicate non-molecular matrices. Dashed line indicates a 1:1 relationship, i.e. where all aligned characters are variable.

contained only 704 variable and 85 parsimony-informative characters - respectively 6% and 0.85% of the dataset (Cronn et al., 2002).

4.3.5 Conclusions and implications. Ranking molecular and morphological matrices according to their number of characters (Fig. 4.1) explains the prevalent perception that molecular datasets are significantly larger than morphological ones and therefore more useful for phylogenetic analysis. More sophisticated measures of potential informativeness such as the minimum number of parsimony-informative character state changes do not show such a clear-cut distinction (Fig. 4.4). Therefore, at present there may be instances in which it would be unwise to assume molecular datasets are more useful because of their greater number of aligned characters. However, this study provides only a "snapshot" of the current situation. While morphological datasets are unlikely to increase much further in size, molecular datasets continue to grow at an ever-increasing rate. The trend towards huge multi-gene molecular analyses seems likely to overwhelm morphological data in the relatively near future (e.g. Graham & Olmstead, 2000; Herniou et al., 2001; Matsuoka et al., 2002; Rokas et al., 2003). A more realistic view of this takeover will be achieved if more accurate measures of dataset informativeness are applied. In addition, it should be noted that potential informativeness may not be the only, or even the most important, feature of a matrix constructed for phylogenetic inference. For instance, data quality, the level and distribution of homoplasy in the dataset, and the accuracy of the phylogeny reconstructed are also crucial parameters (e.g. Jansen et al., 1990).

It was observed during this survey that morphological datasets, at least in world-wide-web-based databases such as TreeBASE, are now much less common than molecular

ones: the 50 molecular datasets compared here were sampled from the 2003 database, but to sample 50 morphological ones required searching back to 1991. It is possible that TreeBASE represents a biased sample, where molecular studies are more likely to be submitted, so whether this is indicative of a general trend away from morphological phylogenetic studies remains debatable (Hillis & Wiens, 2000; Baker & Gatesy, 2002; Scotland et al., 2003). What does seem to be true is that morphological characters are increasingly used in simultaneous analysis with molecular datasets according to the principle of total evidence (e.g. Edgecombe et al., 2002; Giribet et al., 2002; Meier & Wiegmann, 2002; Schulmeister et al., 2002; Stach & Turbeville, 2002; Aagesen & Sanso, 2003; Lundberg & Bremer, 2003; Virtanen, 2003; Wahlberg & Nylin, 2003). The impact of morphological data on simultaneous analyses is investigated in the next section.

4.4 Measuring the actual usefulness of morphological data in total evidence analysis

4.4.1 Introduction. As shown above, the disparity in potential informativeness between morphological and molecular datasets is increasing, and is reflected in a decreasing number of morphological phylogenetic analyses. At the same time, morphological characters are increasingly used in combination with molecular datasets, where they are considered to provide new and useful information over and above that supplied by molecular data alone (Nandi et al., 1998). Underlying this conviction is the assumption that, although they contain fewer potential parsimony-informative state changes, morphological characters compensate for this by their quality. To test this, the actual effect of morphological datasets in simultaneous analysis with molecular data was tested *a posteriori.*

4.4.2 Methods. A sample was compiled comprising simultaneous analyses of molecular sequence and morphological data published during the past three years (2001-2003). This was generated by searching the *Web of Knowledge* database (http://wok.mimas.ac.uk/) to ascertain which journals had published the most simultaneous analyses over that time. From these journals (*Annals of the Missouri Botanical Garden, Cladistics, Molecular Phylogenetics and Evolution, Systematic Botany*, *Systematic Entomology* and *Zoologica Scripta*) were extracted every computer-based parsimony analysis where molecular, morphological and combined datasets were analysed for the same taxa. Twenty-one studies met these criteria.

4.4.3 Results. The results of the survey are summarised in Table 4.4. The number of parsimony-informative characters was compared because it is more often reported than the minimum number of parsimony-informative character state changes, and has been shown to be a good proxy for potential informativeness (Section 4.3). In the 21 simultaneous analyses studied here, morphological datasets contributed 14.44% of the parsimony-informative characters (i.e. approximately 14% of the potentially informative data) overall. This varied between 3% and 30% for individual studies.

Including morphological characters in simultaneous analysis had little effect on the number of equally-parsimonious trees found, compared to analyses of molecular data alone. In 50% of studies the incorporation of morphological data decreased the number of MP trees found. In 22% of studies the number of MP trees increased when morphological data were added. Across all studies, the change in the number of trees found with the addition of morphological data was not statistically significant ($p=0.3014$, paired t-test).

Table 4.4 The effect on phylogeny reconstruction of adding a morphological dataset to a molecular dataset in 21 recent simultaneous analyses. PI, parsimony informative; MP, most parsimonious; CI, consistency index (Kluge & Farris, 1969); RI, retention index (Farris, 1989), CFI, consensus fork index (Colless, 1980; 1981). Not all figures were available for all studies. See text for explanation.

Study	% PI characters supplied by morphology	# MP trees	CI	RI	CFI	% nodes >50%	% nodes >95%
Aagesen & Sanso (2003)	29.53	-964	-0.22	-0.16	+0.33	0	0
Babcocka et al. (2001)	6.78	0	-0.01	0	0	0	0
Cameron & Williams (2003)	9.62	-4	+0.09	-0.01	-	+16.7	0
Caterino & Vogler (2002)	24.38	+28	-	-	-0.09	0	0
Dabert et al. (2001)	-	-6	+0.15	+0.24	+0.08	0	0
Damgaard & Sperling (2001)	15.74	-5	+0.01	+0.05	-	0	0
Edgecombe et al. (2002)	-	0	-	-	-0.09	+2.2	+18
Farmer & Schilling (2002)	30.29	-3	+0.01	-0.11	+0.13	0	-4.2
Fernándeza et al. (2001)	8.11	+207	-0.04	-0.01	+0.16	0	0
Frost et al. (2001)	-	-1	-0.02	-	+0.08	-1.7	+0. 6
Giribet et al. (2002)	-	-	-	-	-0.02	0	0
Meerow et al. (2002)	21.05	-5	0	+0.02	+0.1	+0.2	+2
Murrell et al.(2001)	4.82	+6	+0.002	+0.02	-0.09	0	+1.6
Near et al. (2003)	17.58	0	-	-	0	+20	+6. 7
Pedersen et al. (2003)	9.61	-49,701	-0.09	-0.06	+0.2	+9.7	-3.2
Schulmeister et al. (2002)	18.75	0	-	-	0	0	0
Stach & Turbeville (2002)	3.38	-3063	-0.001	+0.01	+0.03	+14	+2
Wahlberg & Nylin (2003)	11.19	-	-	-	-	0	0
Wiegmann et al. (2002)	19.87	+621	0	0	-0.03	0	0
Winterton et al. (2001)	22.32	0	+0.01	+0.05	0	0	0
Zrzavý (2003)	21.36	-	+0.05	+0.03	+0.12	0	0
Totals/means	14.44	-2938	-0.004	+0.006	+0.05	+2.8	+1.1

Compared to analyses of molecular data alone, adding morphological characters had little effect upon resolution, measured by the consensus fork index, CFI (Colless, 1980; 1981). Resolution increased in 50% of studies and decreased in 28%, with 22% unchanged. Over all studies, the difference in resolution on adding morphological data was not statistically significant (p=0.0708, paired t-test).

Adding morphological characters to an existing molecular dataset did have an effect upon support. The proportion of nodes receiving at least 50% bootstrap support increased in 70% of studies and decreased in 10%, remaining unchanged in 20%. The difference between molecular and total evidence analysis was statistically significant (p=0.0410, paired t-test). The proportion of nodes receiving at least a 95% bootstrap value (i.e. very strong support) increased 67% of studies and decreased in 11%. However the difference between molecular and total evidence analyses at this level was not statistically significant (p=0.2689).

4.4.4 Discussion, conclusions and implications. The impact of morphological data in combined analysis is currently equivocal and still hotly debated (Baker et al., 1998; Poe & Wiens, 2000; Wiens, 2000; Scotland et al., 2003). Morphological datasets contributed approximately 14% of the potentially informative data in this sample of combined analyses. This is both small enough to demonstrate the superiority of molecular datasets, and large enough to fuel the argument for the continued use of morphology in some circumstances, perhaps explaining why the debate over molecular and morphological approaches to phylogenetic inference continues. Another reason for the continued debate is that the effect of including morphological data in simultaneous analysis is very variable between datasets. On average, in this small sample of recent studies, the addition of morphological data had a slight but statistically significant effect upon support, but little effect upon the resolution or decisiveness of the resulting phylogenies. As molecular datasets continue to increase in size, while morphological datasets may be approaching their maximum size, their contribution is likely to become less significant.

4.5 Selection of morphological characters for phylogenetic analysis

The relative contribution of molecular and morphological datasets – in both potential informativeness and actual usefulness – varies greatly between studies. The likely effect of including morphological data in any particular study is therefore difficult to determine before constructing a matrix. Character selection is a crucial part of this process that can have a significant impact upon the resulting phylogeny and should be used to optimise the effects of adding morphological data to an analysis (Pogue & Mickevich, 1990; Poe & Wiens, 2000). Before building a matrix of morphological characters for phylogenetic analysis in Lamiales, I therefore review the processes of character selection that might be applied, to develop a strategy for character selection in this case.

The compilation of a dataset involves excluding data that are ambiguous or problematic (Hillis & Huelsenbeck, 1992). However, phylogenetic analysis is made more powerful by including a greater number of independent pieces of evidence (Smith, 1994; Nixon & Carpenter, 1996; Kluge, 1998; Farris, 2000). Therefore there is a conflict between including as many pieces of data as possible, for a powerful analysis, and excluding characters that will mislead that analysis. The role of a character selection protocol is to provide an objective balance between these conflicting goals, ensuring that the characters in the matrix fulfil all its assumptions while maximising its explanatory power (Nixon & Carpenter, 1996; Poe & Wiens, 2000; Grandcolas et al., 2001; Wiens, 2001; Brooks & McLennan, 2002).

Phylogenetic analysis relies on the assumption that characters are inherited by descent. Therefore selection criteria should exclude characters which are analogies

and not potentially heritable. After selection, a matrix should contain only unambiguous primary homologies (De Pinna, 1991; Grandcolas et al., 2001), because errors in primary homology assessment cannot necessarily be identified after the analysis (Pennington, 2000). In molecular datasets, ambiguous primary homologies are removed through careful alignment and the exclusion of unalignable sequence regions. Morphological character selection is more subjective, and a range of approaches to selection have developed, depending upon whether priority is given to removing ambiguous characters or retaining potentially useful information (Poe & Wiens, 2000).

All approaches to morphological character selection commence with the implicit criteria of observability and variability, in which characters are chosen according to systematists' experience as the most appropriate for the study group. Additional common, but often inexplicit, exclusion criteria include variation within terminal taxa, missing data, continuous variation, unknown polarity, and high levels of homoplasy (Poe & Wiens, 2000). Because these criteria represent an attempt to avoid both coding difficulties and problematic primary homology assessment they may discard some useful data unnecessarily (Poe & Wiens, 2000). At one extreme, strictly applying all criteria may result in a matrix of too few informative characters to resolve the phylogeny (Wenzel & Siddall, 1999). At the other, simply including all observable and variable characters without further selection may produce a matrix containing too many ambiguous primary homologies for reliable phylogeny reconstruction (Patterson & Johnson, 1997; Scotland et al., 2003). Despite practical examples where gains in resolution and support have been provided by large data matrices (e.g. Nandi et al.,

1998; Caddick et al., 2002; Endress, 2003; Gatesy et al., 2003) the debate over the stringency of character selection remains (Poe & Wiens, 2000; Scotland et al., 2003).

Here, for Lamiales, both extremes of the possible range of strategies were tested, to investigate the impact of character selection upon phylogeny. The most stringent strategy invoked explicit criteria for excluding ambiguous primary homology. Primary homology is determined according to the principle of similarity, as defined by Remane (cited in Rieppel & Kearney, 2002), using three tests: topographic correspondence, composition, and ontogeny (continuity of immediate forms). An additional test – conjunction – may also be invoked to identify dissimilar character states. One type of character that frequently fails similarity-testing is the complement relation (presence/absence characters). "Absence" is not a valid character state, since it may arise in a number ways that will be indistinguishable at the morphological level (Pimental & Riggins, 1987). Complement relation characters in which dissimilar transformations were likely to introduce homoplasy were excluded from the stringent matrix in this study.

All character selection strategies agree that individual pieces of evidence should be independent. No morphological characters are totally independent with regard to functional and growth constraints, but may still be logically independent and valid for analysis (Smith, 1994; Poe & Wiens, 2000). Thus characters such as "presence or absence of a structure" and "different forms of that structure" (with absence coded as not applicable) can be logically independent. Characters should be tested for independence once they have been shown to meet all the other criteria for parsimony

analysis, to avoid the unnecessary omission of useful characters due to non-independence with others that may be invalidated by other criteria.

In the next section two extreme strategies for character selection are applied and tested in the construction of a morphological matrix for Lamiales. Both strategies begin with a set of observable and variable characters; both are concluded by the exclusion of non-independent pieces of data. The most lenient strategy, as advocated by Poe & Wiens (2000) includes all characters that fulfil these requirements; the most stringent invokes strict criteria for unambiguous primary homology assessment.

4.6 The application of morphological data to resolve the phylogenetic position of *Thomandersia* in Lamiales

4.6.1 Introduction. The phylogeny of Lamiales has so far been inferred using a two-gene molecular sequence dataset from *rbcL* and *ndhF*. Here, I investigate whether the addition of a morphological dataset for the same taxa can improve resolution and support in Lamiales without compromising accuracy. The potential informativeness (before analysis) and actual usefulness (after simultaneous analysis) of the dataset are characterised. The effect of character selection on the morphological dataset is investigated, and the relative phylogenetic signal provided by two extreme selection regimes is determined by comparison with randomly-generated matrices.

4.6.2 Methods. Taxon sampling. As in Chapter 1, up to three exemplar species were sampled for each family (after Yeates, 1995; Wiens, 1998a; Prendini, 2001), a total of 37 taxa (Appendix 1). Morphological data and sequence data for the two genes were

taken from the same exemplar species, where available, or closely-related species where this was not possible (see Appendix 1).

Selection and coding of morphological characters. An initial list of observable, heritable, variable characters was derived from family-level treatments of Lamiales (Lu, 1990; Watson & Dallwitz, 1992) and my own investigations. Character states were ascertained by anatomical studies of spirit-preserved material (Appendix 11), examination and dissection of herbarium specimens and living material (Appendix 12) and a survey of specific and generic treatments in taxonomic literature and on the world-wide-web (Appendix 13). Where state information was not available for individual species but character states were described by Davis (1966), Carlquist (1992), Metcalfe and Chalk (1979; 1983) or Watson and Dallwitz (1992) as being constant within families, the family state was taken for the species. States were scored if present but coded as "missing" if not recorded, because this was as likely to indicate "not examined" as "absent." Where sources conflicted, taxa were coded as ambiguous, to avoid making assumptions about ancestral states while retaining as much information as possible (Kornet & Turner, 1999).

Meristic characters were treated in the same way as qualitative ones. For continuous characters, the median value for each species was coded as a state; taxa with the same median value shared the same state. This method required no subjective decision regarding significant gap size between ranges and was applicable where no comprehensive distribution data was available (Baum, 1988). It was also preferable to methods, such as gap-weighting, that code each taxon as a unique state but require *a priori* decisions to order the states (Baum, 1988; Thiele, 1993; Wiens, 2001). A

similarly unordered, multi-state approach was taken to coding chromosome number. No attempt was made to split multi-state characters since PAUP* can handle up to 32 states (Swofford, 2002).

Coded characters were filtered to exclude those that were uninformative for parsimony analysis. All remaining characters were included in the largest matrix, which corresponds to the most lenient of character selection methods commonly used in phylogenetic analysis. A second, smaller matrix, to represent the opposite extreme of current practice, was selected from the larger using strict similarity criteria. In this matrix, characters were excluded if they displayed:

1. States not showing topographic correspondence, for example seed wings present at different positions on the seed.
2. States dissimilar in composition or ontogeny, for example domatia of different types (pits, tufts, or pockets), or complement relation characters where absence states might arise through different transformations (e.g. presence/absence of a style cavity).
3. More than one state present in the same individual (fails conjunction test), for example xylem fibre types.
4. Continuous variation, because this is frequently omitted *per se* by selection schemes as being problematic for homology assessment due to difficulties of coding and description (Thiele, 1993; Poe & Wiens, 2000).

Both matrices were then filtered to remove, as far as possible, non-independent characters. Firstly, those characters on which the largest number of others were dependent were removed, followed by those on which the most remaining characters

were dependent, and so on. If two characters had the same number of dependent characters, the one with the most missing data was removed. In this way dependent characters were removed from the datasets while preserving as much information as possible.

Comparison of matrices. To assess whether the same or conflicting signals were present in the molecular and morphological datasets, the partition homogeneity test (Farris et al., 1994) was conducted in PAUP* on the following partitions: *rbcL*-large morphological matrix, *rbcL*-small morphological matrix, *ndhF*-large morphological matrix, *ndhF*-small morphological matrix, combined molecular data set-large morphological matrix, combined molecular data set-small morphological matrix, *rbcL-ndhF*, and large morphological matrix-small morphological matrix. Characters shared by both morphological matrices were excluded from the larger dataset when comparing them. One hundred replicates were performed, using a heuristic search of 100 replicates with a single random-order-entry starting tree, TBR branch-swapping and saving up to 10 trees for each replicate.

Phylogenetic analysis. The two morphological matrices were analysed alone, and in conjunction with the molecular sequence dataset from Chapter 1. Maximum parsimony analyses were performed using PAUP* (Swofford, 2002) with MULPARS on. Characters were unordered and equally weighted. Gaps were coded as missing data, and branches collapsed if their minimum length was equal to zero so only branches with unambiguous support were retained. Two heuristic search strategies were followed: in the first, 10 random addition cycles were conducted using TBR branch-swapping; in the second, 10,000 random addition cycles were conducted using

TBR branch-swapping, but saving only two trees per replicate to reduce the time spent searching trees at sub-optimal levels (Meerow & Snijman, 2001). This strategy was designed to search for shorter or equally-parsimonious islands of trees not found by the first search (Maddison, 1991; Catalan et al., 1997; Olmstead et al., 2001).

Comparison of resulting phylogenies. The trees were compared in terms of resolution and support. Strict consensus trees were constructed from all MP trees and the CFI – the number of nodes in strict consensus divided by the maximum possible number of nodes (Colless, 1980; 1981) – calculated, to measure resolution. The CI and RI were calculated in PAUP*. Internal support was estimated by a nonparametric bootstrap search with 1,000 replicates, each comprising 100 random addition heuristic search cycles, TBR branch-swapping and MULPARS off, saving no more than two trees per cycle (Felsenstein, 1985; De Bry & Olmstead, 2000; Olmstead et al., 2001). The total number of nodes achieving 50%, 75% or 95% bootstrap values was counted, to measure the support across each tree.

Use of artificial matrices to compare phylogenetic signal and the effect of character selection. Fifty replicate datasets were generated from the large morphological matrix by randomising the order of the cells in each column. This preserved the original character state distribution of the data while removing any coordination in the arrangement of states across taxa, i.e. any phylogenetic signal (Archie, 1989; Naylor, 1992). Each randomised dataset was analysed in combination with the molecular data, and a bootstrap heuristic search was conducted. To save computation time, fewer replicates were conducted in these analyses (1,000 replicates for each heuristic search, 100 x 100 for each set of bootstrap searches).

To assess the effect of character selection, 50 replicate datasets were generated, the same size as the smaller morphological matrix, by resampling 27 characters at random from the large matrix. These were analysed in combination with the molecular data, and a bootstrap heuristic search was conducted, using 1,000 replicates for each heuristic search, 100 x 100 for each set of bootstrap searches.

4.6.3 Results. Potential informativeness and comparison of morphological matrices.

The total observed morphological variation comprised 212 non-nucleotide characters. Eighty-four characters were invariable or autapomorphic at the level of this analysis; 127 were informative. Twenty-two characters (Appendix 14) were removed for failing logical independence, leaving a large matrix of 105 characters (Appendices 15-16). Ninety-six ambiguous characters from the larger dataset were excluded by the strict selection criteria (Appendix 17), and four of the remainder for failing logical independence (Appendix 18), leaving a small matrix of 27 characters (Appendices 19-20).

The resulting large morphological matrix of 105 variable, parsimony-informative characters contained 146 potentially parsimony-informative changes. The small matrix of 31 variable, parsimony-informative characters contained 55 potentially parsimony-informative changes. For comparison, the two-gene molecular dataset contained 3,578 aligned characters, 1,371 of which were variable and 749 parsimony-informative, representing 945 potentially parsimony-informative changes.

Partition homogeneity tests for all combinations of data analysed showed significant incongruence: all p-values <0.01. The partition homogeneity test applied to the

relaxed and strict partitions of the morphological dataset gave p=0.956, suggesting the signal contained in the two matrices was congruent.

Phylogenetic analysis. Analysis of the large morphological dataset produced 139 trees of length 530 steps, CI 0.43, RI 0.44. The second search strategy recovered more islands and more trees than the first (139 vs. 62 trees). The strict consensus tree (Fig. 4.10) had CFI 0.21. Only three clades (Martyniaceae, Pedaliaceae and *Thomandersia*) were found in more than 50% of bootstrap searches and only one (*Thomandersia*) in more than 95%. When the analysis was constrained so that the in-group was monophyletic, 10 MP trees two steps longer were found, which were similar to the unconstrained analysis except that *Ligustrum, Retzia* and *Veronica* were drawn to the base of the in-group. When only *Borago* was used as an out-group, 11 MP trees were recovered, and *Hemimeris* was drawn to the base.

Analysis of the small morphological dataset (using the second search strategy; the first did not run to completion) generated 42 trees of length 97 steps, CI 0.52, RI 0.58. The MP trees were not completely resolved. In strict consensus, conflict between the MP trees resulted in a further decrease in resolution: the strict consensus tree (Fig. 4.11) had CFI 0.09. Two clades (*Thomandersia* and *Borago- Hemimeris*; not found in the strict consensus tree) were found in more than 50% of bootstrap searches. When the analysis was constrained so that the in-group was monophyletic, 432 trees two steps longer were found; the strict consensus of these was completely unresolved. When only *Borago* was used as an out-group, two MP trees were recovered; both were similar to the unconstrained analysis except that *Hemimeris* and *Retzia* were drawn to the base of the tree.

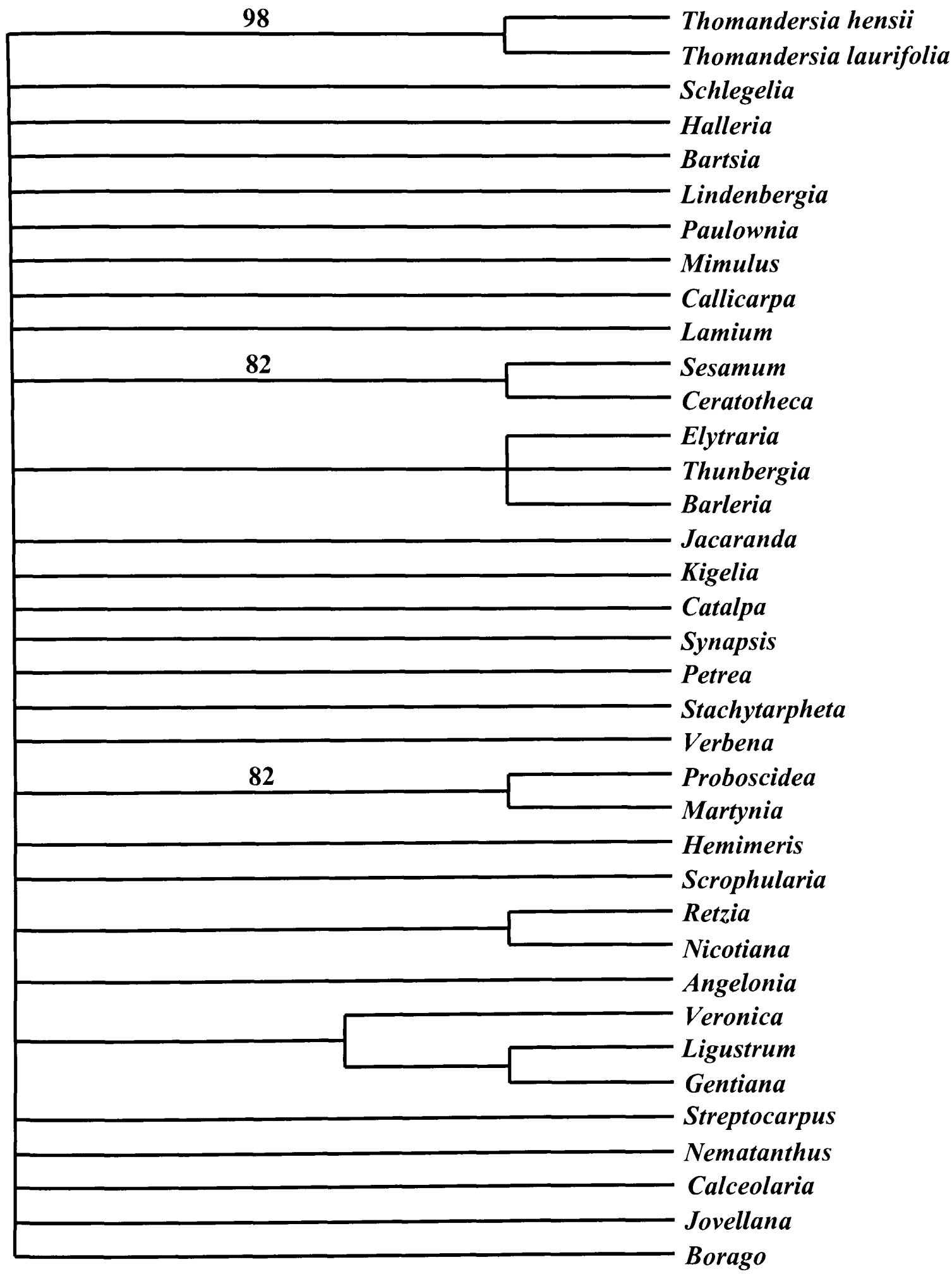


Figure 4.10 Strict consensus of 139 MP trees obtained from analysis of the large morphological matrix, showing bootstrap values greater than 50%.

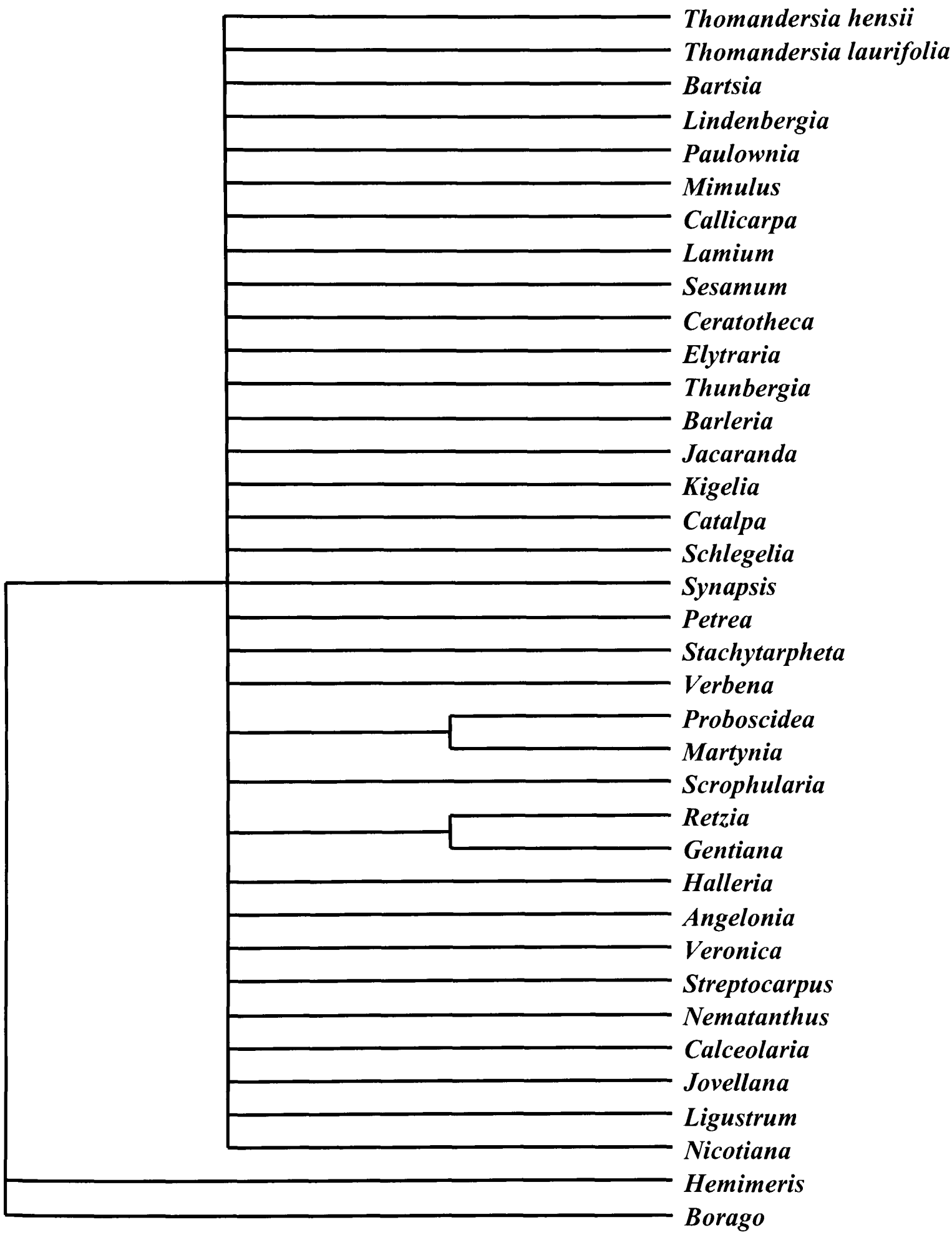


Figure 4.11 Strict consensus of 42 MP trees obtained from analysis of the small morphological matrix.

The combined large morphological and molecular sequence dataset contained 854 parsimony-informative characters. Analysis of this dataset using both strategies produced three MP trees of length 4,181 steps, CI 0.42, RI 0.35. The strict consensus tree (Fig. 4.12) had CFI 0.91. Twenty clades were found in more than 50% of bootstrap searches and 11 in more than 95%.

The combined small morphological and molecular dataset contained 776 parsimony-informative characters. Analysis of this dataset produced 246 trees of length 3,741 steps, CI 0.43, RI 0.35. The second search strategy recovered three more trees than the first. The strict consensus tree (Fig. 4.13) had CFI 0.71. Twenty-one clades were found in more than 50% of bootstrap searches and 10 in more than 95%.

Comparison of phylogenetic signal by use of artificial matrices. Each of the 50 replicate randomised 105-character matrices was analysed in conjunction with the molecular dataset. The resulting phylogenies had CI ranging from 0.408 to 0.422 (mean 0.41, standard deviation 0.002), RI 0.310-0.350 (mean 0.32, standard deviation 0.005) and CFI 0.559-1.0 (mean 0.86, standard deviation 0.12; Fig. 4.14). Between 15 and 21 nodes achieved 50% bootstrap support (Fig. 4.15), 11-15 nodes achieved 75%, and 9-11 nodes achieved 95%.

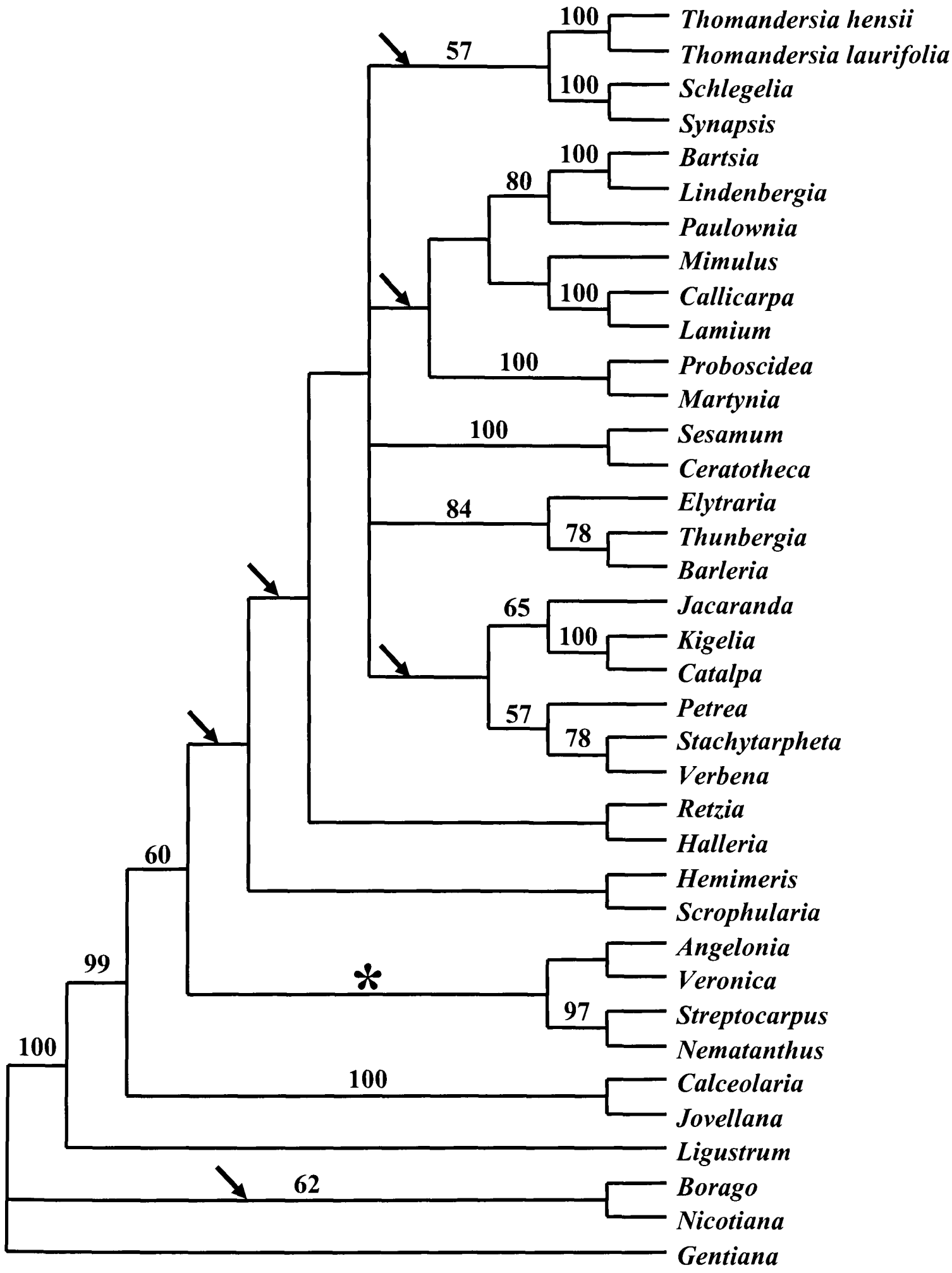


Figure 4.12 Strict consensus of three MP trees obtained from total evidence analysis of combined molecular data and the large morphological dataset, showing bootstrap values greater than 50%. Arrows indicate clades newly resolved over analysis of molecular data alone (Fig. 1.4); asterisk indicates a clade that is incongruent with analysis of molecular data alone.

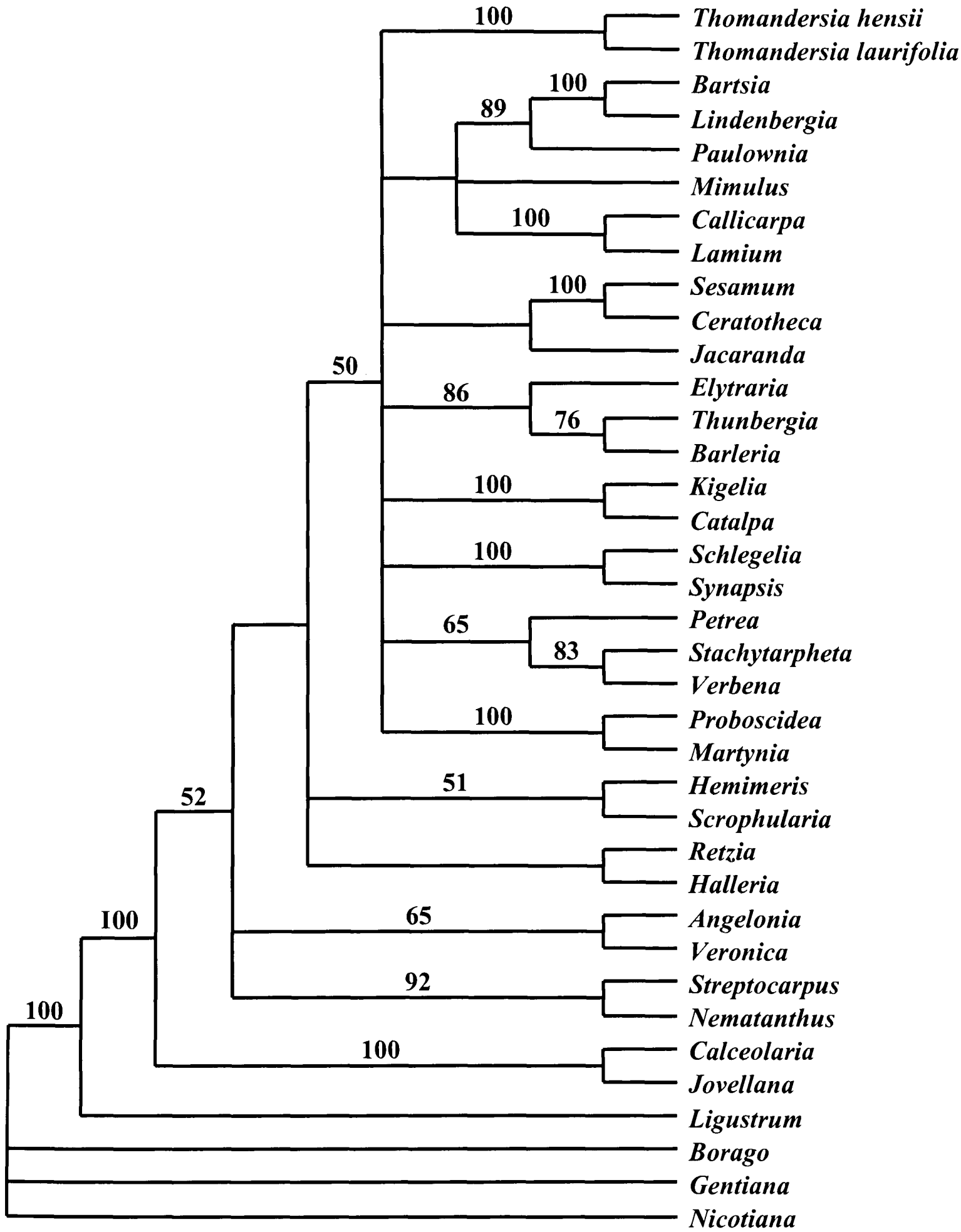


Figure 4.13 Strict consensus of 246 MP trees obtained from total evidence analysis of combined molecular data and the small morphological dataset, showing bootstrap values greater than 50%.

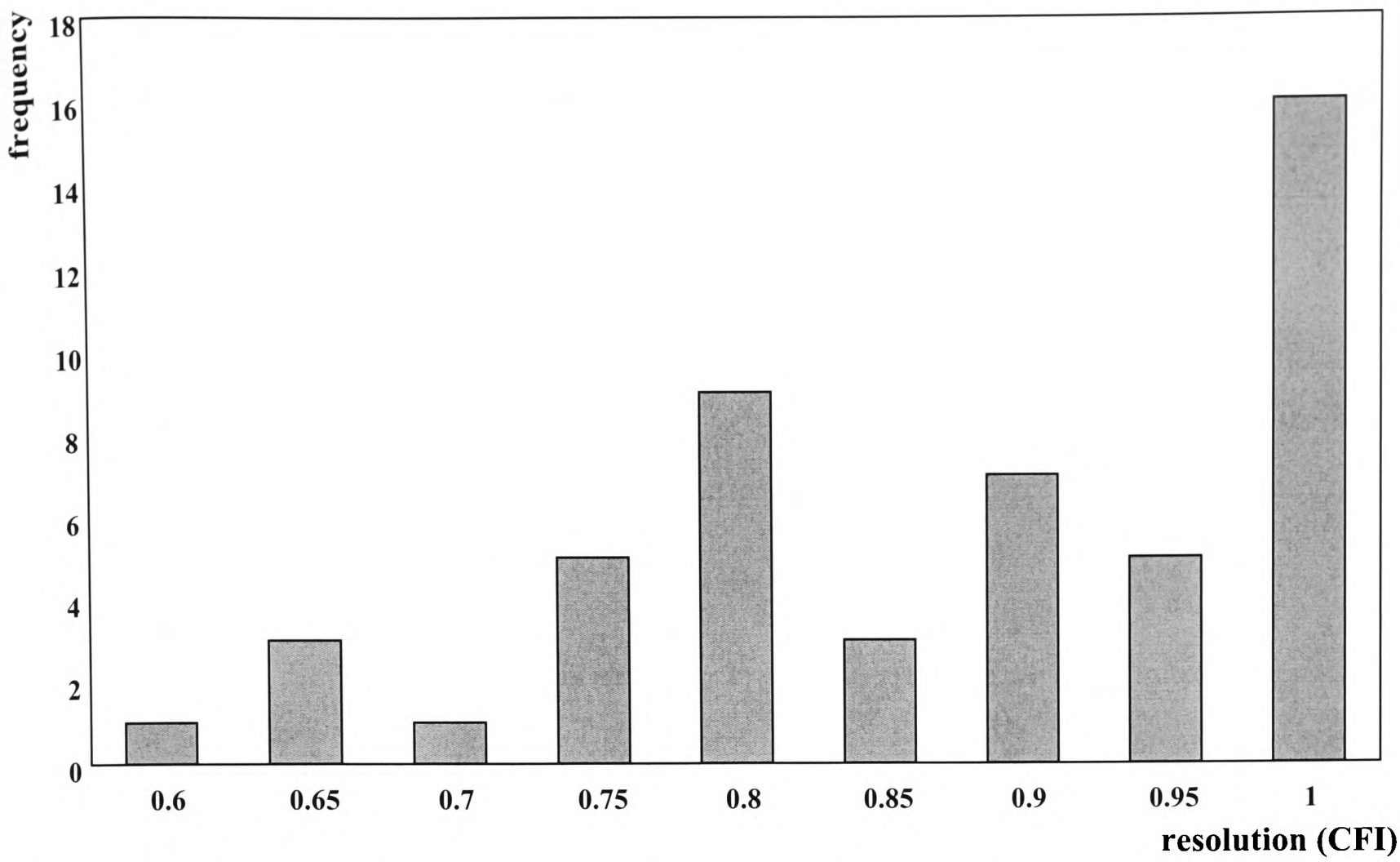


Figure 4.14 Distribution of consensus fork indices (CFI) for phylogenies reconstructed using each of 50 randomised 105-character morphological datasets in simultaneous analysis with molecular data.

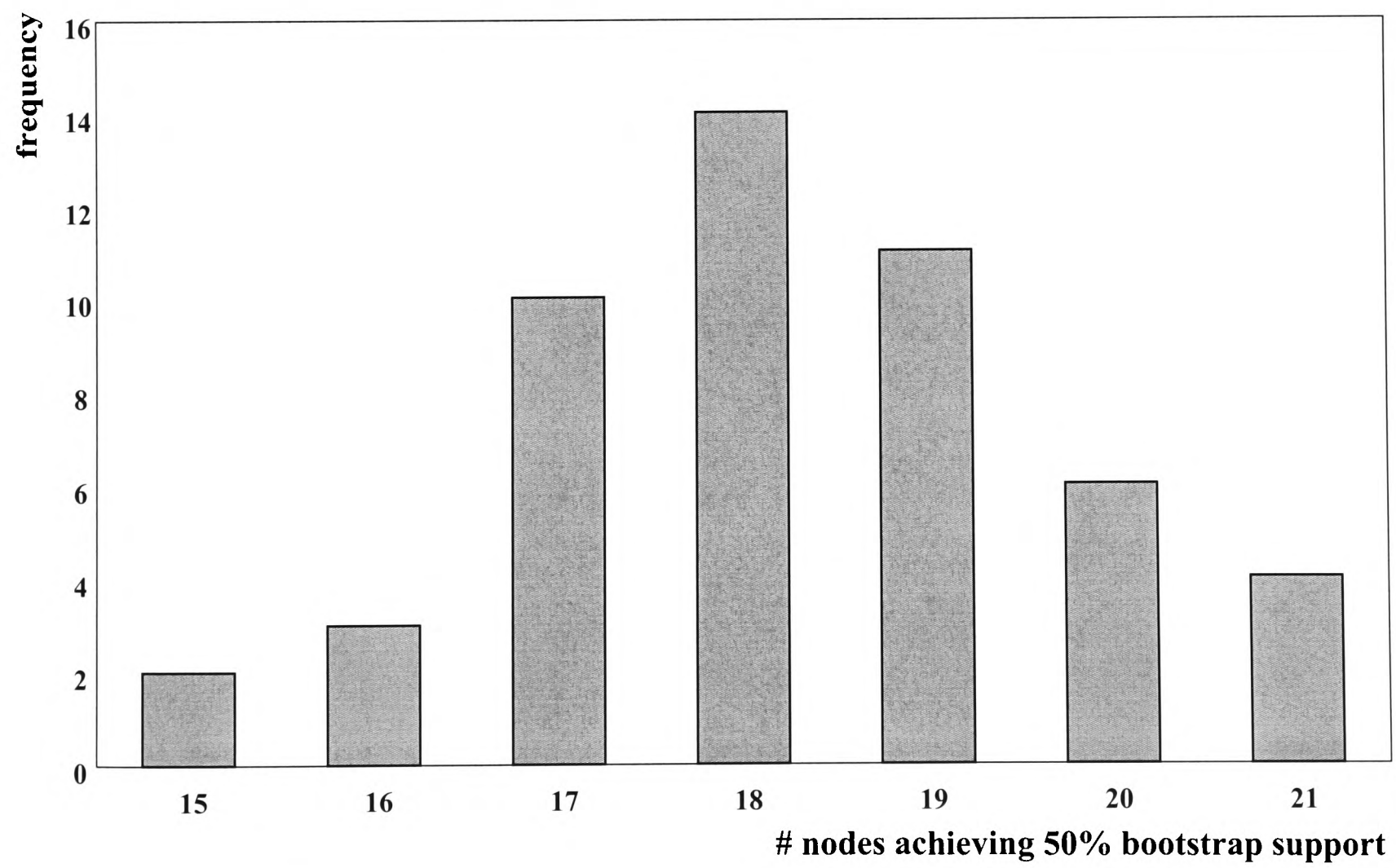


Figure 4.15 Distribution of support (measured as the number of nodes achieving at least 50% bootstrap value) for phylogenies reconstructed using each of 50 randomised 105-character morphological datasets in simultaneous analysis with molecular data.

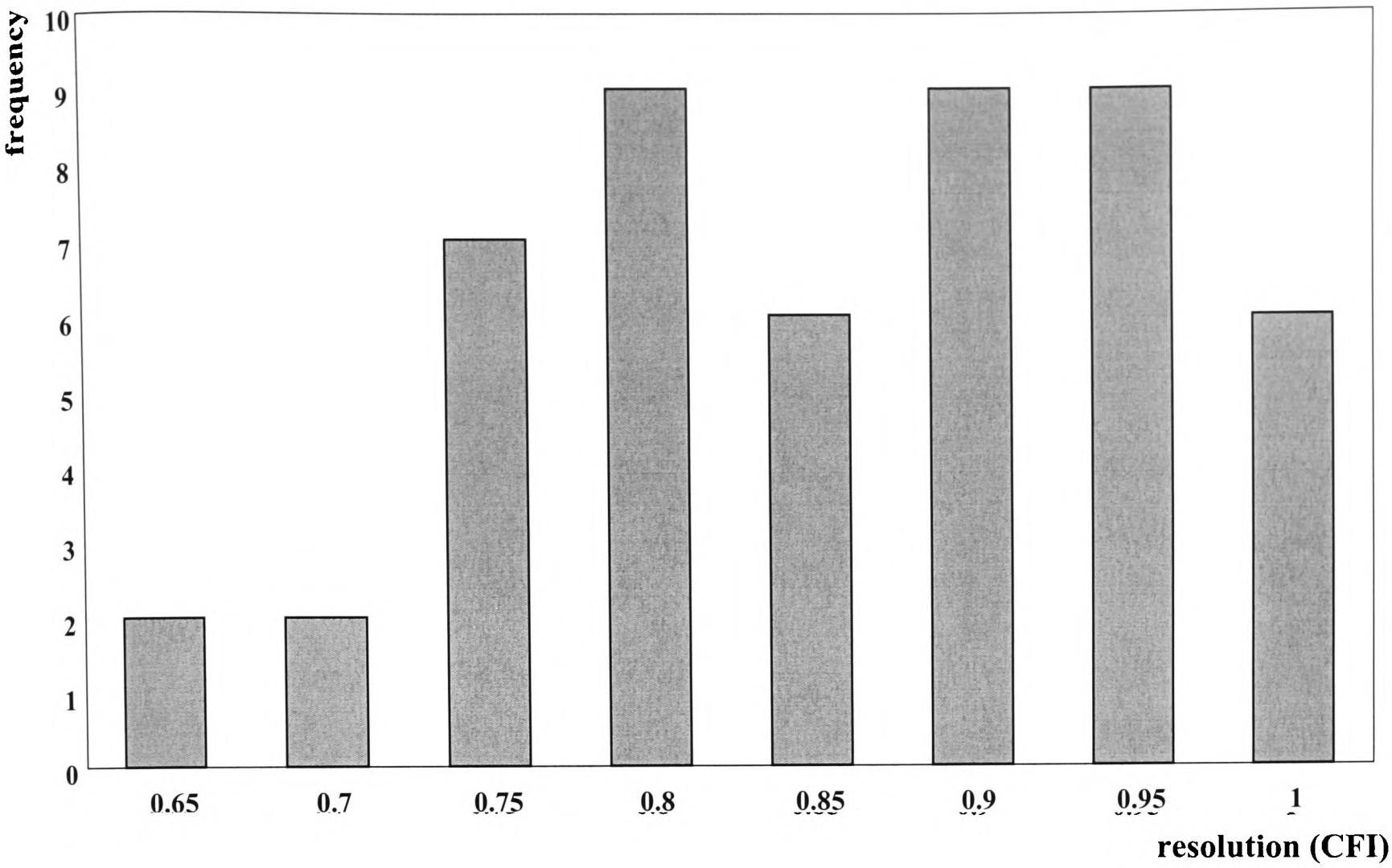


Figure 4.16 Distribution of consensus fork indices (CFI) for phylogenies reconstructed using each of 50 randomly-selected 27-character morphological datasets in simultaneous analysis with molecular data.

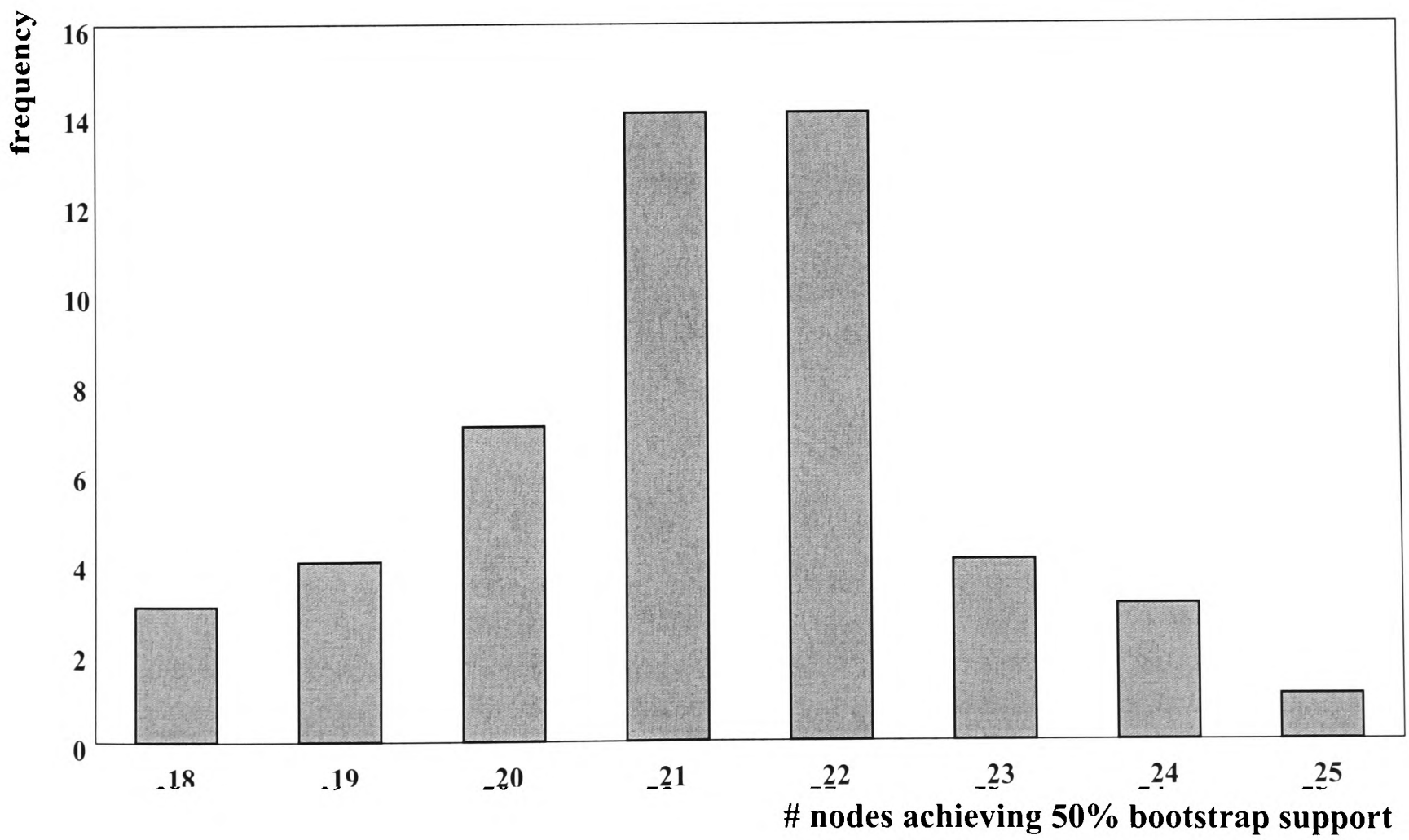


Figure 4.17 Distribution of support (measured as the number of nodes achieving at least 50% bootstrap value) for phylogenies reconstructed using each of 50 randomly-selected 27-character morphological datasets in simultaneous analysis with molecular data.

Effect of strict character selection compared to random selection. Each of the 50 replicate 27-character matrices drawn at random from the relaxed morphological dataset was analysed in conjunction with the molecular dataset. The resulting phylogenies had CI ranging from 0.424 to 0.436 (mean 0.43, standard deviation 0.003), RI 0.345-0.355 (mean 0.35, standard deviation 0.002) and CFI 0.618-1.0 (mean 0.84, standard deviation 0.1; Fig. 4.16). Between 18 and 25 nodes achieved 50% bootstrap support (Fig. 4.17), 12-16 nodes achieved 75%, and 10-11 nodes achieved 95%.

4.6.4 Discussion. Potential informativeness of morphological data in Lamiales. In simultaneous analysis with molecular data, the small morphological dataset provided 5.5% of the total potentially parsimony-informative changes - fewer than most published studies, which generally implement more relaxed selection strategies. The larger morphological dataset provided 13.4%, roughly comparable with other recent simultaneous analyses (Table 4.4), suggesting the expected results in terms of increased support and resolution might be similarly negligible. To test this *a priori* assessment, the actual usefulness of the two morphological datasets was tested in simultaneous analysis with a two-gene molecular dataset.

Actual usefulness of morphological data in Lamiales: resolution. Table 4.5 summarises the key features of all analyses. When analysed alone, the large morphological dataset provided more resolution (CFI 0.21) than the small one (CFI 0.09), and a greater number of nodes was supported by any given bootstrap value. However, neither of the morphological phylogenies was well resolved or supported: even the large dataset recovered only three nodes with a bootstrap value greater than

50%. As expected from their low number of potentially informative characters, parsimony analyses of morphological data alone in Lamiales, whether strictly or leniently selected, were of limited use for phylogeny reconstruction using the characters selected here. Therefore the remainder of this discussion will focus on the simultaneous analysis of morphological and molecular data.

Table 4.5 Summary of results from separate and simultaneous phylogenetic analyses. Figures for the 50 randomised and 50 resampled datasets are given as mean and range.

Dataset	# PI characters	# MP trees	CI	RI	CFI	Nodes with bootstrap value greater than: 50%	75%	95%
Combined molecular data	749	31	0.4306	0.3477	0.76	22	13	10
Large morphology	105	139	0.4264	0.4381	0.21	3	3	1
Small morphology	27	42	0.5155	0.5804	0.09	2	0	0
Molecules & large morphology	854	3	0.4224	0.3500	0.91	20	15	11
Molecules & small morphology	776	246	0.4299	0.3485	0.71	21	15	10
Molecules & randomised morphology	854	8 (1-37)	0.4100 (0.4078-0.4222)	0.3163 (0.3102-0.3500)	0.86 (0.56-1.00)	18.26 (15-21)	12.70 (11-15)	10.04 (9-11)
Molecules & resampled morphology	776	18 (1-192)	0.4287 (0.4241-0.4360)	0.3494 (0.3448-0.3546)	0.84 (0.62-1.00)	21.22 (18-25)	14.02 (12-16)	10.48 (10-11)

In simultaneous analysis with molecular sequence data, the large morphological dataset found fewer MP trees and increased resolution (Table 4.5). Six additional nodes and one incongruent node were resolved compared to the molecular analysis (Fig. 4.12). The new resolution is not likely to be an artefact of differences in character sampling, even though sampling across the morphological dataset was quite variable: newly-resolved groups included some taxa, such as *Synapsis ilicifolia*, for

which little morphological data was available, but also those, such as *Thomandersia hensii*, for which detailed study had completed most of the cells of the data matrix.

To assess the significance of this increased resolution, the results were compared to simultaneous analysis of the molecular data with 50 datasets where the character state assignments were randomly permuted within each character (Archie, 1989). Comparisons were made using a one-sample t-test (where the distribution of the randomised results was normal), and a one-sample sign test (where it was non-normal). The resolution provided by the original simultaneous analysis was not significantly greater than with randomised data ($p=0.0649$) even though seven new groups were resolved (Fig. 4.12; Table 4.6).

The new groups resolved in the simultaneous analysis may be due to phylogenetic signal in the added morphological dataset, or could occur by chance. This can be determined using the randomised datasets which contain no phylogenetic signal (Archie, 1989; Naylor, 1992). The length of the tree reconstructed from the original data was contrasted with those obtained from 50 random matrices, and subjected to a one-sample t-test to determine whether there was a significantly greater signal in the real data than would be found in random matrices of character states. This test suggested significant latent hierarchical structure in the real morphological dataset that is not present in random data ($p<0.0001$). This result is particularly indicative of a strong signal because, in simultaneous analysis with molecular data, the morphological characters account for only a small proportion of the overall tree length. The CI and RI of the real data were also significantly higher than the random

datasets ($p<0.0001$), implying it contained significantly less homoplasy and more synapomorphy than randomised data.

Table 4.6 New groups resolved in simultaneous analysis of molecular and morphological data.

New clade	# included taxa	Bootstrap support value	Unambiguous morphological synapomorphies	Identity of synapomorphies
Schlegeliaceae-*Thomandersia*	4	57	0	-
Lamiaceae-Martyniaceae-*Mimulus*-Orobanchaceae-*Paulownia*	8	11	3	leaf epidermis mucilaginous, branched multicellular hairs present, anthers basifixed
Bignoniaceae-Verbenaceae	6	15	2	synergids hooked, endosperm absent at seed
Core Lamiales-Stilbaceae	25	14	2	axial parenchyma vasicentric, corolla lobes 5
Core Lamiales-Scrophulariaceae-Stilbaceae	27	16	0	-
Gesneriaceae-Veronicaceae	4	12	3	annular disk nectary present, endosperm absent at seed, septicidal dehiscence absent
Borago-Nicotiana	2	62	7	leaf arrangement alternate, leaf epidermis mucilaginous, leaf hairs present, multicellular hairs present, calyx lobes 5, corolla lobes 5, iridoids absent

Actual usefulness of morphological data in Lamiales: support. The new groups that were resolved in total evidence analysis were all at critical interfamilial levels (Table 4.6), yet none of them were very well-supported. Only *Thomandersia*-Schlegeliaceae and *Borago-Nicotiana* had bootstrap values greater than 50%. Overall support values differed little from molecular analysis: approximately the same number of nodes was found with each bootstrap value in both analyses (Table 4.5). Figure 4.18 shows a strict consensus total evidence tree highlighting those nodes for which the bootstrap value increased or decreased compared to the two-gene molecular analysis: both

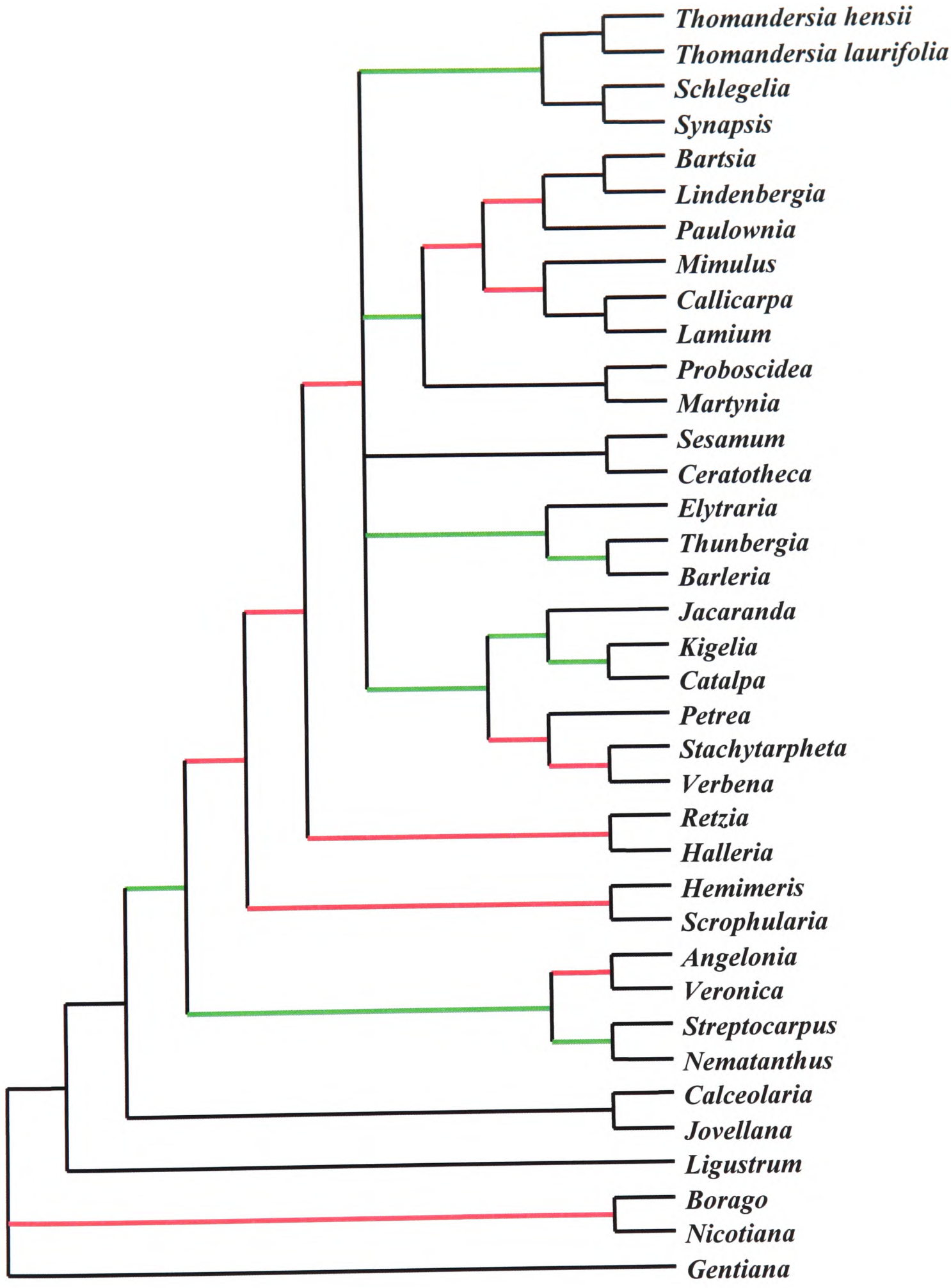


Figure 4.18 Strict consensus of the three MP trees obtained from total evidence analysis of combined molecular data and the large morphological dataset. Red lines indicate clades which have a lower bootstrap value compared to the combined molecular analysis (Fig. 1.4); green lines those with a higher value; black lines those that remained unchanged.

increasingly- and decreasingly-supported nodes were found at all levels in the phylogeny; they were not concentrated towards the base or tips of the tree.

Actual usefulness of morphological data in Lamiales: accuracy. In the 50 total evidence analyses using randomised (signal-free) versions of the larger morphological dataset, only one new grouping was found to have a bootstrap support value of greater than 50%, over and above those from analysis of molecular data alone. This clade was *Thomandersia*-Schlegeliaceae, a clade also found using the real data. It was recovered with support in two out of these 50 analyses, at 54% and 58% bootstrap values. No other new groups were resolved with support, suggesting that adding morphological characters, even if they were to contain no phylogenetic signal, to a molecular dataset would not result in the spurious resolution of supported new clades, contrary to the concern expressed by Givnish & Sytsma (1997).

Effect of character selection. The large, inclusive morphological dataset has been shown to contain structured phylogenetic signal. When combined with molecular data this resolved some new, though mostly unsupported, clades – though not significantly more than might be expected by adding purely random characters. The analysis cannot test whether this signal is accurate or concertedly misleading (e.g. due to unrecognised non-independence between characters).

The partition homogeneity test applied to the two partitions of the morphological dataset gave a non-significant result ($p=0.956$), suggesting that the two matrices did not contain incongruent signals. In the smaller dataset this signal should, in theory, be less obscured by poorly-conceptualised characters. The smaller morphological matrix

did contain fewer homoplasies than the larger, having a CI of 0.52 rather than 0.43. Even in total evidence analysis, the CI using the smaller dataset is greater (0.43) than using the larger (0.42; Table 4.5). However, total evidence analysis using the smaller morphological dataset included no more synapomorphies (RI 0.3485) than the larger (0.35). This is to be expected since no new characters had been added. It also suggests that parsimony analysis could correctly "find" most of the synapomorphies in the large dataset despite the presence of additional, more ambiguous characters. Compared to simultaneous analysis using the large matrix, more MP trees were found and resolution was decreased using the smaller matrix: the CFI of 0.71 was lower than both the analysis of molecular data alone (CFI 0.76), and in combination with the larger morphological dataset (CFI 0.91).

To test the significance of these results they were compared to the analysis of 50 randomly-selected subsets of characters from the large dataset. The addition of the smaller matrix significantly decreased decisiveness ($p<0.0001$; sign test) and resolution ($p<0.0001$) compared to randomly-resampled characters from the large dataset, despite containing a significantly greater signal than randomly-selected characters ($p<0.0001$). Support values were not significantly changed ($p=0.3262$).

Three of the resampled analyses resolved *Thomandersia*-Schlegeliaceae with bootstrap support greater than 50%. This suggests that the signal relating to this clade in the large matrix was removed, by selection, from the smaller matrix but, by chance, passed through into three of the randomly-selected subsets. No morphological character was shared by these three datasets, implying that the characters important for resolution of this clade may not be synapomorphies of the clade itself but a range

of characters supporting other parts of the phylogeny, constraining its topology so that the *Thomandersia*-Schlegeliaceae clade is more likely to be resolved.

4.6.5 Conclusions. The potential informativeness of the larger morphological matrix in Lamiales, which is similar to that of other published studies, suggested that its impact on support and resolution of this problematic phylogeny would be minimal. Parsimony analysis confirmed that a morphological matrix on its own is not sufficient to resolve a supported phylogeny that is congruent with accepted hypotheses. However, both the large and small morphological datasets constructed for Lamiales do contribute a statistically significant phylogenetic signal when they are combined with molecular data, shown by statistical comparison with randomised data. The signals in strictly and leniently selected morphological matrices are congruent, according to the partition homogeneity test, and similar in strength.

In simultaneous parsimony analysis with molecular data, only the larger morphological dataset increased resolution of groups. Overall, the increased resolution, albeit interesting, was small and unsupported by the bootstrap, and its accuracy could not be tested. The addition of a small morphological dataset decreased resolution compared both to molecular data alone and to the addition of randomly-selected morphological characters. This suggests that the small dataset conflicts with the molecular data and that this effect is in some way diluted in the large dataset by the additional characters so that it does not cause the same conflict and consequent decrease in resolution.

There are two possible explanations for this result. Firstly, concertedly misleading characters in the large dataset may conflict with those in the smaller dataset yet coincide with the molecular signal. It is often assumed that concerted homoplasy, generated by convergence in shared ecological conditions affecting multiple traits, is a problem for morphological characters (Givnish & Sytsma, 1997; Givnish et al., 1999; Patterson & Givnish, 2002). However, convergent morphology in detailed studies of distantly-related groups (such as the families of Lamiales) is unlikely to go unnoticed because the characters will differ in detail (Crisp, 1994), and strongly misleading signals are thought to be rare: convergence should instead result in weakly-supported incorrect clades, polytomies, or weakened support for correct clades (Wiens et al., 2003). It requires an amazing coincidence for a set of morphological characters to be both concertedly-misleading and to reinforce a similarly misleading signal from molecular sequences such as to increase resolution of an incorrect phylogeny. In addition, conflict between the two morphological matrices is not suggested by the partition homogeneity test which, if anything, is thought to be too sensitive to incongruence (Yoder et al., 2001; Darlu & Lecointre, 2002).

Alternatively, homoplastic characters in the large dataset may reinforce a signal that was present in the small dataset but too weak to have any noticeable impact in simultaneous analysis. Because these characters are homoplastic they do not show up as a stronger signal in the large dataset in the tree-length based randomisation test. This might be supported by the fact that the length of phylogenies reconstructed from randomly-selected subsets of the large matrix was significantly longer than the length using the real small matrix, indicating higher homoplasy in both the larger dataset and randomly-selected samples from it.

Although neither morphological dataset gave significant benefits for phylogenetic inference in this instance, this explanation implies that stringent selection of a small, less problematic set of morphological characters from a large, ambiguous dataset is counterproductive for total evidence phylogenetic analysis. Moreover, the process of selection by strict similarity criteria was very time-consuming. While stringent selection may reduce homoplasy, as shown by the increased CI and RI values compared to the larger morphological matrix, homoplasy is not necessarily representative of uninformativeness. Instead, apparently ambiguous and homoplastic characters in the larger matrix contribute to the phylogenetic signal it contains. Thus the process of stringent selection results in a loss of phylogenetic information. This loss does not occur, as Poe and Wiens (2000) suggest, through erroneously excluding primary homologies, but by removing useful homoplasies. The finding that homoplastic morphological characters are informative for phylogeny reconstruction is comparable to results found using molecular datasets (Hillis, 1996; Kallersjö et al., 1999).

4.7 Conclusions

Morphological matrices generally contain fewer potentially informative characters for phylogeny reconstruction than molecular ones. The distinction is at present not so great as perceived, and as portrayed by common statistics such as the character-taxon ratio (Hillis, 1987; Sanderson & Donoghue, 1989; Sanderson, 1991; Mishler, 1994; Scotland et al., 2003). However, it is becoming greater as the size and information content of molecular datasets increases.

Incorporating morphological data in a combined analysis generally causes such slight improvements in support and resolution that it is unsurprising that its usefulness is still hotly debated. In Lamiales, the low potential informativeness of even the largest morphological dataset suggested that it would provide negligible effects on support and resolution. The results of total evidence parsimony analyses for Lamiales bear out this prediction.

In Lamiales, morphological data are only marginally useful for phylogeny reconstruction. If they are to be used it is most efficient to employ as large a matrix as possible, subjected to minimal character selection. Most of the additional phylogenetic signal provided by morphological matrices is due to homoplastic characters, so stringent selection of a small morphological dataset is not only time-consuming, but counterproductive. Morphological data are only reliable in a total evidence context: molecular data are needed to provide most of the informative phylogenetic signal, to which morphological characters sometimes add slight improvements in resolution and support. The newly-resolved groups provided by morphological data are poorly-supported, and have not been tested for accuracy. On a positive note, the addition of large morphological datasets rarely lowers the resolution provided by molecular data and seems unlikely to, as has been feared, generate unopposed, incorrect resolution (Donoghue & Sanderson, 1992; Givnish & Sytsma, 1997).

Adding morphological characters to the existing molecular dataset increases support for the relationship of *Thomandersia* with its suggested sister group, Schlegeliaceae. However, the support for this group (57% bootstrap) remains too low to draw firm

conclusions as to the placement of the genus in Lamiales. Chapter 5 will consider whether the alternative strategy of adding a third molecular partition to the existing two-gene molecular sequence dataset could be a more productive method of improving resolution, support and accuracy in the inferred phylogeny of Lamiales.

Chapter 5. The utility of molecular data to resolve the phylogenetic position of *Thomandersia*

5.1 Introduction

The aim of phylogenetic analysis in Lamiales is an accurately-resolved tree, with strongly-supported relationships between all taxa. The previous chapter investigated the potential of morphological data to achieve this goal, and found it to be limited. This chapter investigates the alternative, molecular sequence data. It assesses whether a supported, accurate phylogeny of Lamiales can be resolved using molecular data and, if so, how much and what type of data would be best suited to achieve this.

Kim (1997) defined the term *tree model* to describe the topology, branch lengths and aspects of character evolution associated with a phylogeny. When it is reconstructed using existing molecular sequence data, Lamiales is characterised by a tree model comprising a number of well-supported monophyletic groups separated by unstable internal branches (Fig. 5.1B). The well-supported clades tend to correspond to traditionally-recognised families. The branches between families are short or zero-length, difficult to resolve consistently, with low decay values and bootstrap support typically below 50%. Tree models similar to that of Lamiales are widespread, and often attributed to rapid evolutionary radiations (Conti et al., 1997; Chase et al., 2000; Philippe et al., 2000; Fishbein et al., 2001; Anderberg et al., 2002).

There has been considerable debate over what constitutes the most promising approach to resolving such tree models (Lecointre et al., 1994; Raff et al., 1994; Conti et al., 1997; Renner, 1999; Philippe et al., 2000; Renner & Chanderbali, 2000;

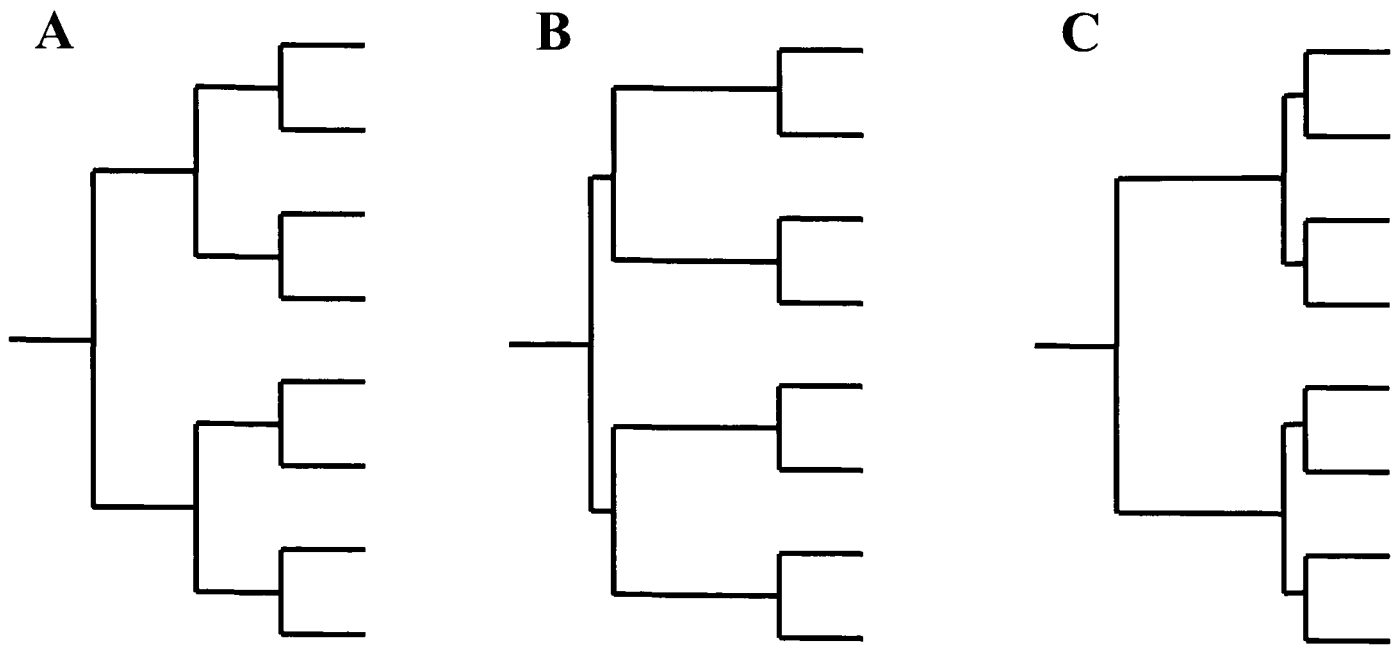


Figure 5.1 Schematic representation of types of ordinal tree model. A. all branch lengths similar. B. family-level clades subtended by long branches; short branches at interfamilial level (as seen in Lamiales). C. family-level clades subtended by short branches; long branches at interfamilial level.

Fishbein et al., 2001; Kress et al., 2001; Cronn et al., 2002; Jenner, 2003; Sanderson & Driskell, 2003). It is widely assumed that increasing the number of nucleotide characters sampled will improve resolution, but in some cases the rate and pattern of evolution in the sequences may be more important than the absolute quantity (Xiang et al., 2002; Borsch et al., 2003). For instance, it may be most productive to sequence slowly-evolving genes, or genes that evolved rapidly at the time of the radiation and have since experienced a slower evolutionary rate (Lecointre et al., 1994; Conti et al., 1997; Goldman, 1998; Fishbein et al., 2001). The best strategy probably varies depending upon the particular phylogeny in question. Therefore, to choose the most effective dataset for successful phylogeny reconstruction in a group such as Lamiales, it is instructive to investigate the effects of different molecular datasets upon the resolution, accuracy and robustness of the inferred phylogeny (Mitchell et al., 2000). One way to approach this is by studying simulated datasets (Nei, 1991; Lecointre et al., 1994; Hillis, 1995; Huelsenbeck, 1995; Hillis, 1996; Huelsenbeck et al., 1996a; Kim, 1996; Rambaut & Grassly, 1997; Abouheif et al., 1998; Wiens, 1998b; De Bry & Olmstead, 2000).

This chapter begins by briefly discussing the prevalence of phylogenies with similar tree models to Lamiales. The prospect of using simulation studies to test the effect of character sampling upon resolution and support is examined. A simulation study is conducted to investigate the impact of additional molecular sequencing on phylogeny reconstruction in Lamiales. In particular, the effects of the number of informative characters and their rate of evolution are compared.

In simulations, it is simplest to increase the number of characters by generating single partitions of increasing length (e.g. Lecointre et al., 1994; Philippe et al., 1994; Huelsenbeck et al., 1996a). In reality, greater numbers of characters can only be obtained by combining sequences from several molecular markers. Therefore, as well as determining the absolute length of sequence that would be necessary to resolve the phylogeny of Lamiales, this chapter investigates the effect of combining separate simulated sequences of the same total length.

Finally, simulated sequences are used to estimate the practical impact of sequencing a particular additional marker (*matK*) for combination with the *rbcL-ndhF* dataset. The use of simulation studies to estimate the effect of adding a sequence dataset prior to sequencing being conducted could be a valuable technique for planning systematic investigations. In combination with the study of morphological datasets in Chapter 4, this will help to determine the most appropriate future strategy for inferring Lamiales phylogeny with maximum resolution and support.

5.2 Brief review

5.2.1 Problematic phylogenies. Lamiales is characterised by a tree model in which well-supported clades are separated by short, poorly-supported branches. This is a common pattern in molecular phylogenetic investigations at many levels and in many taxa. Examples include the interfamilial relationships in other angiosperm orders such as Commelinales (Givnish et al., 1999), Fabales (Kajita et al., 2001), Poales (Bremer, 2002) and Saxifragales (Fishbein et al., 2001), infrageneric relationships such as in *Gossypium* (Cronn et al., 2002), and the radiation of phyla in Metazoa (Halanych, 1998).

A number of factors have been invoked to explain the problems of phylogenetic inference in such groups. These include conflict between characters, insufficient sequence length, poor taxon sampling, rate variation across characters or taxa, compositional bias, paralogy and hybridisation (Renner & Chanderbali, 2000). One issue of debate is whether these problems are best tackled by increasing taxon or character sampling (e.g. Graybeal, 1998; Bremer et al., 1999; Mitchell et al., 2000; Rosenberg & Kumar, 2001). In the case of Lamiales, the existing 37-taxon dataset already spans the root node of all major families (Olmstead et al., 2001; Prendini, 2001). Adding more taxa to this dataset would only increase the density of sampling within well-supported monophyletic groups, as determined by previous family-level studies (Hedren et al., 1995; Olmstead & Reeves, 1995; Scotland et al., 1995; Wagstaff & Olmstead, 1997; Wagstaff et al., 1998; Spangler & Olmstead, 1999; Young et al., 1999; Olmstead et al., 2001; Beardsley & Olmstead, 2002; Schwarzbach & McDade, 2002). Increased taxon sampling is therefore unlikely to help resolve the problematic interfamilial relationships in Lamiales. In contrast, if the tree model results from character conflict or lack of phylogenetic information, support and resolution may be improved by additional character sampling (Qiu et al., 1993; Renner & Chanderbali, 2000; Rokas et al., 2003). Thus, in Lamiales, increased sampling of characters for existing taxa is most likely to improve the resolution of phylogenetic relationships.

Increasing character sampling has already proven beneficial for inferring some problematic phylogenies (Plunkett et al., 1996; Soltis et al., 1999; Renner & Chanderbali, 2000; Anderberg et al., 2002; Bremer et al., 2002]. However, a number of flowering plant orders, including some of the largest and best-studied, remain to

some extent unresolved, even in large-scale analyses using many molecular markers and a range of phylogenetic methods. These include Cornales, Ericales, Laurales, Malpighiales and Apiales {Xiang, 2002 #55). Here, simulation studies are used to determine whether Lamiales is one of those phylogenies that will prove to be resolvable with increased numbers of molecular sequence characters, or not.

5.2.2 Simulation studies. Simulation studies allow the assessment, *a priori,* of the conditions required for supported, accurate resolution of a given phylogeny. Empirically investigating the effects of differing data and methods upon support and resolution in phylogeny reconstruction can be expensive and time-consuming. Furthermore, the effects upon accuracy cannot be tested because the underlying evolutionary relationships are not known. Simulation studies provide a means to investigate resolution, support and accuracy before constructing a real dataset (Hillis, 1996; Graybeal, 1998; Carine, 1999).

The main hindrance to reconstructing Lamiales phylogeny at present is a preponderance of short internal branches (Fig. 5.1B). Short branches result from a lack of phylogenetic information in the datasets used, due to a small number of informative character state changes, and the way they are distributed on the tree. For phylogenies inferred from molecular sequence data, the length of a branch represents the number of nucleotide substitutions that have occurred along it (Swofford et al., 1996), which is a product of the time elapsed between nodes, the rate of nucleotide substitution over that time, and the number of nucleotide sites sampled.

For the branches of a given phylogeny, the time elapsed is fixed, so branch length can be manipulated only through the nucleotide substitution rate and number of sites sampled. Branch lengths can be increased by sampling a greater number of sites, or sites which have experienced a greater rate of substitution. Since this requires expensive and time-consuming DNA extraction and sequencing for a large number of taxa, it would be useful to know *a priori* which of these methods is likely to yield the best resolved, supported and accurate phylogeny. Simulation studies can be used to address this question (Lecointre et al., 1994; Philippe et al., 1994; Berbee et al., 2000). Here, they are used to investigate how much data might be needed to resolve relationships in Lamiales, contrasting the effects of increasing the number of informative characters or using sequences with a faster evolutionary rate.

Simulation studies operate by comparing an inferred phylogeny to a starting tree established under idealised conditions and an artificial evolutionary process (Nei, 1991; Huelsenbeck, 1995; Rydin & Kallersjö, 2002). They typically comprise a four-step procedure (Lamboy, 1994; Pol & Siddall, 2001). Firstly, a starting tree (topology and branch lengths) is selected. Secondly, character state data are simulated upon the tree according to a specified model of substitution. Thirdly, phylogenetic trees are reconstructed from the simulated data; and finally the reconstructed phylogenies are compared to the original tree, according to criteria such as accuracy, support, resolution, internal consistency or robustness (Lecointre et al., 1993; Huelsenbeck, 1995; Bremer et al., 1999).

5.3 Methods

A simulation study was conducted, following the basic four step procedure outlined above, using starting trees and parameters derived from *rbcL* and *ndhF,* and generated for *matK*. The tree model reconstructed using *rbcL* and *ndhF* was assumed to be representative of the underlying evolutionary history of Lamiales, based on the observations that similar relative branch lengths were obtained whether the phylogeny was reconstructed using *rbcL* or *ndhF*, and that this form of tree model is very prevalent amongst other orders of angiosperms.

The simulation of *rbcL*- and *ndhF*-like sequences was based upon parameters derived from existing sequence data. *MatK* sequences are not yet available for many Lamiales, so its parameters were derived from other existing studies in order to give as realistic as possible an *a priori* assessment of the likely usefulness of this marker for phylogeny reconstruction. *MatK* was chosen as a potentially useful marker because it has previously been employed in combination with *rbcL* and *ndhF* to investigate interfamilial relationships in other orders of plants, including a similar tree model in Saxifragales (Hilu & Liang, 1997; Hilu et al., 1999; Fishbein et al., 2001; Bremer et al., 2002; Xiang et al., 2002; Hilu et al., 2003). Simulations were conducted by parametric bootstrapping, a stochastic Markovian method that employs an evolutionary model and parameters derived from existing data (Nei, 1991; Adell & Dopazo, 1994; Hillis, 1995; Huelsenbeck et al., 1996a; Wollenberg & Atchley, 2000). After phylogeny reconstruction, comparisons with the starting tree were made using the criteria of accuracy, support and resolution (Lecointre et al., 1993; Huelsenbeck, 1995; Bremer et al., 1999).

Starting phylogenies. Fully resolved un-rooted tree topologies were generated for Lamiales from *ndhF* (Fig. 5.2) and *rbcL* (Fig. 5.3) using the neighbour-joining method in PAUP* (Swofford, 2002). Branch lengths (number of inferred substitutions between nodes) were estimated for these trees using uncorrected distances.

A starting tree was also designed to represent the phylogeny that might be reconstructed from *matK*. The topology of the tree was provided by a combined analysis of *rbcL* and *ndhF* (Fig. 5.4). Employing this topology assumes minimal conflict between the three partitions and therefore means the results will tend to overestimate the potential usefulness of *matK*. Thus, if *matK* fails to provide additional resolution and support in this simulation, it is unlikely to be worthwhile sequencing for empirical investigation, at least on its own. Branch lengths were generated for the *matK* tree using those from *rbcL,* correcting for the relative rate of evolution of the two genes (no comparative data were available for *matK* and *ndhF*). The average number of nucleotide substitutions per site for *matK* is consistently about twice that of *rbcL* (Steele & Vilgalys, 1994; Fishbein et al., 2001; Lopez et al., 2002). Therefore branch lengths for the *matK*-like starting tree were calculated by doubling the branch lengths of the *rbcL* tree. Since the relative branch lengths in the *rbcL* and *ndhF* phylogenies were similar to one another, it was assumed that those of *matK* would also be similar.

Sequence simulation. To determine the most realistic evolutionary model and parameters for sequence simulation based on these trees, each dataset was tested against 56 possible models using MODELTEST 3.06 and MODELBLOCK 3 (Posada & Crandall, 1998). For *rbcL*, the most suitable model, according to the hierarchical

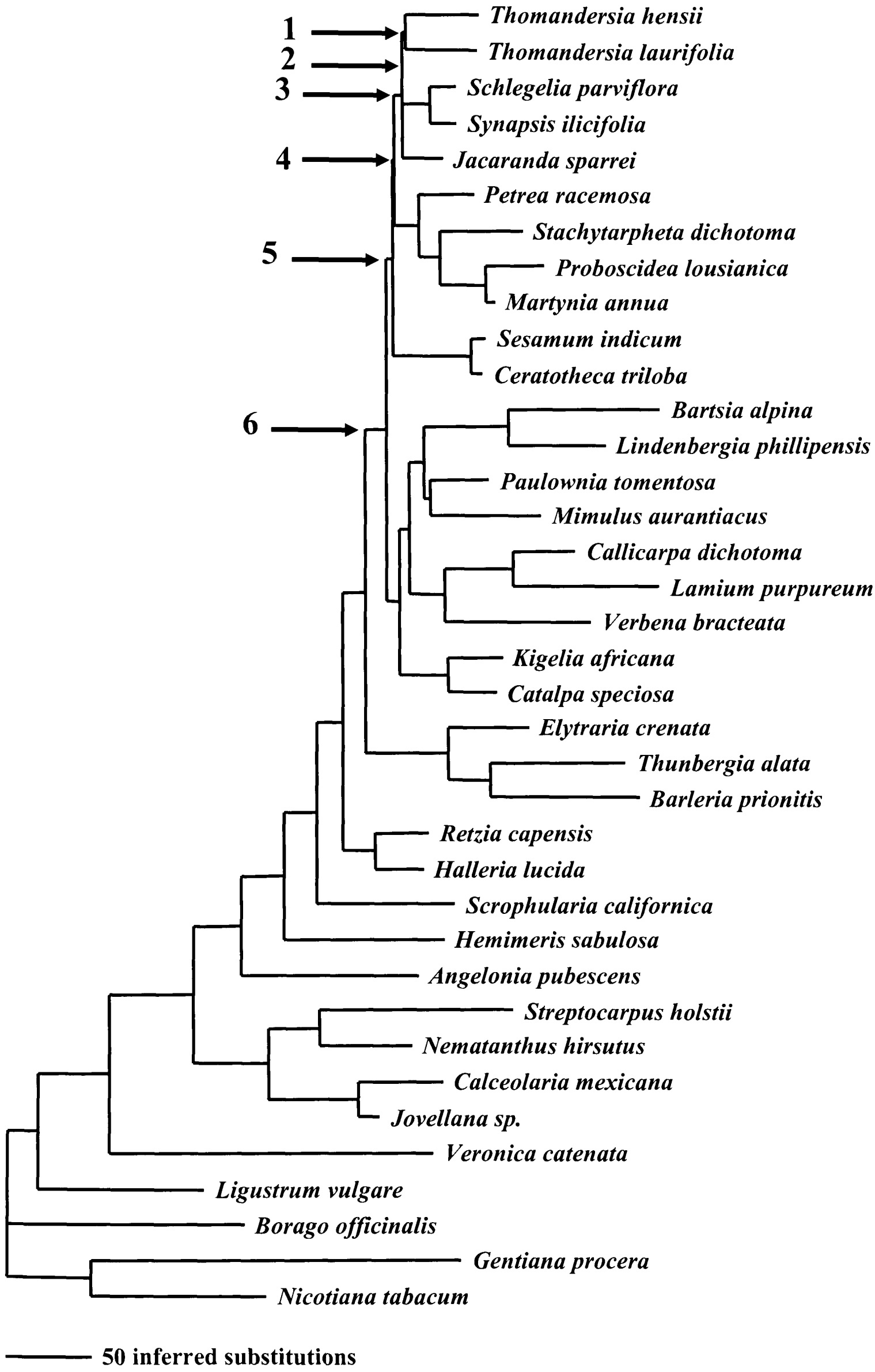


Figure 5.2 Starting phylogeny of Lamiales based on neighbour-joining analysis of *ndhF* sequences, with branch lengths from uncorrected data, and six key nodes highlighted.

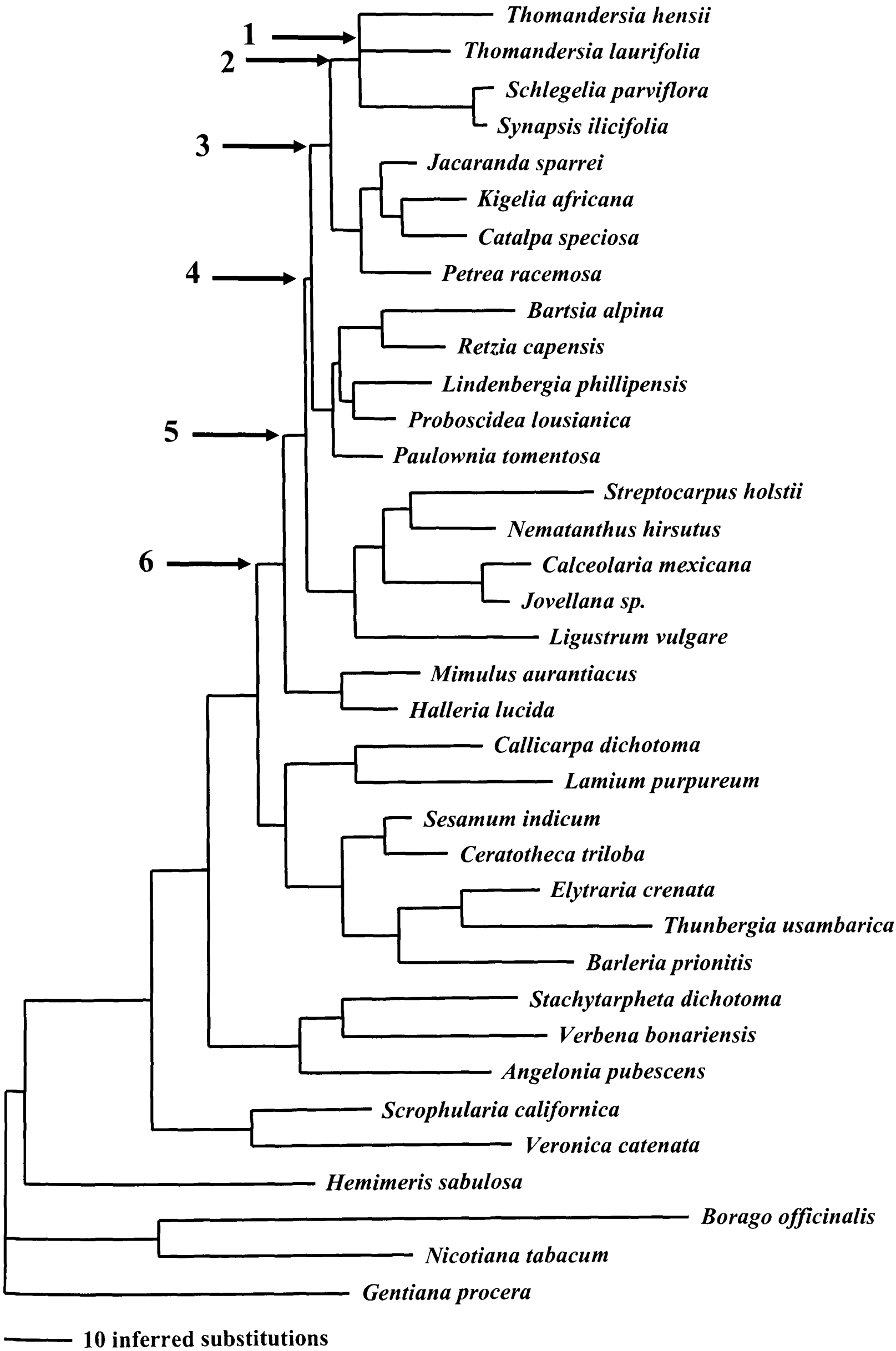


Figure 5.3 Starting phylogeny of Lamiales based on neighbour-joining analysis of *rbcL* sequences, with branch lengths from uncorrected data, and six key nodes highlighted.

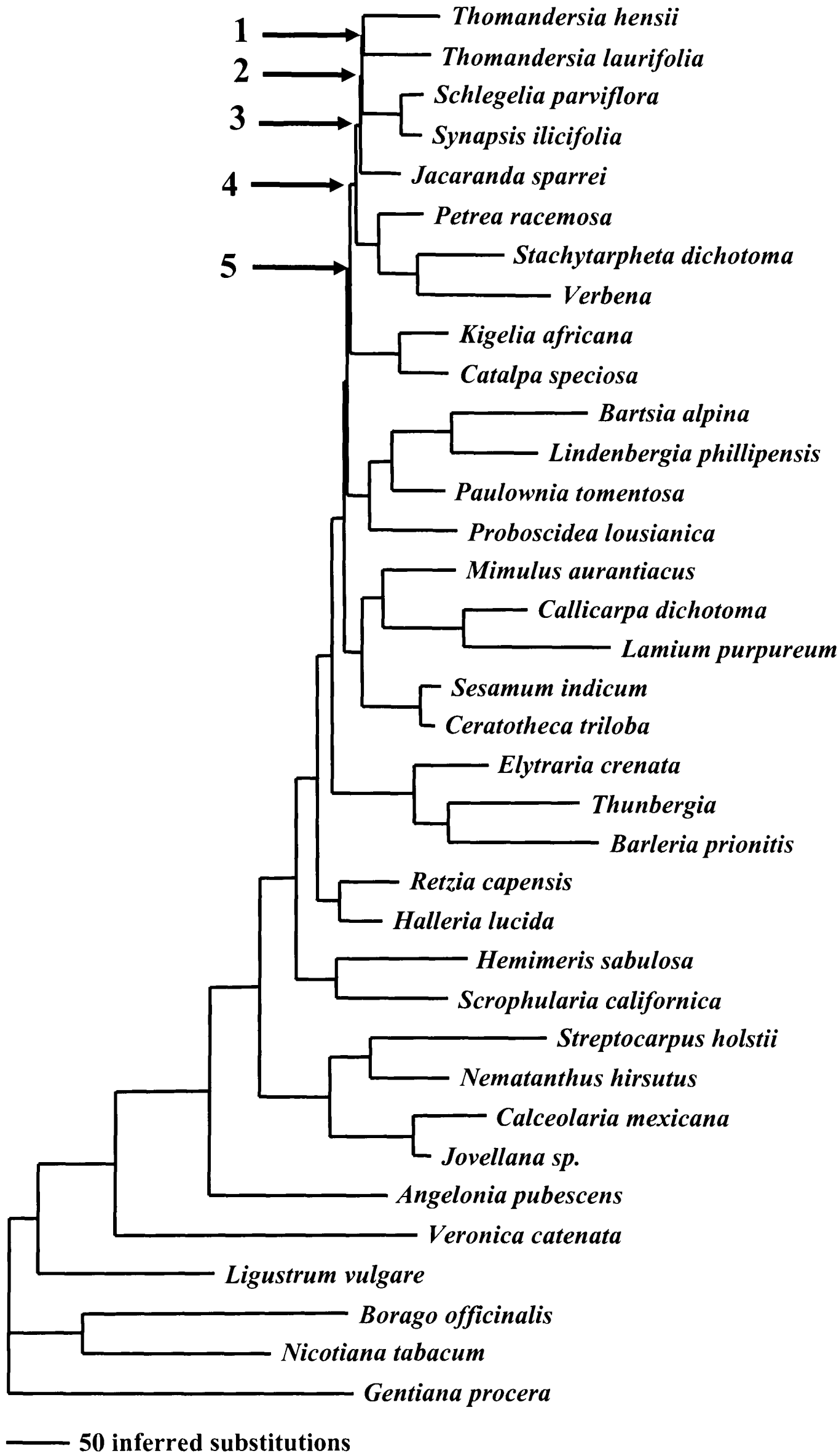


Figure 5.4 Starting phylogeny of Lamiales based on neighbour-joining analysis of combined *ndhF* and *rbcL* sequences, with branch lengths from uncorrected data, and five key nodes highlighted.

likelihood ratio test, was the general reversible model, GTR+I+G, with relative base frequencies A=0.2723, C=0.1927, G=0.2357, T=0.2993, relative substitution rates A-C=1.4734, A-G=2.7252, A-T=0.3801, C-G=0.9138, C-T=3.4541, G-T=1, and gamma distribution shape parameter 0.8023. For *ndhF*, the model selected was also GTR+I+G, with relative base frequencies A=0.2949, C=0.1476, G=0.1606, T=0.3969, relative substitution rates A-C=2.1962, A-G=3.2938, A-T=0.2243, C-G=2.5359, C-T=3.2724, G-T=1, and gamma distribution shape parameter 1.0267.

A model and parameters for simulation of *matK* sequences in Lamiales were taken from an existing dataset for *Diploxylon* (Lopez et al., 2002). This model was assumed to be applicable to *matK* in Lamiales because the evolutionary model for *rbcL* in *Diploxylon* was very similar to that of *rbcL* in Lamiales. The evolutionary model selected for *matK* was the Hasegawa-Kishino-Yano model, HKY85 (Lopez et al., 2002), which is equivalent to the GTR model when only transition and transversion substitution rates differ (transition/transversion ratio 1.68 in this case) and no gamma shape parameter is specified. Base frequencies were also taken from Lopez (2002), since they did not differ greatly from those obtained for *rbcL* and *ndhF* in Lamiales: A=0.31, C=0.18, G=0.18, T=0.33.

Replicate sequence datasets were simulated by parametric bootstrapping, in NEXUS format, using Seq-Gen 1.2.5.1 (Rambaut & Grassly, 1997), employing the starting parameters determined above (Table 5.1).

One hundred replicate matrices were simulated for each set of parameter values. Sixty-four sets of replicates were used to investigate the effect of altering substitution

Table 5.1 Simulated molecular datasets and parameters (continued overleaf).

Dataset	Topology and parameters as:	# substitutions per site	Sequence length (bp)	# replicate matrices	Starting seed
1	*ndhF*	0.1	2,000	100	15448376
2	*ndhF*	0.2	2,000	100	47709730
3	*ndhF*	0.5	2,000	100	78399216
4	*ndhF*	1	2,000	100	79842335
5	*ndhF*	2	2,000	100	52711674
6	*ndhF*	5	2,000	100	2486395
7	*ndhF*	10	2,000	100	40019257
8	*ndhF*	20	2,000	100	50255930
9	*rbcL*	0.1	2,000	100	84624646
10	*rbcL*	0.2	2,000	100	19788860
11	*rbcL*	0.5	2,000	100	67529069
12	*rbcL*	1	2,000	100	24128070
13	*rbcL*	2	2,000	100	82906566
14	*rbcL*	5	2,000	100	46522473
15	*rbcL*	10	2,000	100	18348872
16	*rbcL*	20	2,000	100	25410028
17	*ndhF*	0.1	1,000	100	73024013
18	*ndhF*	0.2	1,000	100	89971506
19	*ndhF*	0.5	1,000	100	31302977
20	*ndhF*	1	1,000	100	47677141
21	*ndhF*	2	1,000	100	58334587
22	*ndhF*	5	1,000	100	25115300
23	*ndhF*	10	1,000	100	78492019
24	*ndhF*	20	1,000	100	91255506
25	*rbcL*	0.1	1,000	100	41461212
26	*rbcL*	0.2	1,000	100	11702582
27	*rbcL*	0.5	1,000	100	52191925
28	*rbcL*	1	1,000	100	98032014
29	*rbcL*	2	1,000	100	93555486
30	*rbcL*	5	1,000	100	29566716
31	*rbcL*	10	1,000	100	95852382
32	*rbcL*	20	1,000	100	87387046
33	*ndhF*	1	100	100	49319467
34	*ndhF*	1	200	100	87626389
35	*ndhF*	1	500	100	99908045
36	*ndhF*	1	1,000	100	18054075
37	*ndhF*	1	2,000	100	30201192
38	*ndhF*	1	5,000	100	27046649
39	*ndhF*	1	10,000	100	46273839
40	*ndhF*	1	20,000	50	76508394
41	*rbcL*	1	100	100	10078655
42	*rbcL*	1	200	100	3605281
43	*rbcL*	1	500	100	28595320
44	*rbcL*	1	1,000	100	55770326
45	*rbcL*	1	2,000	100	41513186
46	*rbcL*	1	5,000	100	22136409
47	*rbcL*	1	10,000	100	22808617
48	*rbcL*	1	20,000	50	24561367
49	*ndhF*	2	100	100	38322705
50	*ndhF*	2	200	100	88423710
51	*ndhF*	2	500	100	30393937
52	*ndhF*	2	1,000	100	19995511
53	*ndhF*	2	2,000	100	97044384
54	*ndhF*	2	5,000	100	94042089
55	*ndhF*	2	10,000	100	8494918

56	*ndhF*	2	20,000	50	49726076
57	*rbcL*	2	100	100	80927635
58	*rbcL*	2	200	100	11440646
59	*rbcL*	2	500	100	79718335
60	*rbcL*	2	1,000	100	34909131
61	*rbcL*	2	2,000	100	63707149
62	*rbcL*	2	5,000	100	89050894
63	*rbcL*	2	10,000	100	89970723
64	*rbcL*	2	20,000	50	23612088
65	*rbcL*	1	500	100	88936737
66	*rbcL*	1	1,000	100	48334848
67	*rbcL*	1	2,000	100	17673847
68	*rbcL*	1	5,000	100	95982113
69	*rbcL*	1	10,000	100*	61466965
70	*ndhF*	1	500	100	88191445
71	*ndhF*	1	1,000	100	49317736
72	*ndhF*	1	2,000	100	70621389
73	*ndhF*	1	5,000	100	93790617
74	*ndhF*	1	10,000	100*	38780095
75	*matK*	1	1,034	100	68743963
76	*ndhF*	1	2,176	100	7104129
77	*rbcL*	1	1,402	100	90779718

*only 50 replicate matrices were used when datasets 69 and 74 were combined, to maintain a manageable file size for analysis.

Table 5.2 Combined simulated datasets.

Combined dataset	***rbcL* dataset (Table 5.1)**	***rbcL* sequence length (bp)**	***ndhF* dataset (Table 5.1)**	***ndhF* sequence length (bp)**	**Total sequence length (bp)**
A	65	500	70	500	1,000
B	65	500	71	1,000	1,500
C	65	500	72	2,000	2,500
D	65	500	73	5,000	5,500
E	65	500	74	10,000	10,500
F	66	1,000	70	500	1,500
G	66	1,000	71	1,000	2,000
H	66	1,000	72	2,000	3,000
I	66	1,000	73	5,000	6,000
J	66	1,000	74	10,000	11,000
K	67	2,000	70	500	2,500
L	67	2,000	71	1,000	3,000
M	67	2,000	72	2,000	4,000
N	67	2,000	73	5,000	7,000
O	67	2,000	74	10,000	12,000
P	68	5,000	70	500	5,500
Q	68	5,000	71	1,000	6,000
R	68	5,000	72	2,000	7,000
S	68	5,000	73	5,000	10,000
T	68	5,000	74	10,000	15,000
U	69	10,000	70	500	10,500
V	69	10,000	71	1,000	11,000
W	69	10,000	72	2,000	12,000
X	69	10,000	73	5,000	15,000
Y	69	10,000	74	10,000	20,000

Table 5.3 Combined *matK, ndhF* and *rbcL* datasets.

Combined dataset	*matK* dataset?	*ndhF* dataset?	*rbcL* dataset?	Total sequence length (bp)
matK	Y	N	N	1,034
ndhF	N	Y	N	2,176
rbcL	N	N	Y	1,402
ndhF-rbcL	N	Y	Y	3,578
matK-ndhF	Y	Y	N	3,210
matK-rbcL	Y	N	Y	2,436
matK-ndhF-rbcL	Y	Y	Y	4,612
matK-real data	Y	(real)	(real)	4,612

rate and sequence length for a single partition with the properties of either *rbcL* or *ndhF*. Ten further sets, with a limited range of substitution rates and sequence lengths, were used to investigate the effects of combining two different partitions of varying lengths. These datasets were interleaved in all possible *rbcL-ndhF* combinations (Table 5.2). Three datasets were simulated to the specifications of the real *matK, ndhF* and *rbcL* data, and were interleaved to investigate the effect of combining a third molecular marker with the existing two-gene dataset (Table 5.3).

Phylogeny reconstruction. Heuristic tree searches were conducted using PAUP* (Swofford, 2002), with 1,000 random-addition replicates, retaining a maximum of two trees at each step, using TBR branch-swapping and saving a maximum of 100 trees (to reduce computation time). Bootstrap searches were conducted with 100 replicates, each comprising a 10-replicate heuristic search using random addition and holding only two trees at each step, with TBR branch-swapping and MULPARS off.

Comparison of resulting phylogenies. The PAUP* output files were inspected automatically using a C++ program (A. South, pers. comm.). The following details were recorded for comparison with the starting tree:

- Tree length

- Ensemble consistency index (CI), corrected for invariable sites
- Ensemble retention index (RI)
- Number of MP trees containing each of 5-6 key nodes (see explanation below)
- Presence/absence of 5-6 key nodes in a strict consensus of MP trees
- Consensus fork index (CFI, Colless, 1980; 1981)
- Bootstrap support value for key nodes
- Total number of nodes with bootstrap value of at least 50%, 75%, 90% or 95%.

Accuracy and support were assessed for five or six nodes on each starting tree (labelled on Figs. 5.2-5.4). These were selected to encompass a range of hierarchical levels, by taking successively nested nodes. The nodes chosen all contain *Thomandersia* and are subtended by some of the shortest branches on the tree, thereby being relevant to two issues of interest: the sister group of *Thomandersia* and the resolution of problematic interfamilial relationships in Lamiales.

Because PAUP* displays only bootstrap values greater than 5%, any absent values (i.e. lower than 5%) were taken as 2.5%. The effect of this approximation was tested, and found to have a negligible effect on the resulting distribution of support values: whether the missing values were set at 0% or 5% made no discernable difference. Therefore taking 2.5%, the mean of 0% and 5%, for these nodes was justified.

5.4 Results

This study investigated the effect of two variables – nucleotide sequence length and nucleotide substitution rate – upon phylogeny reconstruction in Lamiales. Firstly, sequences were constructed under two sets of starting parameters derived from

existing datasets for *rbcL* and *ndhF*. Then the effects of combining these two datasets, and of adding a third molecular marker, *matK*, were considered. All inferred phylogenies were compared to the starting tree in terms of resolution, support and accuracy. Resolution was measured by the CFI. Support was measured in two ways, as the number of nodes achieving a certain bootstrap value, or the support value achieved by certain selected nodes. Two measures of accuracy were employed: the percentage of MP trees containing each of the selected nodes, and the percentage of strict consensus trees in which each node was found.

5.4.1 Effect of sequence length. Resolution increased with the length of sequence analysed (Fig. 5.5). The rate of increase was greatest for short lengths of sequence. High resolution (taken as a CFI of at least 0.95) was achieved using approximately 10,000 base pairs (bp) of sequence data, for datasets based on *ndhF*-type sequences. When nucleotide substitution rates were doubled, high resolution was achieved with 5,000 bp. A similar result was found for *rbcL*-type datasets.

Accuracy, measured as the mean percentage of MP trees containing each of six nodes, increased with the length of sequence analysed (Fig. 5.6). For *ndhF*-type sequence datasets, Node 1 was found in all MP trees when at least 500 bp of sequence were analysed, regardless of substitution rate. Other nodes required a greater length of sequence to be accurately resolved: Nodes 3 and 4 were found in all trees with 10,000 bp; Nodes 5 and 6 required 20,000 bp to be accurately reconstructed almost all of the time. Node 2 was the least likely to be accurately resolved: 20,000 bp were required to recover it in 90% of MP trees. The length of sequence needed to provide accurate resolution was shorter when the substitution rate was doubled.

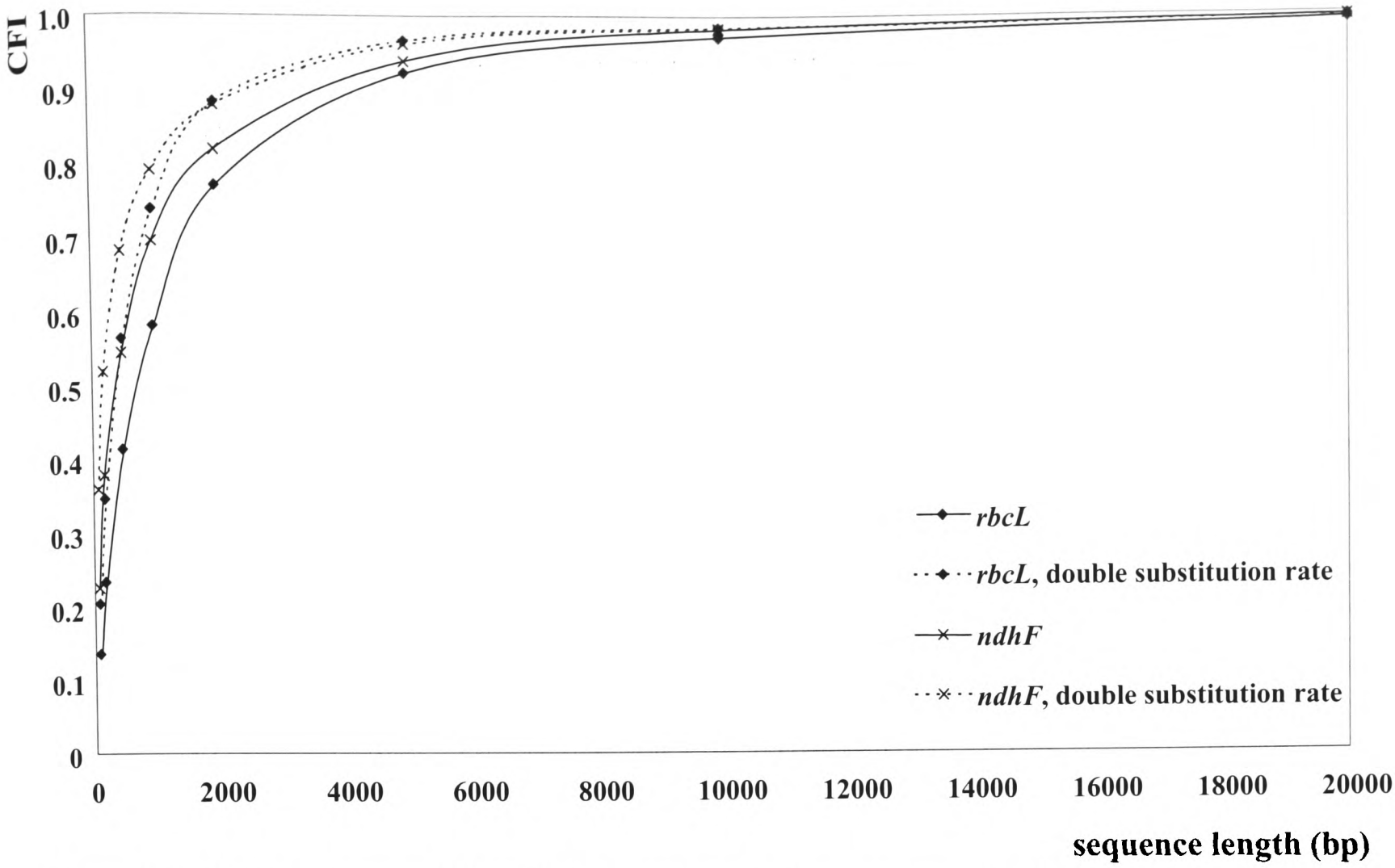


Figure 5.5 Graph to show relationship between resolution, measured by the consensus fork index (CFI), and length of aligned sequence analysed, for *ndhF*- and *rbcL*-type sequences simulated at normal, and twice-normal, substitution rates.

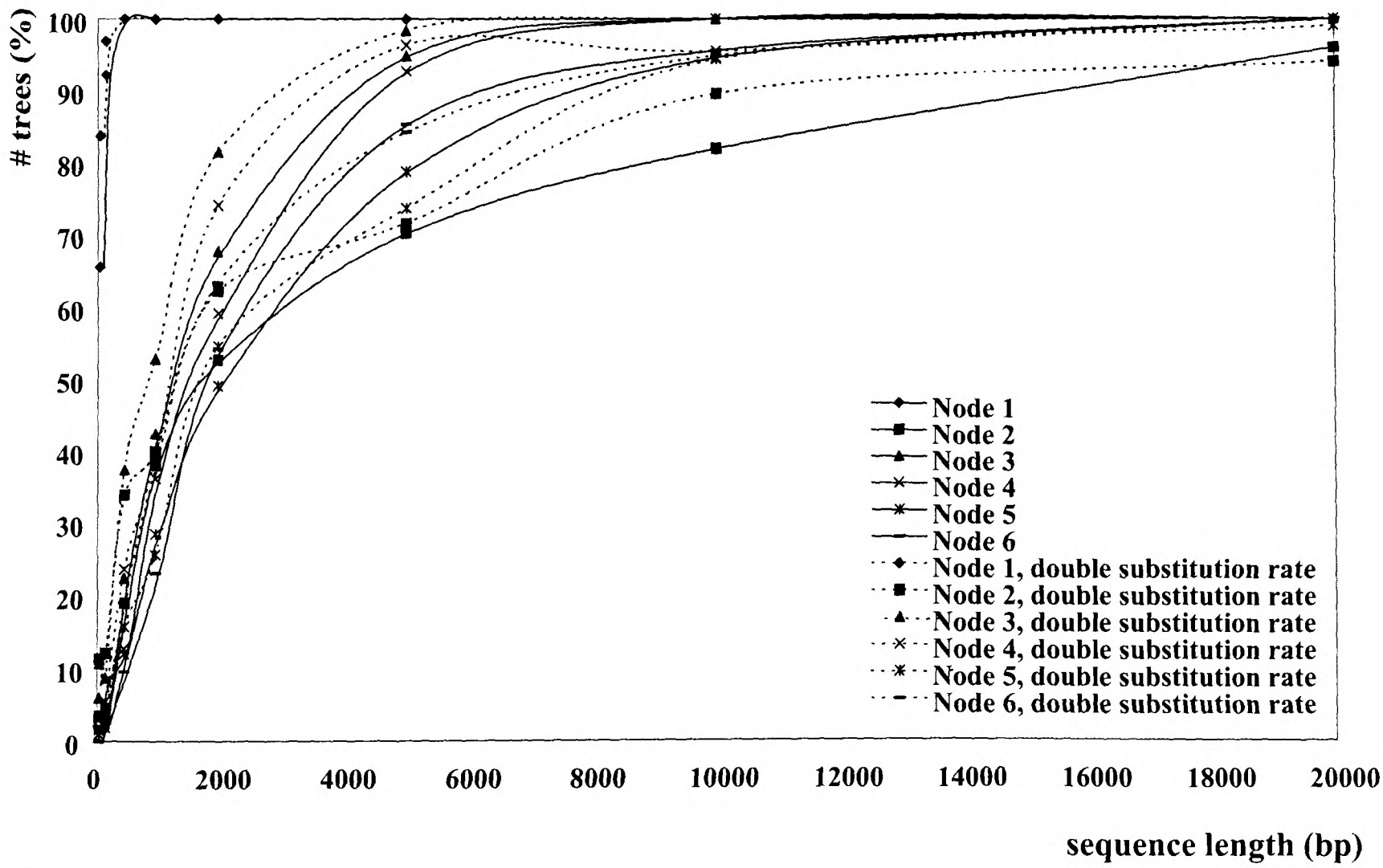


Figure 5.6 Graph to show relationship between accuracy, measured as the mean percentage of MP trees containing Nodes 1-6, and length of aligned sequence analysed, for *ndhF*-type sequences simulated at normal, and twice-normal, substitution rates.

The accuracy of phylogeny reconstruction according to the second measure followed a similar pattern. A node was considered to be accurately-reconstructed if it was found in 95% of strict consensus trees (a 5% type II error rate). Five thousand bp of *ndhF*-type sequence were needed to accurately construct Node 1, 6,000 bp for Nodes 3 and 4, and 20,000 bp for Nodes 2, 5 and 6. Nodes 3 and 4 required slightly fewer nucleotides when the substitution rate was doubled. For *rbcL*-type sequences, 95% accuracy was achieved with just over 1,000 bp for Node 1, about 5,000 bp for Nodes 3 and 6, 10,000 bp for Node 2, and 15,000 bp for Nodes 4 and 5 (Fig. 5.7). Almost 100% accuracy was achieved for all nodes with sequences 20,000 bp long. The length of sequence needed for resolution was shorter when substitution rates were doubled.

Support, measured as the number of nodes attaining a certain bootstrap value, increased with the length of sequence analysed. For *ndhF*-type data, 31 out of a maximum 34 nodes achieved at least 50% support when 10,000 bp were analysed, regardless of substitution rate (Fig. 5.8). The number of nodes achieving 90% bootstrap support was fewer, and was more responsive to increasing sequence length at a lower nucleotide substitution rate. *RbcL*-type datasets gave similar results, except that higher support values were seen when the nucleotide substitution rate was doubled. No further increase in support was found with sequence lengths longer than 10,000 bp.

Support values for individual nodes were also compared. With *ndhF*-type sequences, Node 1 achieved 100% bootstrap support with 1,000 bp of sequence. Nodes 3 and 4 achieved at least 70% support when 5,000 bp were analysed and 90% with 10,000 bp. Nodes 5 and 6 achieved at least 80% support with 10,000 bp and about 95% with

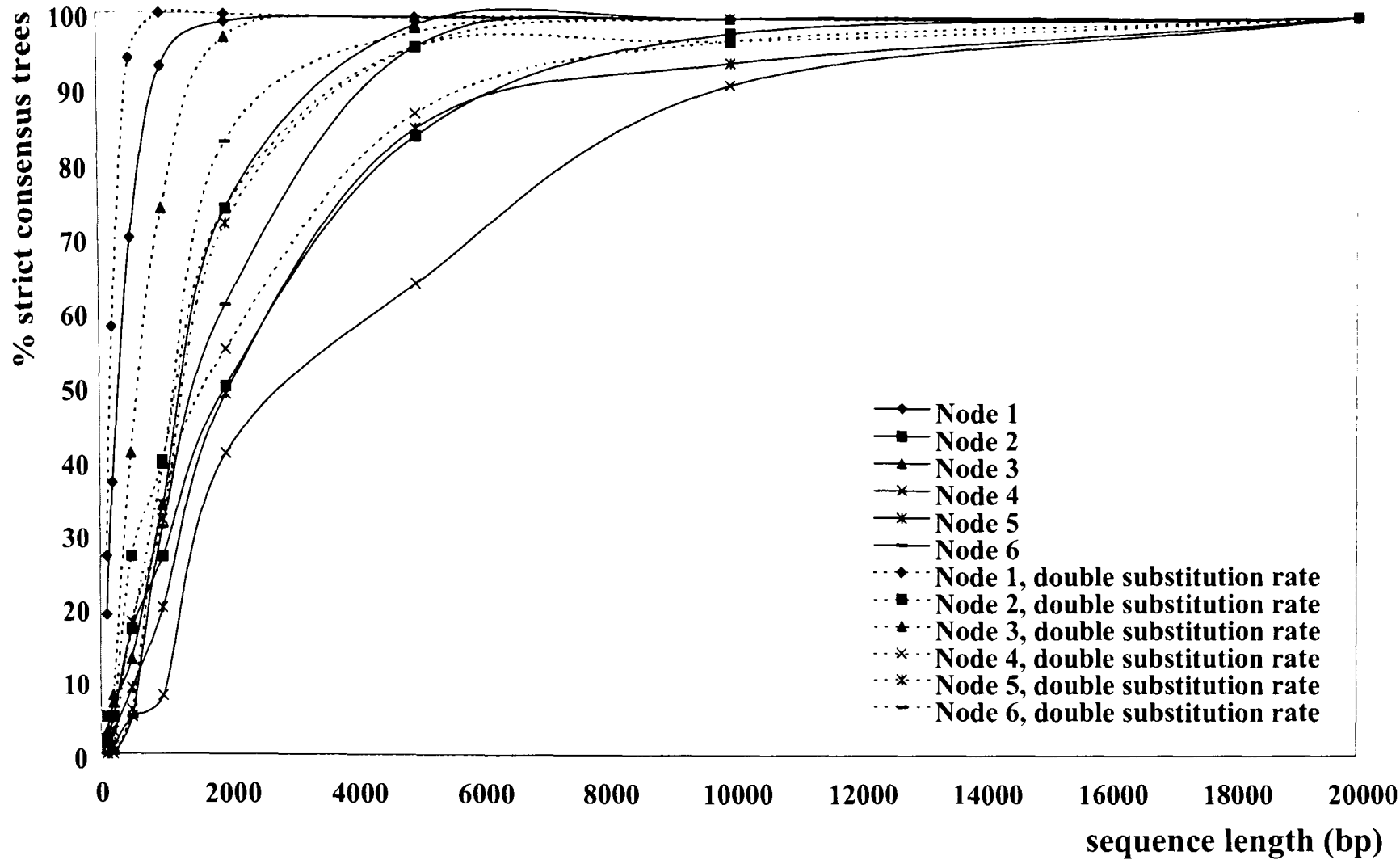


Figure 5.7 Graph to show relationship between accuracy, measured as the percentage of strict consensus trees containing Nodes 1-6, and length of aligned sequence analysed, for *rbcL*-type sequences simulated at normal, and twice-normal, substitution rates.

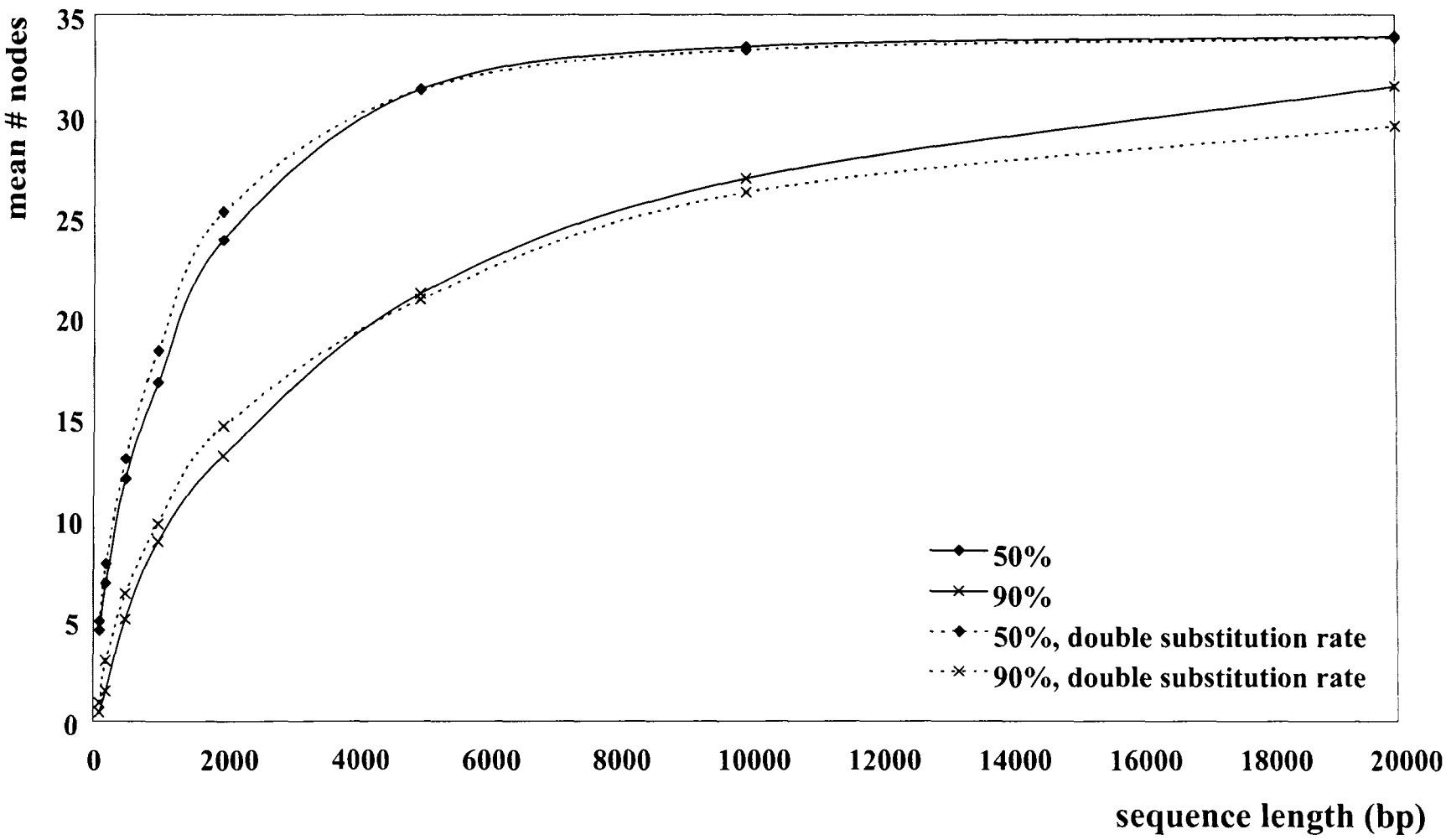


Figure 5.8 Graph to show relationship between support, measured as the number of nodes attaining a bootstrap value of at least 50% or 90%, and length of aligned sequence analysed, for *ndhF*-type sequences simulated at normal, and twice-normal, substitution rates.

20,000 bp. Node 2 was the poorest supported, achieving less than 90% using 20,000 bp, even when sequences with a doubled nucleotide substitution rate were used. For this node the relationship between sequence length and support reached an asymptote: 90% was the maximum support achieved. *RbcL*-type sequences provided at least 95% bootstrap support for all six nodes with 20,000 bp of data (Fig. 5.9). All nodes except 4 and 5 achieved at least 70% support with 5,000 bp of sequence. Node 1 required longer sequences than under the *ndhF* model to achieve the same level of support: 5,000 bp for 100%.

5.4.2 Effect of substitution rate. The effect of substitution rate was tested for two lengths of sequence, 1,000 and 2,000 bp, which are approximately the length of real single gene partitions. Resolution increased with substitution rate, but the relationship soon became asymptotic (Fig. 5.10). When 2,000 bp were used, a maximum CFI of 0.95 was achieved at substitution rates of approximately double those in real *rbcL* and *ndhF* sequences. When 1,000 bp were used, maximum resolution was achieved at approximately five times the real substitution rate of these genes.

Both measures of accuracy displayed similar relationships to substitution rate: a rapid increase followed by a gradual decline. For *ndhF*-type sequences, Node 1 was resolved accurately in 100% of strict consensus trees with substitution rates of at least 0.2 times the real rate (with 2,000 bp, or 0.5 times with 1,000 bp; Fig. 5.11). For all other nodes, accurate reconstruction was not achieved in more than 80% of trees. Maximum accuracy occurred at between once and twice the original substitution rate (with 2,000 bp, or twice and five times with 1,000 bp). For *rbcL*-type sequences, highest accuracy occurred at between twice and five times the original substitution

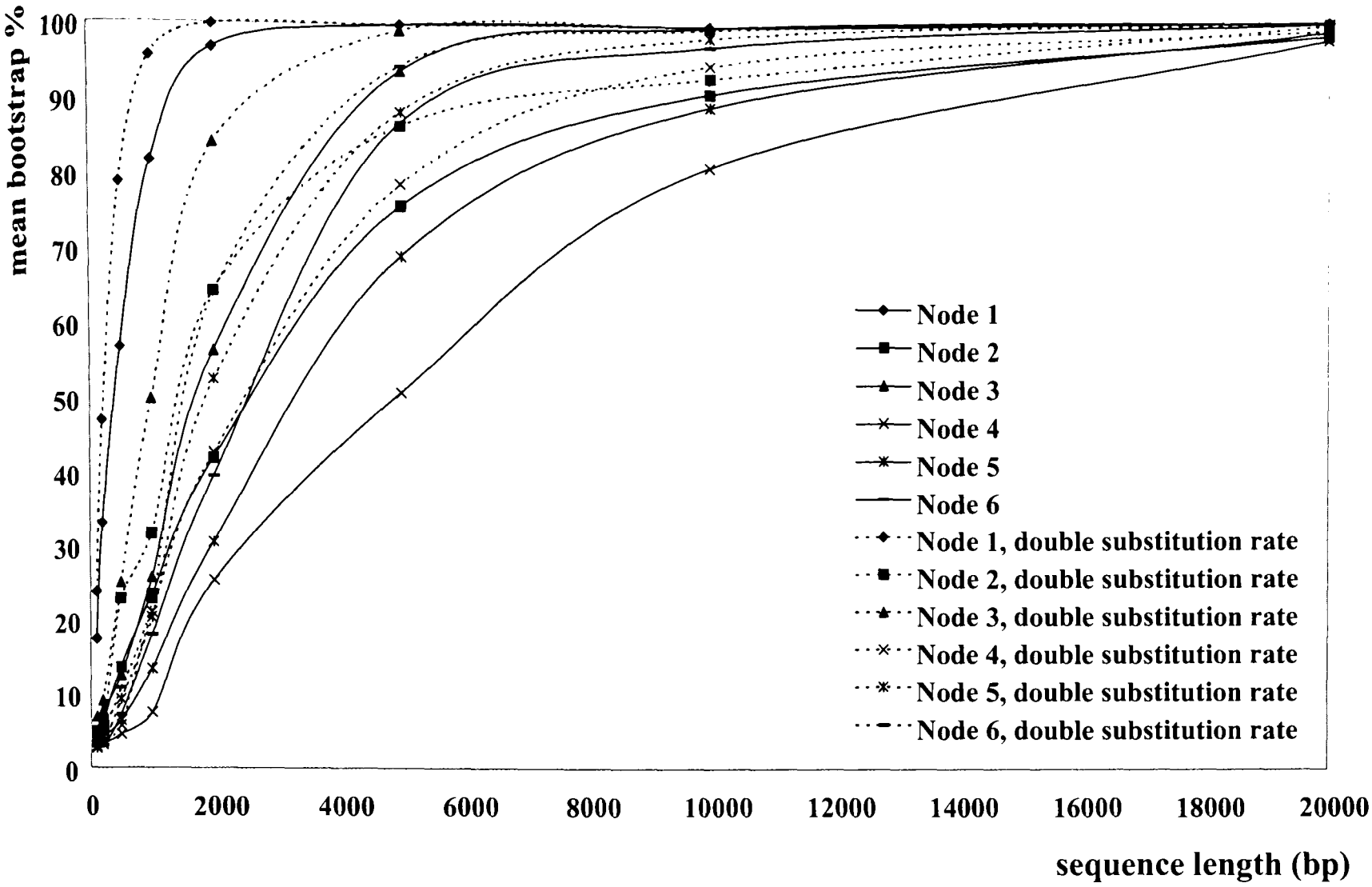


Figure 5.9 Graph to show relationship between support, measured as the mean bootstrap support value attained by Nodes 1-6, and length of aligned sequence analysed, for *rbcL*-type sequences simulated at normal, and twice-normal, substitution rates.

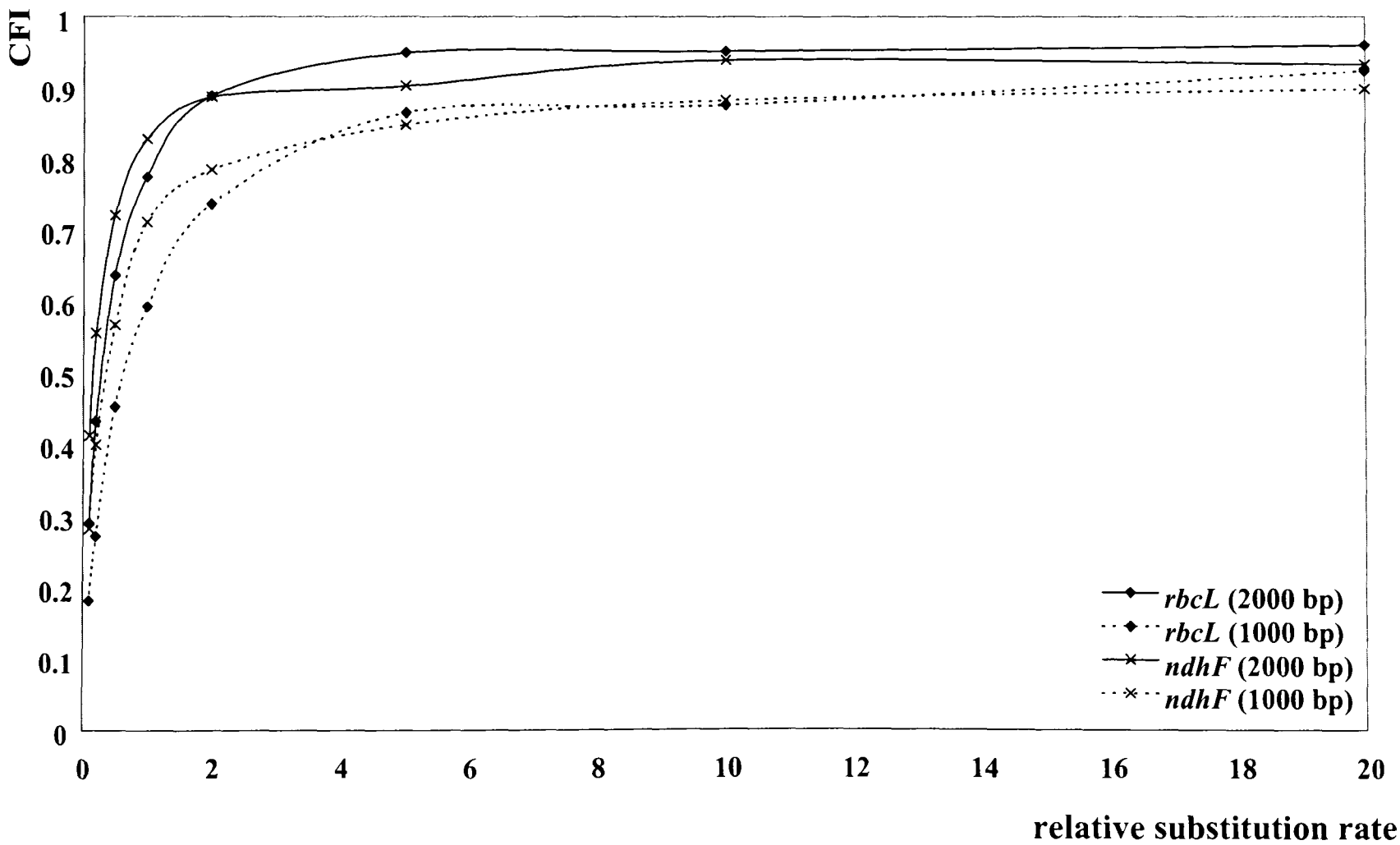


Figure 5.10 Graph to show relationship between resolution, measured by the CFI, and relative nucleotide substitution rate in simulation, for *ndhF*- and *rbcL*-type sequences of 1,000 and 2,000 aligned bp.

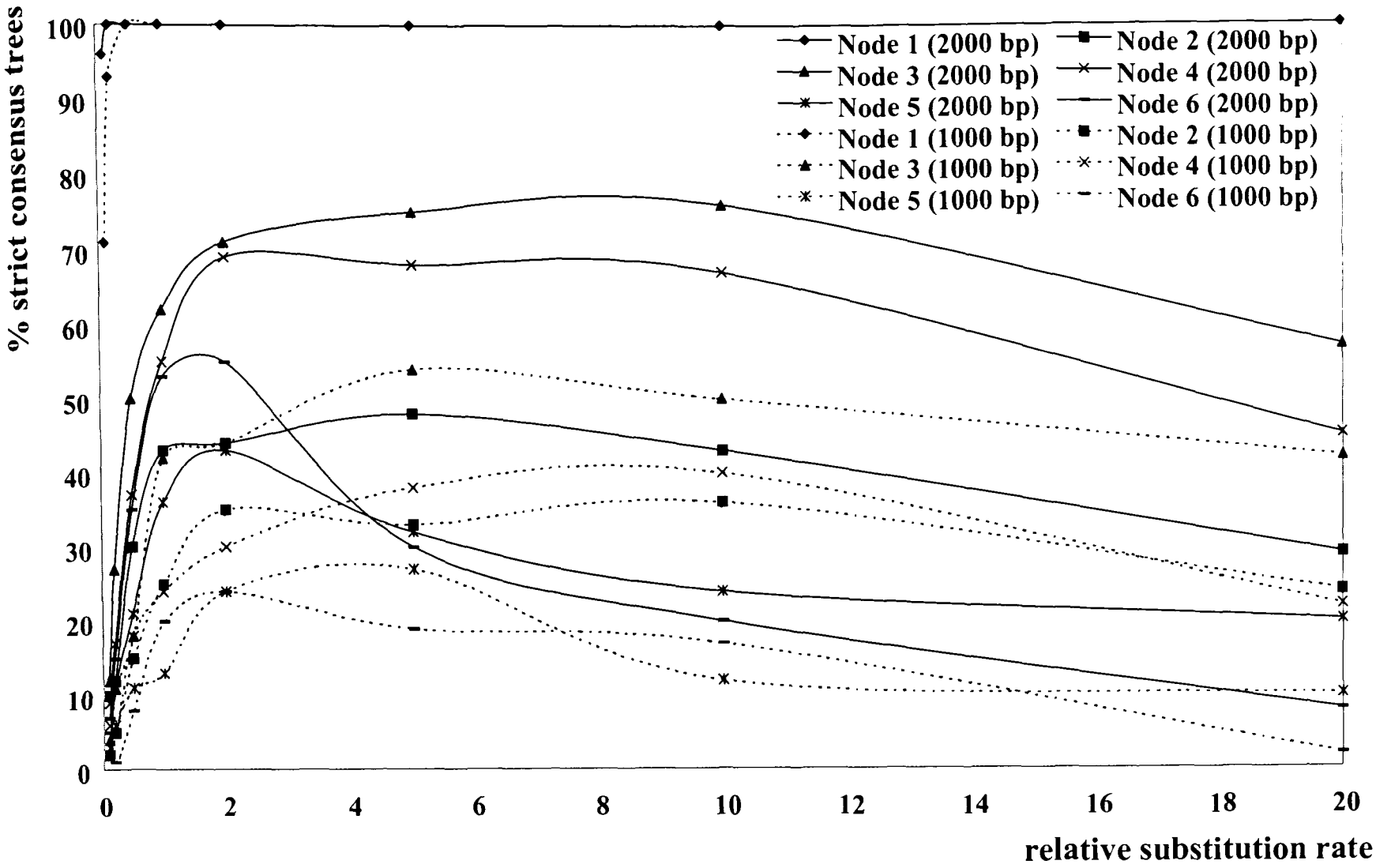


Figure 5.11 Graph to show relationship between accuracy, measured as the percentage of strict consensus trees containing Nodes 1-6, and relative nucleotide substitution rate in simulation, for *ndhF*-type sequences of 1,000 and 2,000 aligned bp.

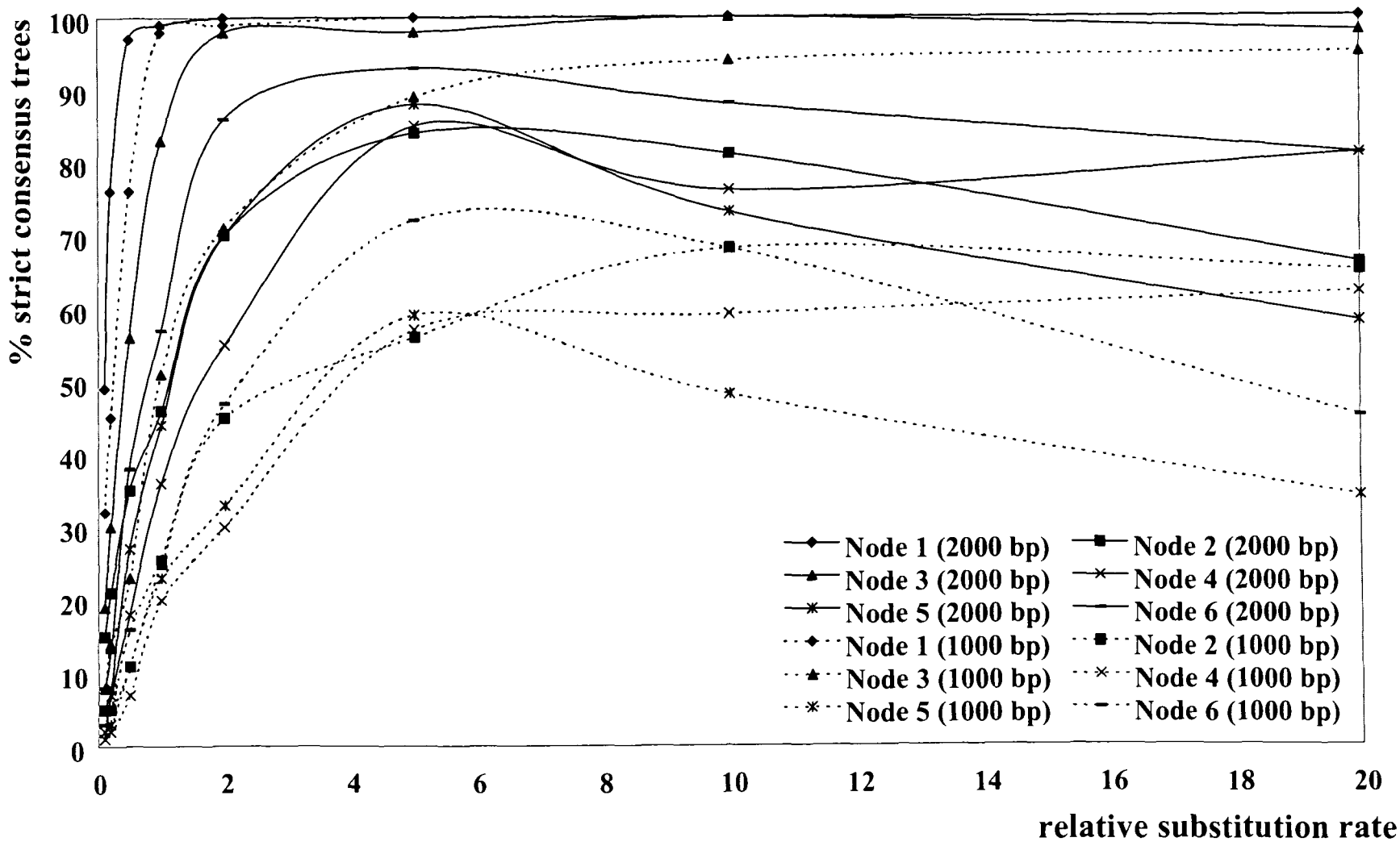


Figure 5.12 Graph to show relationship between accuracy, measured as the percentage of strict consensus trees containing Nodes 1-6, and relative nucleotide substitution rate in simulation, for *rbcL*-type sequences of 1,000 and 2,000 aligned bp.

rate (with 2,000 bp) or five and 10 times (with 1,000 bp; Fig. 5.12).

Support values showed a rapid increase followed by a gradual decline as nucleotide substitution rate increased, for both lengths of sequence and both types of partition tested. For *ndhF*-type datasets, strongest support was achieved at a substitution rate about double that of real sequences (Fig. 5.13). For *rbcL*-type, it was found at about five times the real rate. With *ndhF*-type data only Node 1 received high bootstrap support, with a nucleotide substitution rate of at least the real rate. For all other nodes, support peaked at between twice and five times the real substitution rate and thereafter declined. For *rbcL*-type sequences a similar relationship was observed. Support values with the *rbcL*-type data were generally higher than with *ndhF*, and peak support occurred at a faster substitution rate (twice normal for Node 1, 10 times normal for other nodes; Fig. 5.14).

5.4.3 Effect of combining gene partitions. The preceding results show the effect of substitution rate and sequence length on phylogenetic inference for either *rbcL*-type or *ndhF*-type datasets. Figure 5.15 shows the resolution achieved against the length of sequence analysed, when the total length of sequence was provided by combinations of *rbcL* and *ndhF*-type sequences in varying proportions. Greatest resolution was achieved when the entire length of sequence was simulated according to an *ndhF*-type model. Combined *ndhF* and *rbcL*-type datasets, of the same total length, achieved lower resolution.

Accuracy was greater with a single partition than with most combined datasets of the same total sequence length. Contrary to resolution, the greatest accuracy was achieved

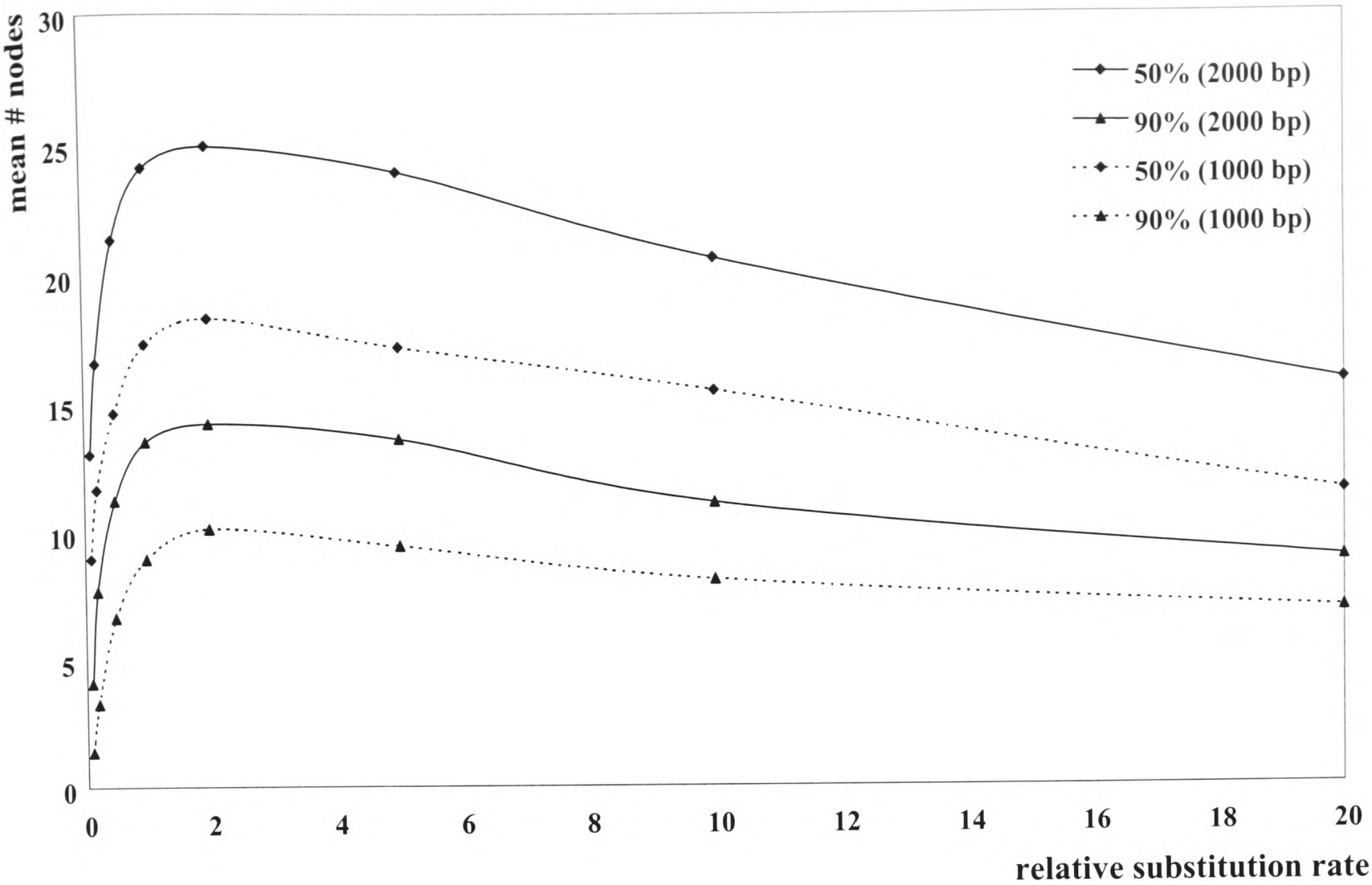


Figure 5.13 Graph to show relationship between support, measured as the number of nodes attaining a bootstrap value of at least 50% or 90%, and relative nucleotide substitution rate in simulation, for *ndhF*-type sequences of 1,000 and 2,000 aligned bp.

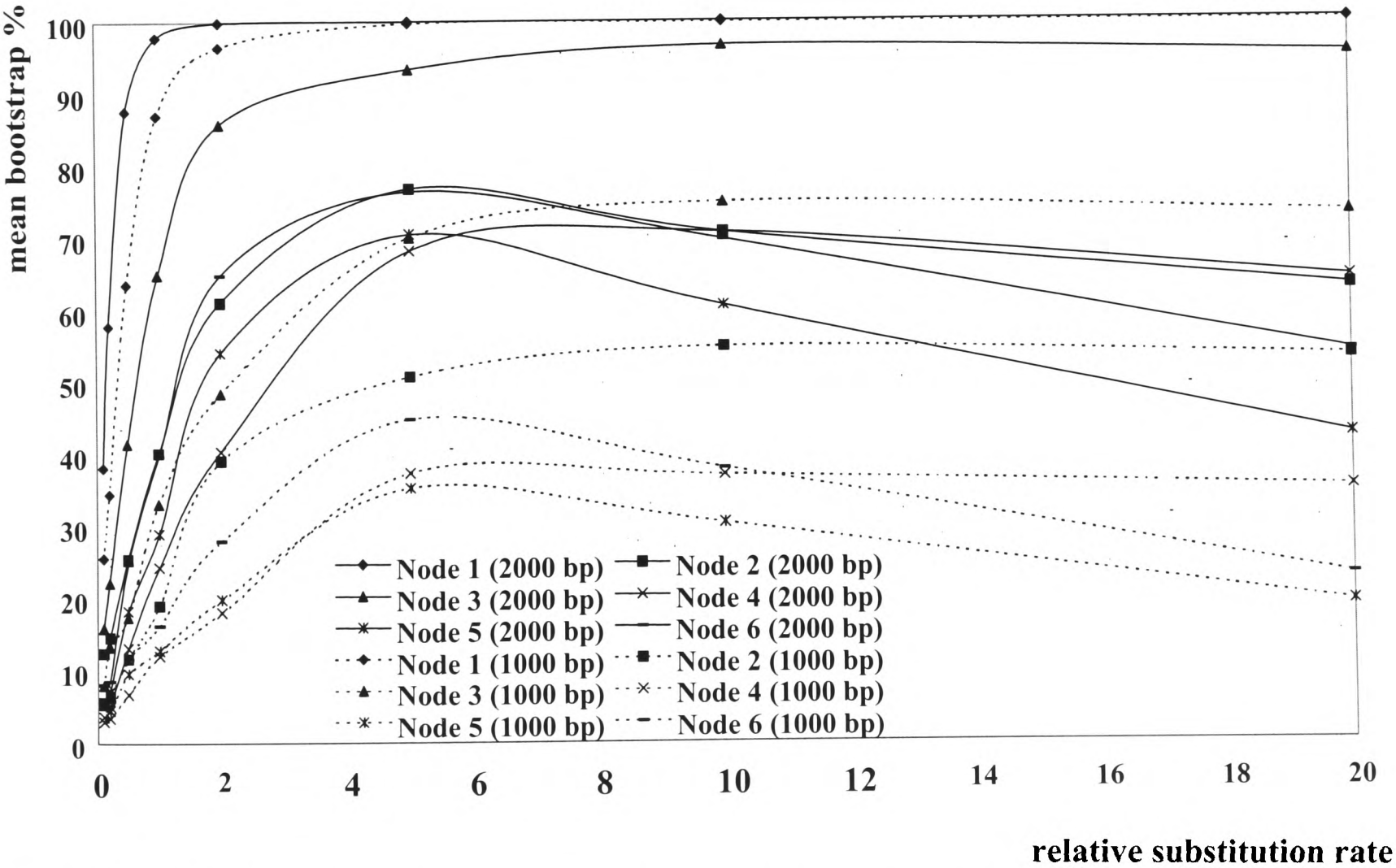


Figure 5.14 Graph to show relationship between support, measured as the mean bootstrap support value attained by Nodes 1-6, and relative nucleotide substitution rate in simulation, for *rbcL*-type sequences of 1,000 and 2,000 aligned bp.

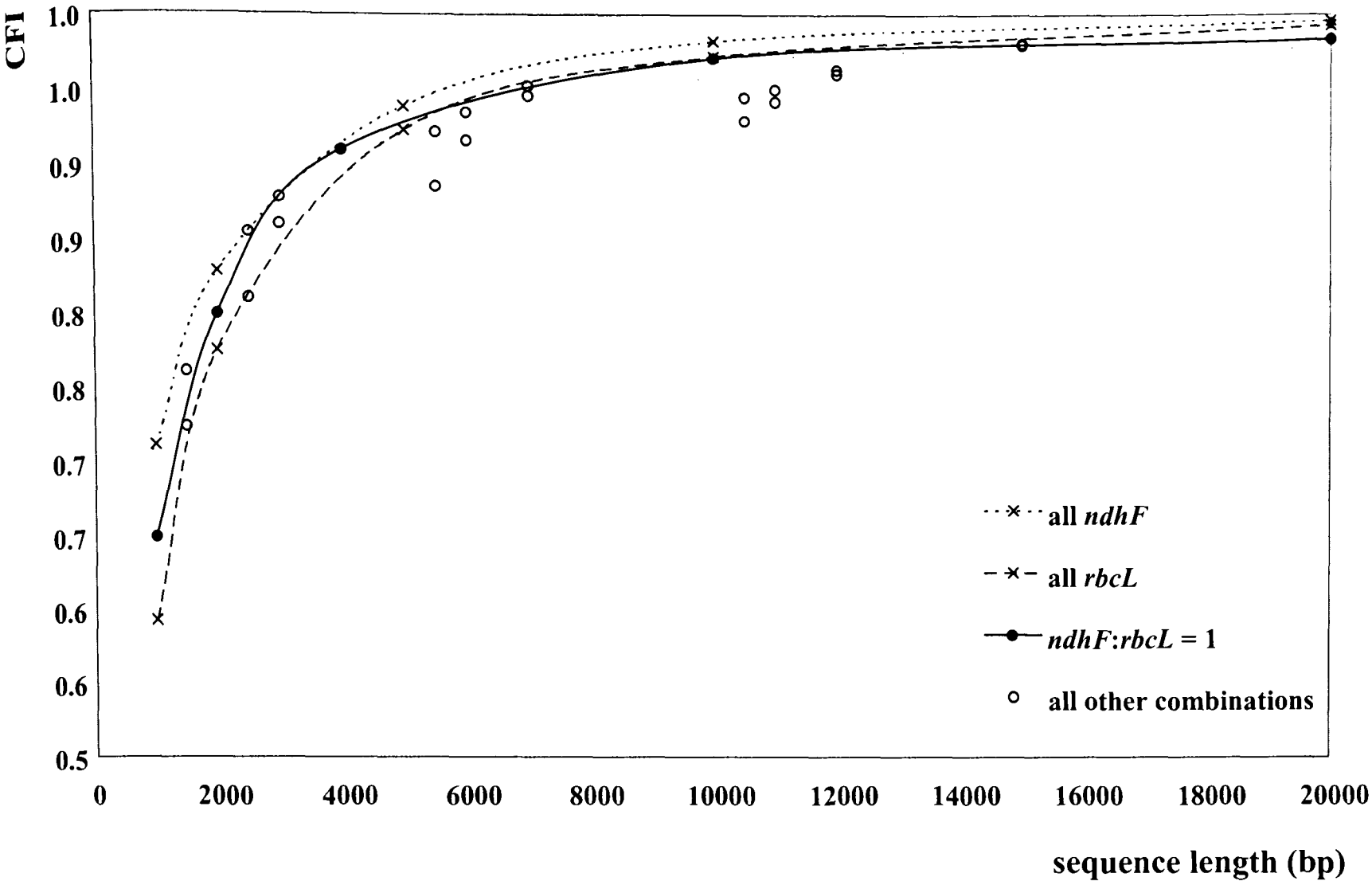


Figure 5.15 Graph to show relationship between resolution, measured by the CFI, and total length of aligned sequence analysed, for *ndhF*- and *rbcL*-type sequences and their combinations in various proportions.

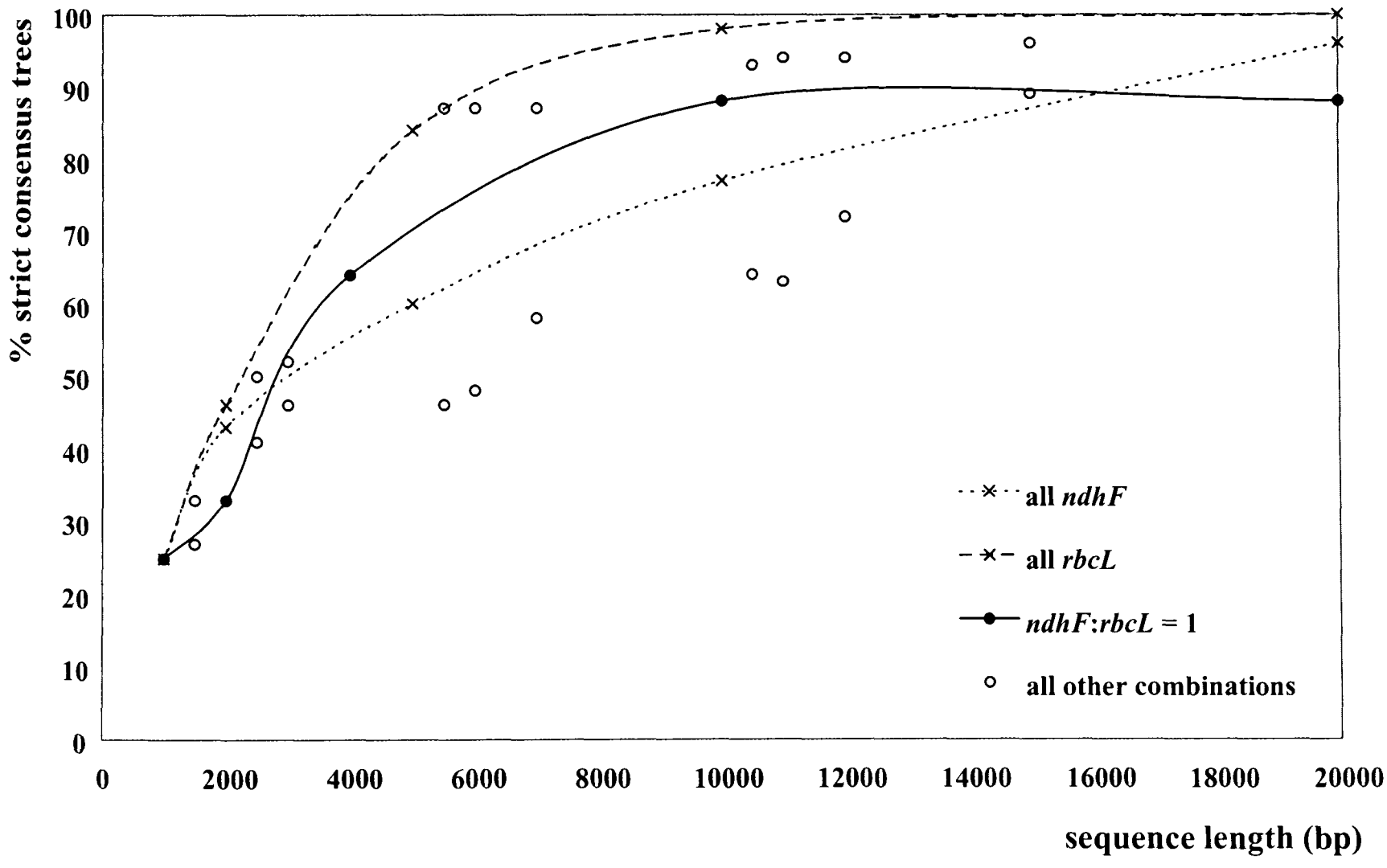


Figure 5.16 Graph to show relationship between accuracy, measured as the percentage of strict consensus trees containing Node 2, and total length of aligned sequence analysed, for *ndhF*- and *rbcL*-type sequences and their combinations in various proportions.

when the entire length of sequence was simulated according to an *rbcL*-type model. This relationship is shown for Node 2, the most difficult node to resolve, in Fig. 5.16.

Support, measured as the average number of nodes achieving a given bootstrap value, showed a slightly different pattern to accuracy and resolution (Fig. 5.17 shows the number of nodes with 95%). Combined datasets provided a better-supported phylogeny than single gene partitions of the same length, especially at total lengths below about 7,000 bp. Above this length, single-partition *rbcL*-type sequences provided the greatest number of supported nodes. At 50% bootstrap values (not shown), some combinations of partitions provided greater support than single partitions, but for a smaller range of total sequence lengths, between 2,500 and 4,000 bp.

5.4.4 Effect of adding matK. The resolution obtained in phylogenetic analysis of the *matK*-type simulated dataset alone was greater than that obtained with *rbcL* but less than that with *ndhF* (Fig. 5.18). Combining the *matK*-type dataset with *rbcL* and *ndhF* increased resolution over that of only *rbcL* and *ndhF* combined. This increase in resolution, from 0.89 to 0.93, was statistically significant ($p<0.0001$, two-sample t-test).

The accuracy of reconstructing Node 2, measured as the percentage of strict consensus trees in which the node was recovered, followed a similar trend (Fig. 5.19): there was a significant increase in accuracy when the three datasets were combined. However, when accuracy was measured as the percentage of MP trees in which the

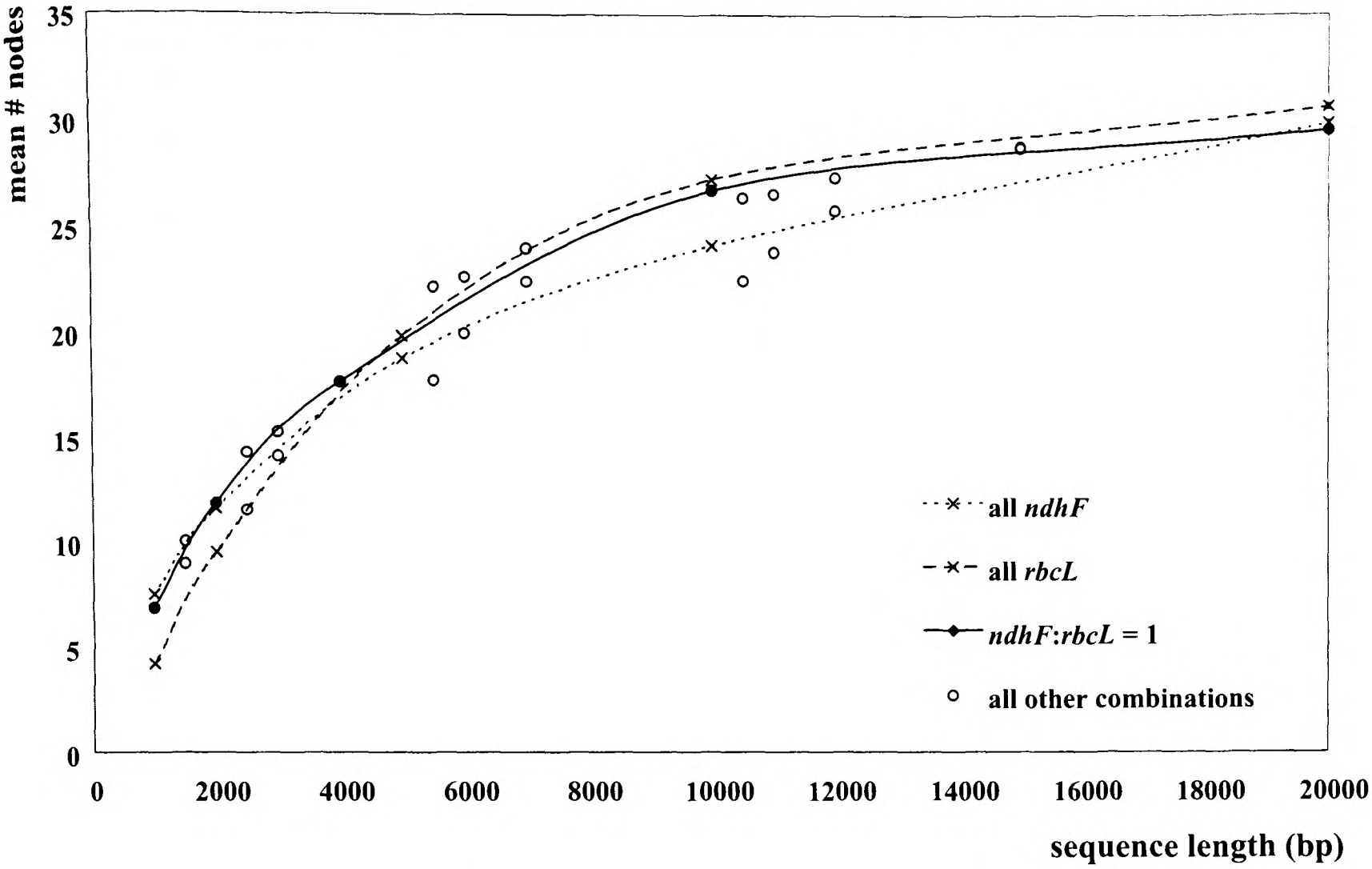


Figure 5.17 Graph to show relationship between support, measured as the number of nodes attaining a bootstrap value of at least 95%, and total length of aligned sequence analysed, for *ndhF*- and *rbcL*-type sequences and their combinations in various proportions.

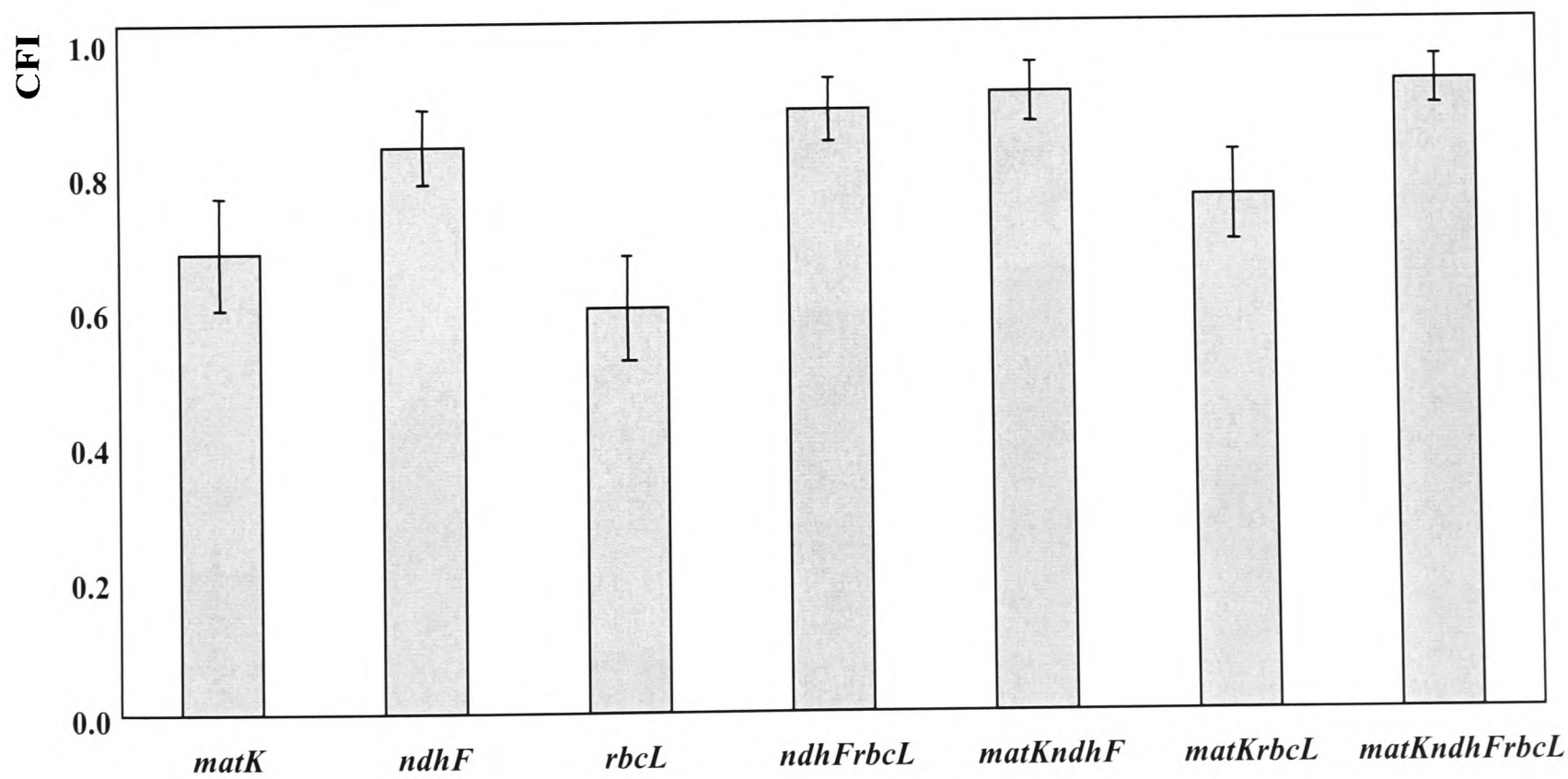


Figure 5.18 Chart to show resolution, measured by the CFI, obtained in analyses of simulated datasets based on *rbcL*-, *ndhF*- and *matK*-type models. Error bars show standard deviation of 100 replicate datasets.

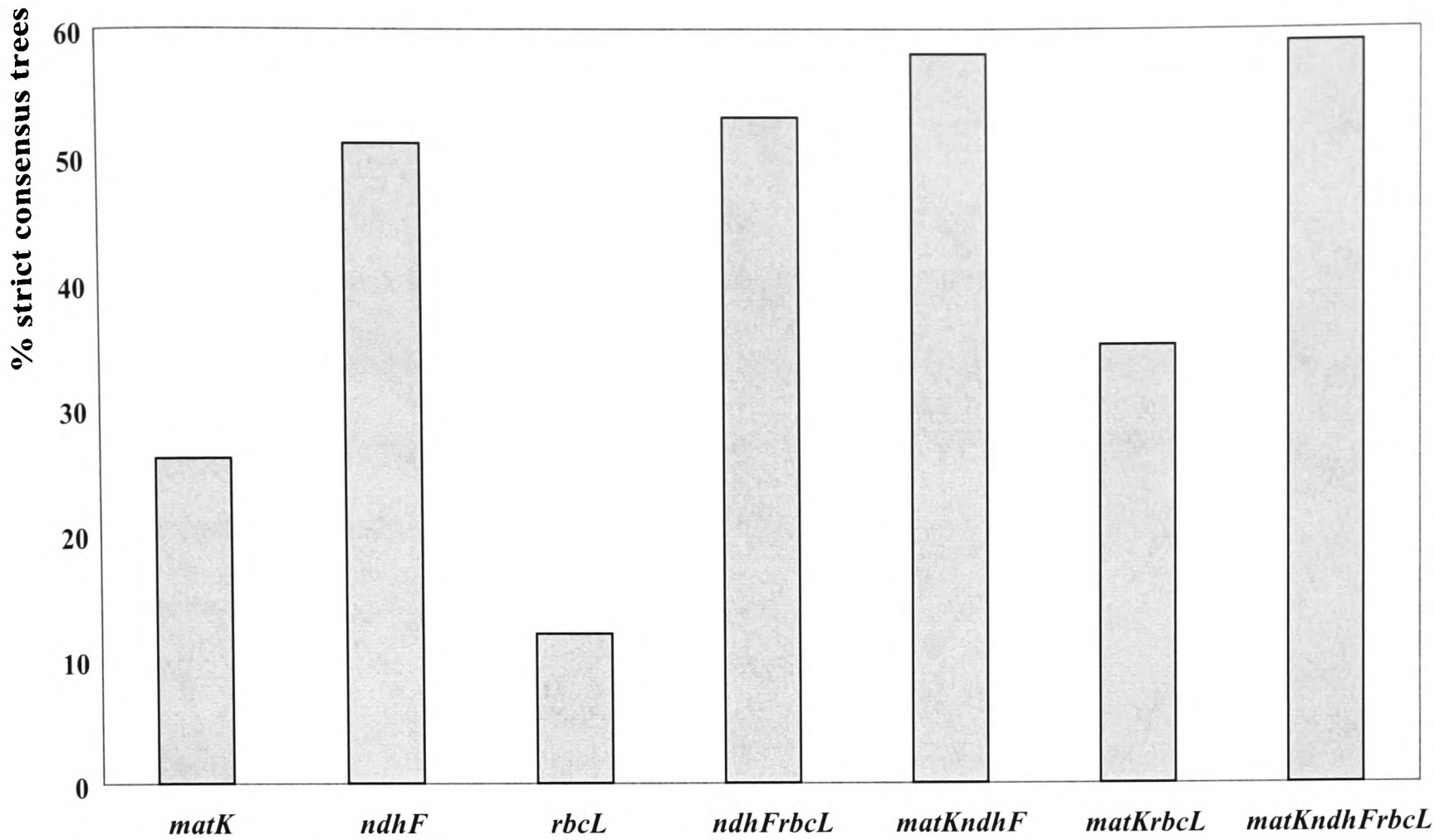


Figure 5.19 Chart to show accuracy, measured as the percentage of strict consensus trees containing Node 2, obtained in analyses of simulated datasets based on *rbcL*-, *ndhF*- and *matK*-type models.

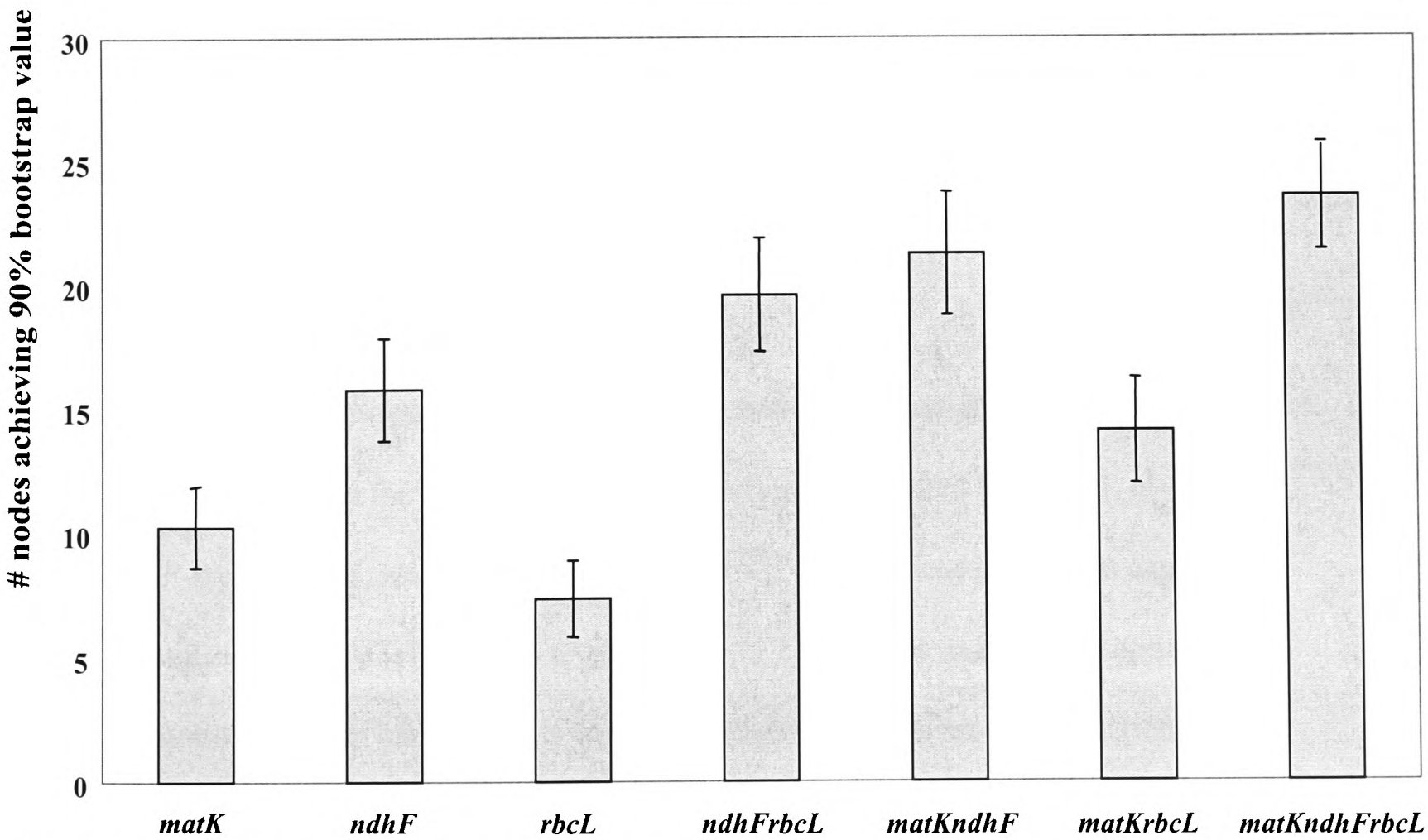


Figure 5.20 Chart to show support, measured as the number of nodes attaining a bootstrap value of at least 90%, obtained in analyses of simulated datasets based on *rbcL*-, *ndhF*- and *matK*-type models. Error bars show standard deviation of 100 replicate datasets.

node is found, the improvement in accuracy compared to only *rbcL-ndhF* was not significant (p=0.578).

Support levels across the phylogeny, at all bootstrap values tested, showed a similar pattern (Fig. 5.20 shows the number of nodes achieving 90% bootstrap support). The three-gene dataset provided a significantly greater number of supported nodes than all other combinations ($p<0.0001$). Support for Node 2, however, was not significantly greater with three datasets than with *rbcL* and *ndhF* alone (p=0.0908).

5.5 Discussion

Sequences simulated upon a known phylogeny can be used to test the potential of molecular data to resolve that phylogeny. In this study, the type and amount of sequence data that would be needed to improve resolution, support, and accuracy in the phylogeny of Lamiales were investigated. The implications for future molecular phylogenetic analysis in the order were explored by considering the effect of combining different datasets, in particular the addition of a third molecular partition to the existing two-gene dataset.

In all analyses there was a strong correlation between bootstrap support for a node and the likelihood that the node has been accurately reconstructed (Fig. 5.21; r=0.99, *ndhF*-type; r=0.98, *rbcL*-type). A similar correlation was found between support and resolution for the phylogeny as a whole (Fig. 5.22). In almost all cases, optimal resolution, support and accuracy were provided by a single set of simulation conditions. Likewise, poorest support, resolution and accuracy were all found under one set of parameters. These strong correlations between support, resolution and

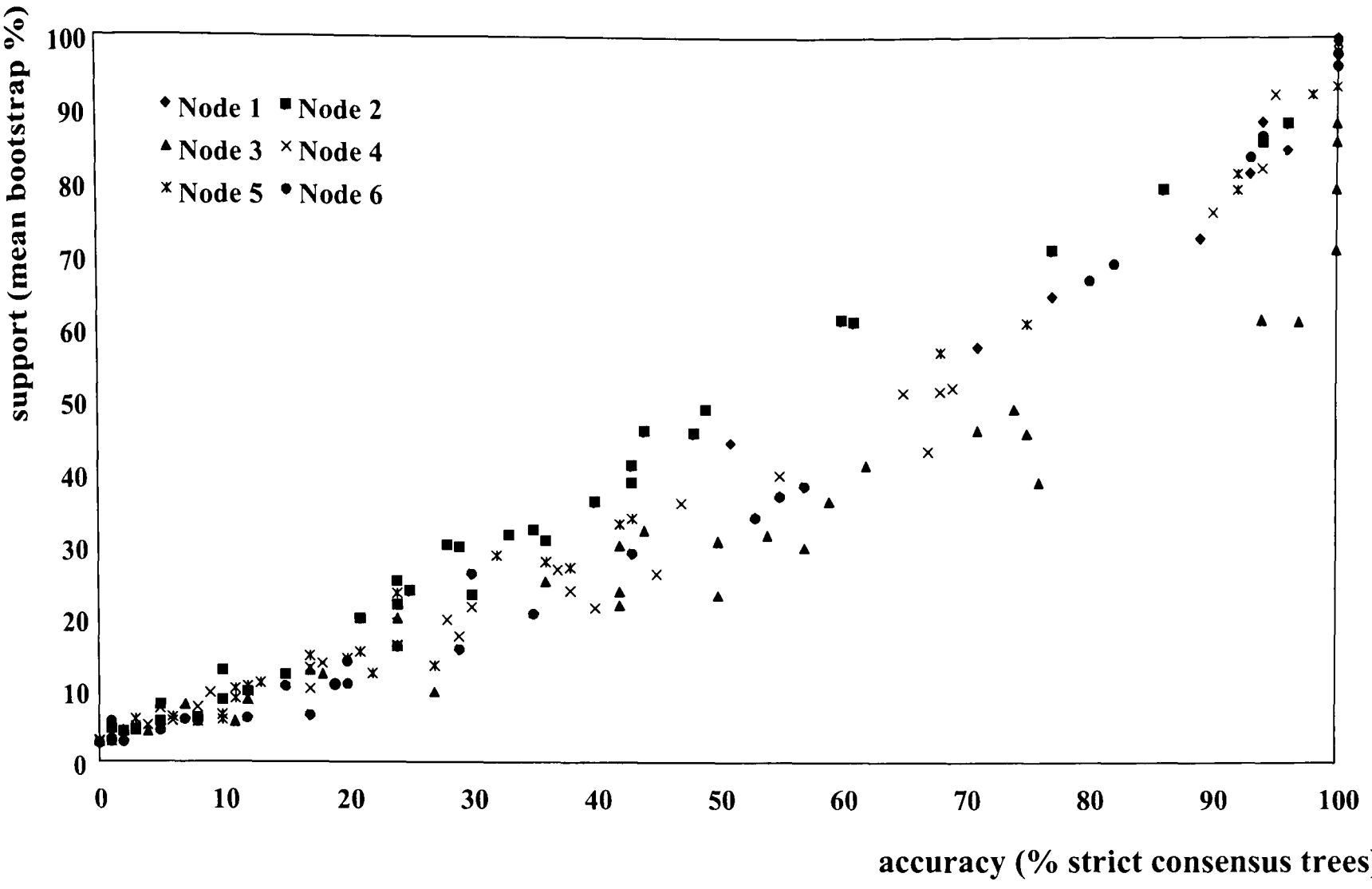


Figure 5.21 Graph to show relationship between support, measured as the mean bootstrap value attained by Nodes 1-6, and accuracy, measured as the percentage of strict consensus trees containing those nodes, for phylogenies reconstructed from simulated *ndhF*-type sequences of varying lengths, simulated at various substitution rates.

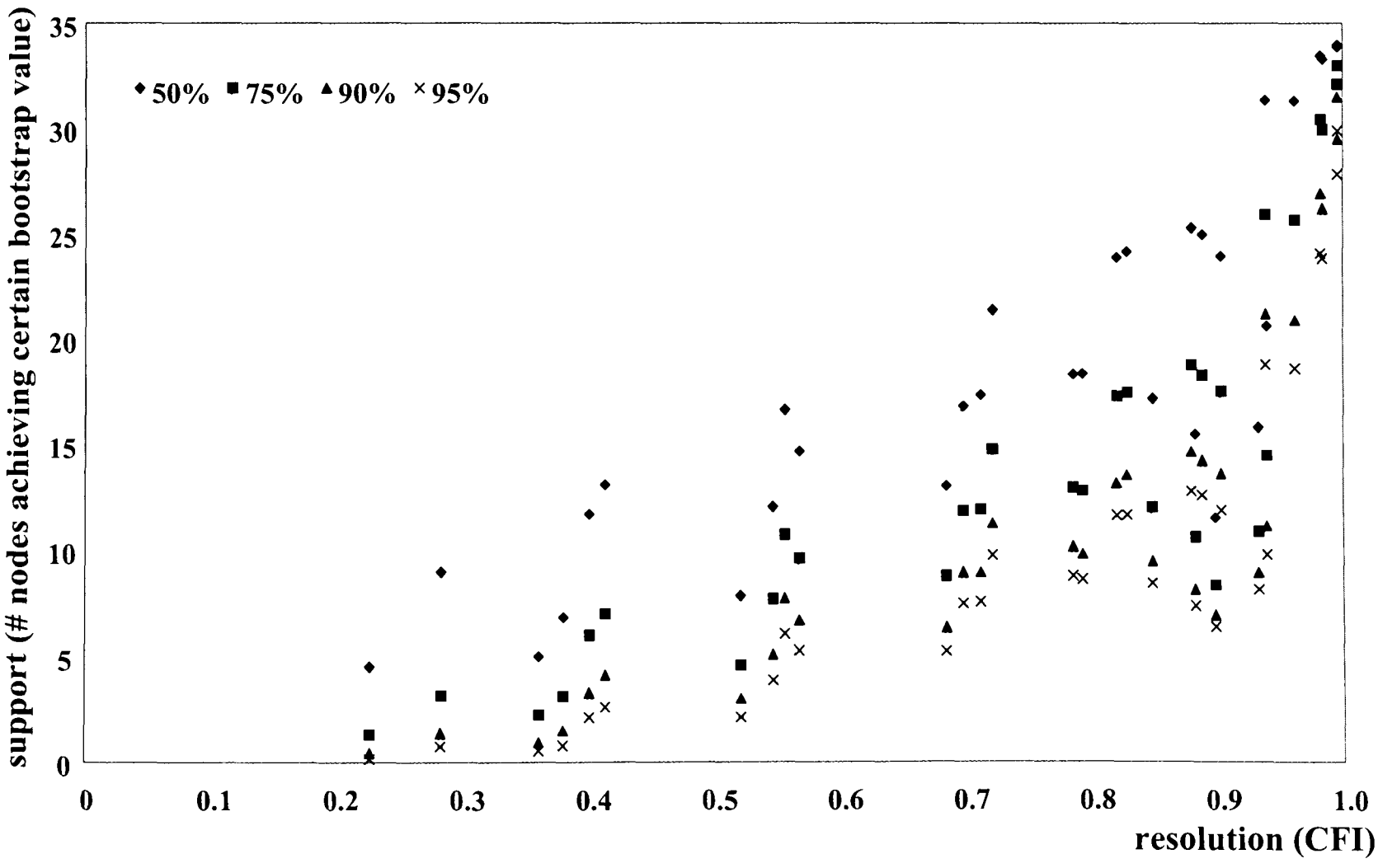


Figure 5.22 Graph to show relationship between support, measured as the number of nodes attaining a bootstrap value of at least 50%, 75%, 90% or 95%, and resolution, measured by the CFI, for phylogenies reconstructed from simulated *ndhF*-type sequences of varying lengths, simulated at various substitution rates.

accuracy imply that if a phylogeny is well-resolved and its nodes are well-supported, it is likely that the reconstruction is accurate.

5.5.1 Effect of sequence length and substitution rate. All measures of resolution, support and accuracy showed a rapid, then slowing improvement with increasing molecular sequence length (Figs. 5.5-5.9). The asymptotic effect of increasing sequence length on resolution is due to the fact that, as clades are resolved, a decreasing number remain unresolved, and therefore the total number of newly-resolved clades decreases for each further increment in character sampling. Moreover, once a certain level of sampling is reached, only very recalcitrant clades remain to be resolved. Previous empirical studies have indicated that support values asymptote in a similar way (De Queiroz et al., 2002).

Resolution also followed an asymptotic pattern with increasing nucleotide substitution rate (Fig. 5.10), while support and accuracy showed a rapid increase followed by a slow decline (Figs. 5.11-5.14).

5.5.2 Optimum conditions for phylogeny reconstruction. The most informative datasets (considered to be those providing greatest resolution, highest accuracy, and strongest support) were the four longest partitions simulated on an *rbcL*-type model. The best *ndhF*-type datasets were also the longest. Maximum support and resolution were achieved by maximising the absolute number of substitutions (total branch length) across the phylogeny, a product of the number of nucleotide sites and the nucleotide substitution rate. Greatest improvements in resolution, support and accuracy were achieved by increasing the number of sites rather than the nucleotide

substitution rate. This is shown in Fig. 5.23 for *ndhF*-type datasets: for the same total number of substitutions, all nodes were more likely to be accurately reconstructed when 2,000 bp were used than they were with 1,000 bp; i.e. doubling the sequence length had a greater effect than doubling the substitution rate. The same pattern was seen with *rbcL*-type datasets. For example, of two datasets that provided the same total number of substitutions, dataset 64 (20,000 bp at double the observed rate of substitution) provided maximum support and accuracy, but dataset 16 (2,000 bp simulated at 20 times the observed rate of substitution) gave much lower support and accuracy. The only exception to this rule was Node 1 which, with *rbcL*-type data, was reconstructed slightly more frequently at higher substitution rates and lower sequence lengths, but only for total numbers of up to about 2,000 substitutions.

One possible explanation for the superiority of longer, rather than faster-evolving, sequences for phylogenetic inference is that faster rates of nucleotide substitution may result in multiple substitutions (Moritz et al., 1987; Griffiths, 1997; Yang, 1998). The most useful datasets for supported, accurate phylogeny reconstruction in Lamiales were those containing a large number of slowly-evolving sites. In these datasets nucleotide substitutions are distributed across many sites, minimising the likelihood of multiple substitutions at the same site. Node 1 does not conform to the same pattern, which might be because it is the most recent node in the phylogeny and therefore the least likely to have experienced multiple substitutions at the same nucleotide sites.

RbcL and *ndhF*-type sequences differed in the hierarchical level at which they provided best resolution. Using 2,000 bp, for instance, *ndhF*-type sequences resolved

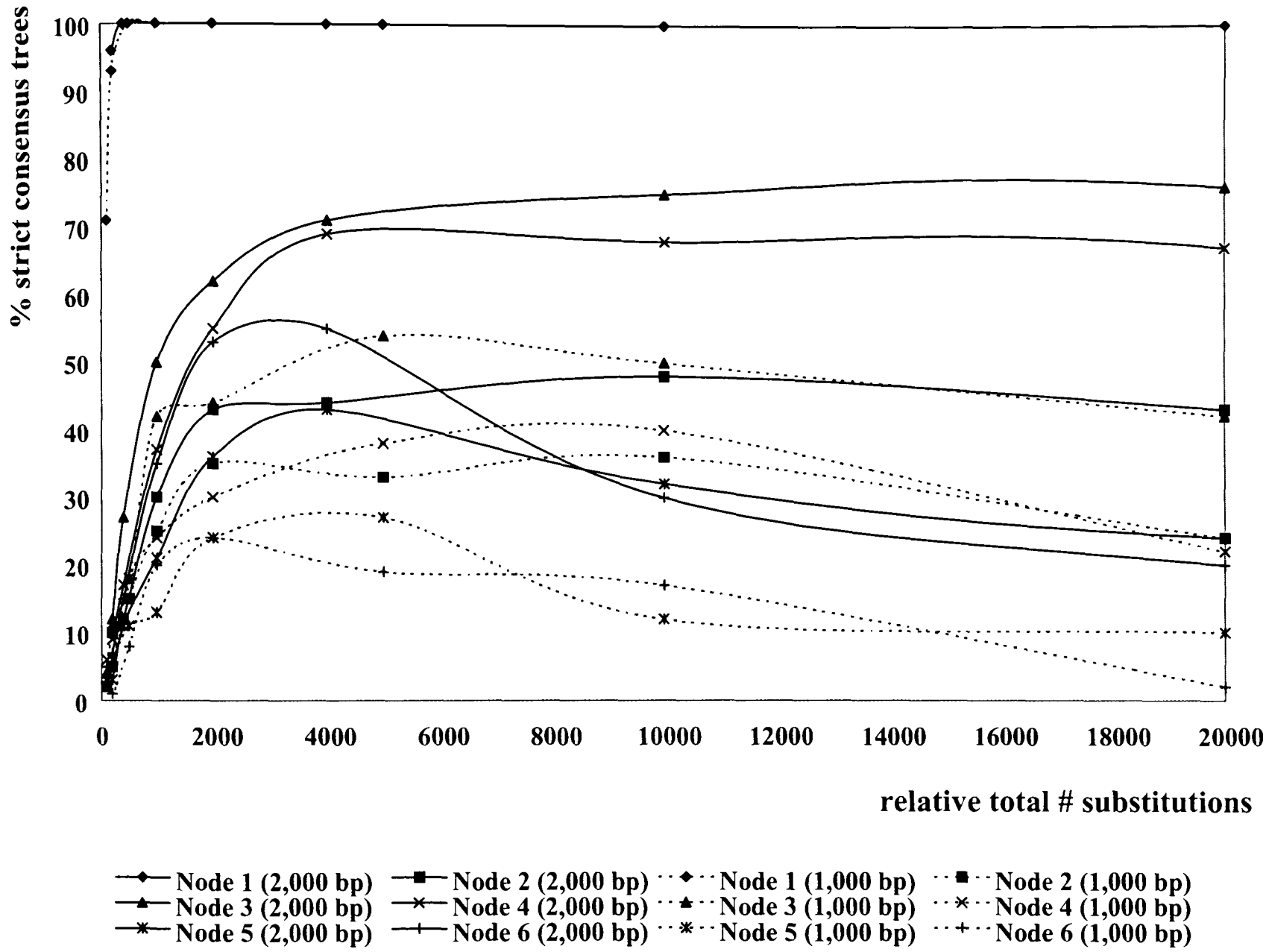


Figure 5.23 Graph to show relationship between accuracy, measured as the percentage of strict consensus trees containing Nodes 1-6, and relative total number of nucleotide substitutions in phylogenetic analysis, for simulated *ndhF*-type sequences of 1,000 and 2,000 aligned bp.

Nodes 1, 3 and 4 accurately in at least 70% of MP trees, while *rbcL*-type resolved to this accuracy Nodes 1, 2, 3 and 6. To resolve Node 1 accurately in 95% of strict consensus trees required only 500 bp of *ndhF*-type but 2,000 bp of *rbcL*-type sequence data. These comparisons are hampered by the fact that only Nodes 1 and 2 subtend identical clades in the two tree topologies investigated here, but the differences are still apparent when comparisons are restricted to these nodes: *ndhF*-type sequences were best for accurately resolving Node 1, *rbcL*-type for Node 2.

As discussed above, greatest improvements in phylogeny reconstruction were achieved by increasing the length of nucleotide sequence analysed. Exactly what length of sequence needs to be analysed to infer the phylogeny of Lamiales depends upon the levels of support, accuracy, and resolution demanded. These are considered in turn:

1. Resolution. No phylogenetic analysis can be expected to provide 100% accurate, supported resolution (but c.f. Rokas et al., 2003). However, for both *rbcL*- and *ndhF*-type datasets, good resolution (CFI>0.9) can be achieved with relatively small amounts of sequence data (c.5,000 bp; Fig. 5.5).
2. Accuracy. Employing conventional confidence limits, a method could be considered accurate if correctly-reconstructed clades are found in 95% of MP trees. Extrapolating from Fig. 5.6, for *ndhF*-type data, approximately 19,000 bp of sequence are required to accurately resolve Node 2 at normal substitution rates, while only 300 bp are needed for Node 1, and 5,000-10,000 bp for the remaining nodes. For *rbcL*-type data, Node 4 requires around 12,000 bp, other nodes between 1,000 and 10,000 bp.

3. Support. Taking an arbitrary level of acceptable bootstrap support (75%) and extrapolating from Fig. 5.9, approximately 8,600 bp of *rbcL*-type sequence are needed to support Node 4, with 800-6,000 bp for the other five nodes. For *ndhF*-type sequences, 12,000 bp are needed to support Node 2, and 200-7,500 bp for other nodes.

Currently, the nucleotide sequence data available for a wide range of Lamiales taxa are limited to *rbcL* (1,402 bp) and *ndhF* (2,176 bp as aligned in this study). Sequences of additional partitions such as *rps2* are available for a smaller range of families (e.g. Olmstead et al., 2001). Thus the total sequence data available for large-scale phylogenetic studies in Lamiales amount to less than 3,600 bp at present. To resolve most nodes with the support and accuracy described above requires about three times this amount, 10,000 bp. However, sequencing this much data is not inconceivable over the next few years, given the increasing ease of extracting and sequencing DNA (Doolittle, 1999; Rokas et al., 2003; Sanderson & Driskell, 2003).

One major caveat of this estimate is that it requires 10,000 bp of sequence that has experienced a similar pattern of evolution to *rbcL*. In practice, increasing the number of nucleotide sites available for analysis is achieved by combining discrete datasets, since existing partitions are of finite length. Fortunately, combining separate molecular datasets is thought to be beneficial compared to increasing the length of a single partition (Mitchell et al., 2000; Philippe et al., 2000). As shown above, two gene partitions may provide optimal resolution and support at different hierarchical levels (Pennington, 1996). This suggests that simultaneous analysis of combined sequence partitions might give greater improvements in phylogeny reconstruction

than would be expected simply from the number of additional nucleotides they provide. That is, combined molecular datasets might provide synergistic rather than additive improvements in resolution and support. The second part of the investigation was designed to determine whether combining molecular sequence data from different gene partitions does have a synergistic effect, or whether conflict between datasets negates this.

5.5.3 Effect of combining gene partitions. Combining two partitions simulated according to models generated from *rbcL* and *ndhF* sequence data reduced the effectiveness of phylogenetic analysis in Lamiales: there were no synergistic improvements in phylogeny reconstruction. All combined datasets gave lower resolution than single-partition analyses of the same length (Fig. 5.15). Most combined datasets reconstructed fewer accurate nodes than the same length single partitions (Fig. 5.16). In terms of support, the effect of data combination was equivocal: combined data may provide support for a greater or lesser number of nodes than a single partition, depending upon the total length of sequence analysed (Fig. 5.17).

It is sometimes assumed that simultaneous analysis will improve support and resolution by at least as much as the analysis of the individual datasets would suggest (Hillis, 1987; Reed & Sperling, 1999); i.e. "the whole will exceed the sum of the parts." In fact, combining molecular partitions is *less* beneficial than the sum of their individual contributions. Thus, increasing data by combining partitions may necessitate a much larger sequencing project than the 10,000 bp suggested above. This may be because combined partitions are bound to contain some contradictory

signals. Partition homogeneity tests (Chapter 4) did show significant incongruence between *ndhF* and *rbcL* sequence datasets in Lamiales ($p<0.01$). Although the potential usefulness of combining molecular datasets may therefore have been overestimated, simultaneous analysis is recommended as the only realistic way to increase the length of sequence data available, and to provide independent genetic corroboration of phylogenies.

5.5.4 Effect of adding matK. The third part of this investigation was designed to extend the results based on existing data (*ndhF* and *rbcL*) to predict the effect of sequencing one additional molecular marker. The simulations suggested that adding *matK* to the existing partitions would provide, at best, marginal improvements in resolution (Fig. 5.18) and no significant improvement in accuracy (Fig. 5.19). The biggest gains were seen in support values, which were increased for all nodes except Node 2. This is consistent with the fact that adding *matK* to the existing data produces a total dataset of around 4,612 bp, which is within the range of lengths found to increase support (Fig. 5.9), but shorter than would be needed to significantly improve accuracy (Fig. 5.6).

Comparing the characteristics of the simulated datasets with the real *rbcL* and *ndhF* partitions suggests they were a reasonable approximation to reality. Whether the simulated *matK* sequences, produced using a model and parameters derived from outside Lamiales, are so realistic is difficult to assess. With this caveat, the results confirm that expectations of synergistic interactions during combined analysis of three separate genes may be misplaced. If anything, conflict between partitions results in lower resolution and support than would be expected from their total sequence length.

Because the *matK* sequences were simulated upon a starting phylogeny whose topology was derived from the *ndhF* and *rbcL* datasets, conflict between datasets should be minimal in this case. Yet combining datasets was not as successful as expected. Thus, the prospects for resolving a supported phylogeny for Lamiales by adding new molecular datasets under real conditions may be limited.

On the other hand, *matK* was assumed to have evolved along a phylogeny with the same relative branch lengths as that of *rbcL* and *ndhF*, i.e. to contribute a similar number of character state changes on any given branch. This would increase support to those branches that are already resolved but not greatly improve resolution of those that are extremely short in the two-gene molecular morphology (Fig. 5.4). A real molecular partition might provide fewer improvements in support but greater improvements in resolution than seen here, depending upon whether it tends to conflict with the existing data or to follow a similar evolutionary pattern.

5.6 Conclusions

Simulated sequences provide a means to test potential approaches to phylogenetic questions *a priori*, in the absence of a large empirical dataset. Taking a model phylogeny for Lamiales, this method can be used to investigate the nucleotide sequence data needed to generate a required level of resolution and support. The results suggest that support and resolution tend to be correlated with accuracy. Thus if a reconstructed phylogeny is highly-resolved and well-supported, it is likely to be an accurate reconstruction of the underlying tree.

The answer to the question posed at the start of this chapter – is it possible to resolve an accurate, supported phylogeny of Lamiales using molecular sequence data? – is "yes," given sufficient molecular sequence data and minimal conflict between datasets. Greatest improvements in resolution and support were achieved by sampling more nucleotide sites (at least 10,000 bp), rather than by sampling sites with an increased substitution rate.

Ten thousand aligned bp corresponds to perhaps six genes, three times the size of the current two-gene dataset. Combining multiple molecular markers in a single analysis should be an effective way to improve resolution and support for all nodes, because different nodes were best resolved using different markers. In practice, however, combining sequence datasets is problematic: any conflict or incongruence between datasets further increases the length of sequence needed to resolve an accurate, supported phylogeny. This is in agreement with a recent study (Rokas et al., 2003) which suggested that at least twenty unlinked genes may need to be sequenced in order to overcome incongruence and infer a supported, accurate phylogeny.

It is potentially feasible to generate such quantities of sequence data for Lamiales within the next few years. However, this requires a sequencing project of an order of magnitude larger than can be attempted here. Sequencing more than one new molecular partition for a suitable sample of Lamiales taxa (since the position of *Thomandersia* cannot yet be pinned down to any smaller group within the order) is beyond the scope of a D.Phil. research project. The addition of sequences for one gene, *matK* was tested using an evolutionary model from taxa in which it had already been sequenced, and found to provide only marginal increases in resolution and

bootstrap support for relationships within Lamiales. Thus while Lamiales, like other problematic angiosperm orders such as Cornales, Apiales and Ericales (Plunkett et al., 1996; Anderberg et al., 2002; Xiang et al., 2002), should in the near future become resolvable, this is not an immediate prospect.

Chapter 6. Conclusions

6.1 Introduction

This thesis has explored three aspects of the systematics of *Thomandersia*: a taxonomic account, anatomical investigation, and exploration of potential ways to determine its phylogenetic relationships in Lamiales. The sister group of *Thomandersia* has not yet been conclusively determined, but the investigations presented here contribute to the future resolution of that question, by ascertaining the type and quantity of data that will be required to generate a robust phylogeny of Lamiales. This prospect should be achieved in the near future.

This chapter summarises the advances that have been made during my research project, and their implications for *Thomandersia*. The taxonomic status of *Thomandersia* is discussed, from species to family level. The present state of Lamiales phylogeny is outlined, and the current best estimate of the sister group to *Thomandersia* is considered.

Molecular data have been shown to be central to further resolution of phylogenetic relationships in Lamiales. The role of morphological data in phylogenetic inference remains more controversial (e.g. Hillis & Wiens, 2000; Wiens, 2000; Baker & Gatesy, 2002; Endress, 2003; Freudenstein et al., 2003; Scotland et al., 2003). Some possible applications of morphological data, apart from phylogenetic analysis, are discussed here for Lamiales. Finally, the broader implications of the thesis are considered, with reference to interfamilial relationships in other angiosperm orders and the systematics of other isolated genera.

6.2 Taxonomy of *Thomandersia*

6.2.1 Infrageneric taxonomy. Heine's (1966a) delimitation of *Thomandersia* into six species was upheld in Chapter 2, and an improved key, descriptions and distribution data were presented. Although the species display a wide range of phenotypic characters, each is easily distinguished from the others in a number of respects:

- *T. anachoreta* is confined to the coastal forests of southern Côte d'Ivoire and Liberia. It is unique in having two large, abaxial calycine glands where the other species have only one.
- *T. butayei* has smaller leaves than the other species; they are distinctively emarginate at the tip and have pronounced tuft domatia beneath. It has been collected in Gabon, the Democratic Republic of Congo and the Republic of Congo, but only south of the equator. It is the most light-demanding species, inhabiting shady savannah, scrubland or forest edges, usually on sandy soils or by water.
- *T. congolana* has downy, compacted inflorescences and a characteristically-striped corolla. Its leaves are strongly anisophyllous, with petioles much shorter than the other species. *T. congolana* is found in primary forest or forest remnants, shadier and wetter habitats than the other species. It occurs in two disjunct areas, one in the eastern Democratic Republic of Congo, the other in Cameroon, the western Democratic Republic of Congo, Gabon and the Republic of Congo.
- *T. hensii* is the most widespread and widely-used species. It has robust, erect, pyramidal inflorescences, a white corolla and very thick-walled capsules. It is found throughout western central Africa from south-eastern Nigeria to the

eastern Democratic Republic of Congo, in a variety of forest types, usually at the edges or in clearings.

- *T. laurentii* is distinguished by its downy ovary and pubescent, thin-walled capsules. It is restricted to wet gallery and riverine forests around Kinshasa and Brazzaville.
- *T. laurifolia* has long, slender, lax inflorescences and greenish flowers. The capsules are thin-walled and the seeds black. This is the only species where the surface of the seed has a spiral pattern of rounded warts, rather than triangular scales. It is found in the west of the central African region: Gabon, Cameroon, Equatorial Guinea and the western Democratic Republic of Congo and Republic of Congo, in disturbed areas of forest such as by roads, coasts, and on slopes.

6.2.2 Generic and familial status. The six species of *Thomandersia* form a coherent genus. Morphological and anatomical characteristics unite them as a monophyletic group which can be distinguished from other Lamiales by its large calycine nectaries and retinacula, and from Acanthaceae (with which it shares retinacula) by its minute bracts and bracteoles and absence of articulated stems, cystoliths, acicular crystals, and explosively-dehiscent capsules.

In molecular and total evidence analyses *Thomandersia* lies outside all currently-circumscribed families of Lamiales, most of which are well-supported, monophyletic groups. Lamiales is characterised by a tree model with well-supported family-level clades separated by short, unstable branches. *Thomandersia*, which is also well-supported but of uncertain position, thus seems to behave as a family-level taxon in Lamiales. There is no strongly-supported, consistently resolved sister family to

Thomandersia, in which it could be classified. The phylogenetic and phenotypic distinctiveness of *Thomandersia* raises the question of whether it should be elevated to familial rank.

If classifications are to be based on monophyly (Hennig, 1966; Backlund & Bremer, 1998; Carine & Scotland, 2002), *Thomandersia* should not be classified in Acanthaceae. However, it is against the spirit of a rank-based code such as the ICBN (IBC et al., 2000) for a taxon not to belong to a group at each formal taxonomic rank in the hierarchy. The ICBN does not categorically rule against genera *incertae sedis*, stating "[E]ach species is assignable to a genus, each genus to a family, etc." (IBC et al., 2000: Article 3). But although this assignation is not compulsory, it is implicitly recommended. Thus, since *Thomandersia* cannot confidently be placed within any existing angiosperm family, it should itself be ranked as a family.

Thomandersiaceae Sreem. was erected in 1976 (Sreemadhaven, 1976; 1977). It was distinguished from related families by a shrubby habit, simple leaves, pinnate-camptodromous venation, absence of cystoliths, non-articulated stems, poorly-developed bracts, capsular fruits with retinacula, and oblate, 5-6-colpate pollen. Resurrecting Thomandersiaceae from synonymy would highlight the exclusion of *Thomandersia* from Acanthaceae and emphasise its morphological and phylogenetic distinctiveness.

There are several recent precedents of families that have been resurrected from synonymy in order to maintain the monophyly of a well-supported family in which they were previously classified (e.g. Chase et al., 2000; APGII, 2003). Examples

include Barbeuiaceae and Gisekiaceae (excluded from Phytolaccaceae), Brunelliaceae (from Cunoniaceae), Curtisiaceae (Cornaceae), and Mackinlayaceae and Myodocarpaceae (Araliaceae) (Plunkett & Lowry, 2001; Cuenoud et al., 2002; APGII, 2003). Under these circumstances, the elevation of a genus to familial status is desirable when it has an isolated phylogenetic position, distinctive morphology, and large number of molecular or morphological autapomorphies (i.e. is subtended by a long branch); this reduces the probability that the genus will later be found to lie within another family (Bakker et al., 1998). Another recent example is Biebersteiniaceae (Bakker et al., 1998). Like *Thomandersia* this is a small (five species), well-defined, monophyletic group now considered to be in an isolated phylogenetic position (previously thought to be a member of Geraniaceae). *Biebersteinia* has a unique set of morphological autapomorphies and is also at the end of a long branch in molecular sequence-based phylogenies, so has been promoted to family rank (Bakker et al., 1998).

The branch subtending *Thomandersia* is long in all analyses, regardless of its exact position in the phylogeny (e.g. Figs. 1.2, 1.5, 6.1). Two species of *Thomandersia* were included in the analysis, so the length of its subtending branch is unlikely to be due to autapomorphies of individual species (this might be the case for Biebersteiniaceae where only a single species was sampled). The broad sampling of other families in Lamiales, spanning their root nodes (Olmstead et al., 2001; Prendini, 2001), allowed a realistic reconstruction of their subtending branch lengths for comparison with that of *Thomandersia.* In both maximum likelihood (Fig. 1.5) and total evidence maximum parsimony phylogenies (Fig. 6.1) the branch leading to *Thomandersia* was at least as long as those of most other families in Lamiales. This long branch represents a large

number of inferred nucleotide substitutions in the genes *rbcL* and *ndhF*, coincident with a high bootstrap support value (100%). Furthermore, it is as long as the branches subtending other Lamiales families in terms of inferred morphological character state changes (Fig. 6.2). Thus *Thomandersia* complies with the criteria for familial status – isolated phylogenetic position outside of any existing family, a distinctive combination of morphological characters, and a large number of molecular autapomorphies.

Monotypic groups such as Thomandersiaceae tend to be avoided by systematists because they contain no information about taxonomic relationships and are therefore considered superfluous in a predictive, diagnostic classification (Farris, 1980; Backlund & Bremer, 1998; Scotland & Sanderson, 2004). Nonetheless, monogeneric families are common, and placing *Thomandersia* in Thomandersiaceae does convey the significant information that it is isolated in position and distinctive in character. In general, monogeneric families are tolerated where there is no clear sister group or there are great morphological differences from the most closely related taxon (Bakker et al., 1998; APGII, 2003), both conditions that are true for Thomandersiaceae. Thus the combined implications of anatomical investigations and phylogenetic analysis support the resurrection of Thomandersiaceae from synonymy. The next section summarises what is now known about the relationships of Thomandersiaceae within Lamiales.

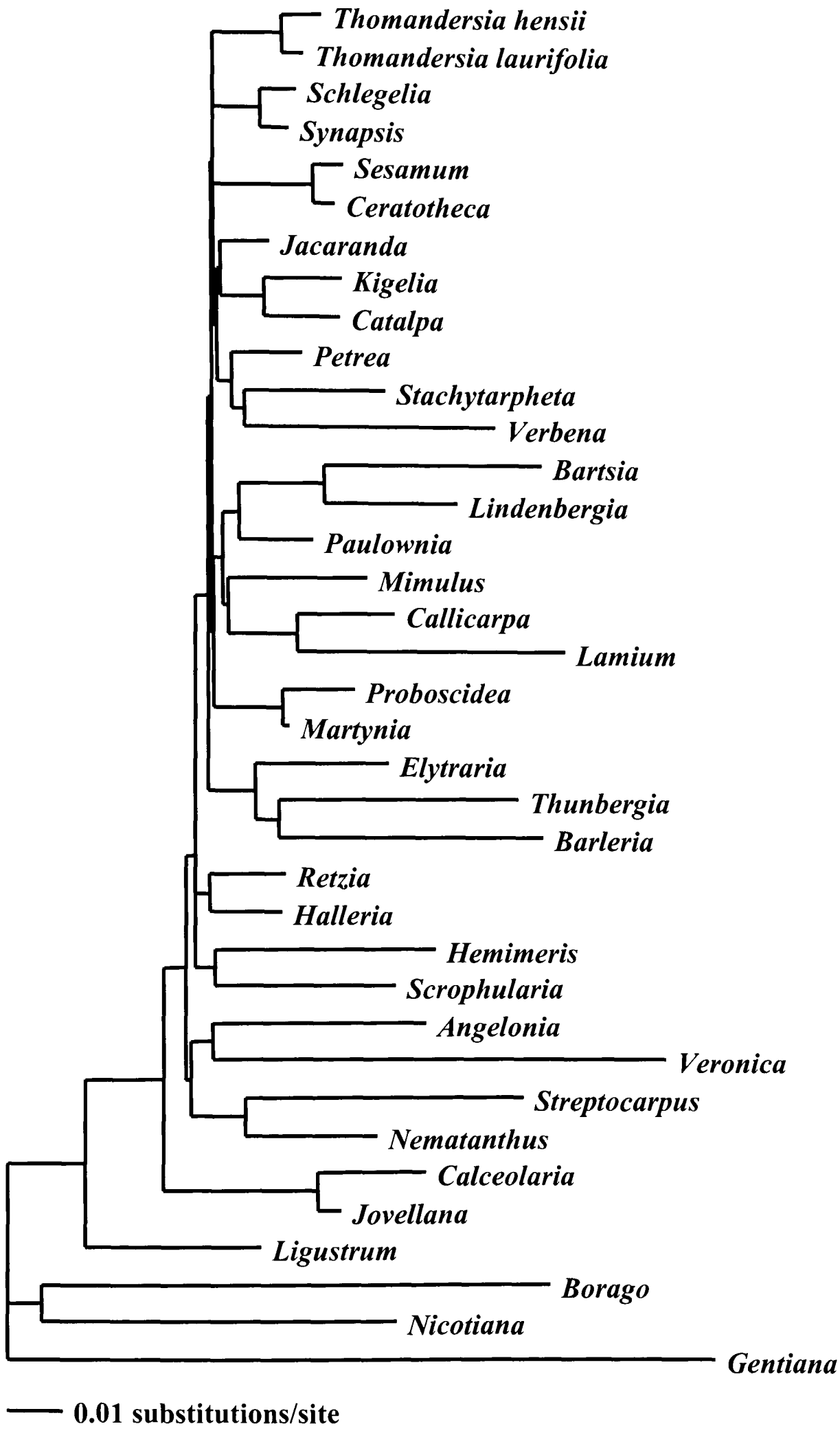


Figure 6.1 One of the three MP trees obtained from parsimony analysis of the combined molecular and morphological dataset for 37 taxa in Lamiales. Branch lengths are proportional to number of nucleotide and morphological changes implied upon them (ACCTRAN transformation).

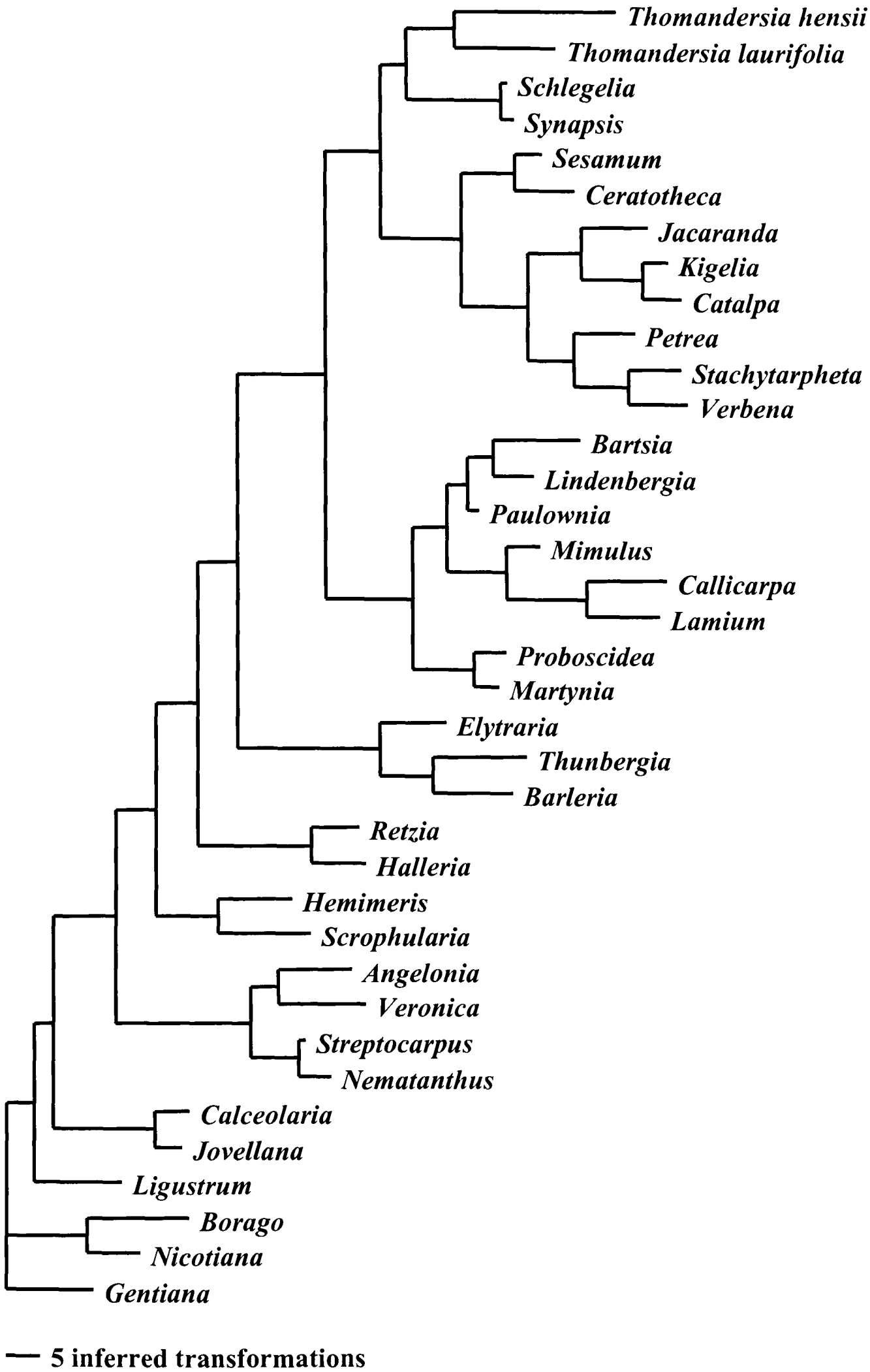


Figure 6.2 One of the three MP trees obtained from parsimony analysis of the combined molecular and morphological dataset for 37 taxa in Lamiales. Branch lengths are proportional to the number of morphological character state transformations implied by this topology (ACCTRAN transformation).

6.3 Phylogeny of Lamiales

The outcome of a phylogenetic analysis depends upon the data available, the taxa for which those data are sampled and the methods of analysis to which they are subjected. This thesis focused on the impact of sampling additional molecular or morphological characters in Lamiales, which exemplifies a tree model comprising strongly-supported family-level clades and short internal branches that are difficult to resolve with existing molecular datasets.

Simulation studies showed that a large number of molecular characters (at least 10,000 bp) should generate a well-resolved, supported and accurate phylogeny of Lamiales. However, adding a single molecular partition to the existing two-gene dataset increased support only marginally, and resolution not at all. Sequencing more than one new molecular marker across a suitable number of exemplar taxa in Lamiales was beyond the scope of this project. Therefore the best estimate for Lamiales phylogeny in this study was provided by total evidence analysis of the morphological and two-gene molecular dataset.

The partition homogeneity test indicated significant incongruence between the *rbcL, ndhF* and morphological datasets. However, this test is often considered over-sensitive, particularly when data is noisy (Dolphin et al., 2000; Yoder et al., 2001). The two molecular datasets were drawn from the same single-copy, non-recombinant plastid genome, and it is common practice to combine them in a single analysis (e.g. Olmstead & Reeves, 1995; Oxelman et al., 1999; Spangler & Olmstead, 1999; Graham & Olmstead, 2000; Soltis et al., 2000; Fishbein et al., 2001; Olmstead et al., 2001; Bremer et al., 2002; Cuenoud et al., 2002; Patterson & Givnish, 2002; Virtanen,

2003). The addition of a large dataset of morphological characters might also produce a "false positive" result in the test because homoplastic (noisy) characters will disproportionately increase the length of the MP trees found, despite contributing useful phylogenetic information to total evidence analysis (Dolphin et al., 2000). Combining all three datasets in a total evidence approach generated little improvement in support over the existing molecular datasets, but did find fewer alternative MP trees, and the strict consensus tree was marginally better-resolved (Table 6.1).

Table 6.1 Summary of predicted effects of adding a morphological or molecular (*matK*) dataset to the existing two-gene molecular dataset (from results of Chapters 4 and 5).

	# PI sites	CI	RI	Resolution (CFI)	# nodes with bootstrap support value greater than:		
					50%	75%	95%
rbcL-ndhF	749	0.43	0.35	0.76	22	13	10
***rbcL-ndhF*-morphology**	854	0.42	0.35	0.91	20	15	11
***rbcL-ndhF-matK* (mean of 100 simulations)**	1019	0.46	0.37	0.85	25	19	11

Parsimony analysis of the total evidence dataset generated three MP trees; a strict consensus was shown in Chapter 4 (Fig. 4.12). The likelihood of the MP trees can be compared by implementing a model and parameters selected using the hierarchical likelihood ratio test in MODELTEST (Posada & Crandall, 1998); the most likely MP tree is shown in Fig. 6.1.

The total evidence dataset was analysed using parsimony, because model-based methods are not generally considered to be applicable to morphological data (but c.f. Lewis, 2001). However, the topology was similar to that from maximum likelihood

analysis of molecular data presented in Chapter 1 (Fig. 1.5). The most significant differences between phylogenies were found between molecular-only and total evidence analyses, rather than between the different methods. Parsimony and likelihood-based methods, when both conducted on the same dataset, produced similar phylogenies; for example, the maximum likelihood and parsimony phylogenies reconstructed using molecular data for 41 taxa in Lamiales (Figs. 1.1, 1.2).

All phylogenies were poorly supported by bootstrap and decay analysis at interfamilial level. Low bootstrap and decay values could imply that the data contains a variety of phylogenetic signals (Renner & Chanderbali, 2000), or may be attributed to the presence of rogue taxa (Sanderson & Shaffer, 2002). Two well-established families, in particular, were poorly supported in total evidence analysis: Bignoniaceae (60% bootstrap support) and Verbenaceae (54%), both with a decay value of two steps. In both cases, a smaller clade within the family *was* well supported, and the low support might be due to the inclusion of one rogue taxon: *Jacaranda*, in the case of Bignoniaceae, or *Petrea* (Verbenaceae). Another rogue taxon, that takes up a variety of positions in Lamiales, is Pedaliaceae (represented by *Sesamum* and *Ceratotheca*). Parsimony analyses were conducted excluding some or all of these taxa, to investigate whether they might be responsible for some of the problems of resolution and support in Lamiales.

Analysis of the total evidence dataset excluding *Jacaranda* and *Petrea* generated two MP trees of 4,073 steps. The strict consensus tree was more resolved than the full Lamiales analysis, but support values were little changed. Removing Pedaliaceae also

decreased the number of MP trees found, resulting in a single MP tree of 4,068 steps, but bootstrap support values were again little changed. This tree was congruent with the most likely MP tree (Fig. 6.1), suggesting that the inclusion or exclusion of Pedaliaceae had no significant effect upon the topology of the inferred phylogeny. Removing all four possible rogue taxa generated four MP trees of 3,960 steps, again with little change in support values. One bootstrap support value that *was* significantly increased on exclusion of Pedaliaceae or *Jacaranda* and *Petrea* was for core Lamiales, which increased from 5% to 52% or 47% respectively, and 52% when all four taxa were excluded. These values are similar to that obtained with molecular data alone (51%; Fig. 1.4), suggesting that the decreases in support associated with including these taxa might be due to morphological characters. The results imply that the membership of these taxa in core Lamiales should be regarded with some caution, but that their inclusion does not have a detrimental effect on the phylogenetic relationships of other taxa within that clade. The relationships of more basal clades of the order remained stable in all analyses.

The total evidence analysis was broadly congruent with previous molecular-based results, including the resolution of a core Lamiales clade (Olmstead et al., 2001; Bremer et al., 2002). In total, seven new groupings were recovered in total evidence analysis (Table 6.2; Fig. 4.12), over and above the baseline molecular phylogeny from Chapter 1 (Fig. 1.4). One clade found in total evidence analysis – Veronicaceae and Gesneriaceae – was incongruent with the two-gene molecular tree. Although the phylogeny differed slightly from previous molecular results, the differences, such as in the positions of Martyniaceae, Verbenaceae and Veronicaceae compared to Olmstead et al. (2001), and of Lamiaceae, Martyniaceae, Pedaliaceae and

Veronicaceae compared to Bremer et al. (2002), involved groups on which past molecular analyses have also conflicted with one another, confirming that the addition of morphological data did not compromise the accuracy of the analysis.

Table 6.2 New clades resolved in total evidence parsimony and maximum likelihood analyses of Lamiales, showing variation in support values.

	Bootstrap support value (%)				Decay value	# unambiguous morphological synapomorphies
	Maximum likelihood	37-taxon maximum parsimony	Maximum parsimony excluding Pedaliaceae	Maximum parsimony excluding *Jacaranda* and *Petrea*		
Schlegeliaceae-*Thomandersia*	6	57	48	56	3	0
Lamiaceae-Martyniaceae-*Mimulus*-Orobanchaceae-*Paulownia*	<5	11	18	6	1	3
Bignoniaceae-Verbenaceae	<5	15	28	<5	1	2
Core Lamiales-Stilbaceae	<5	14	15	12	1	2
Core Lamiales-Scrophulariaceae-Stilbaceae	9	16	18	14	2	0
Gesneriaceae-Veronicaceae	<5	12	12	17	2	3
Borago-Nicotiana	<5	62	62	68	2	7

Clades that are resolved using a variety of methods and datasets, have high bootstrap support and decay values, do not break down easily in constraint analyses, and remain stable when taxon-sampling is altered, are considered to be reliable representations of evolutionary relationships according to the available evidence. However, such clades are few and far-between in Lamiales. Only three interfamilial clades were consistently resolved across all MP trees and maximum likelihood analyses. These were Lamiales

itself, all Lamiales except Oleaceae (represented by *Ligustrum*) and core Lamiales plus Scrophulariaceae and Stilbaceae.

As in previous studies (Olmstead et al., 2001; Bremer et al., 2002), there were few well-supported interfamilial branches in Lamiales. The families Acanthaceae, Calceolariaceae, Gesneriaceae, Lamiaceae, Martyniaceae, Orobanchaceae, Pedaliaceae, Schlegeliaceae and Thomandersiaceae were all well supported by bootstrap and decay values. The only other well-supported branches were those subtending Lamiales as a whole, and Lamiales excluding *Ligustrum* (Oleaceae), which was already known to be an early-diverging branch of the order (Bremer et al., 2001). Core Lamiales is consistently recovered but with little support. The position of some relatively basal groups such as Gesneriaceae and Veronicaceae remains unstable; others, such as Carlemanniaceae and Tetrachondraceae (Bremer et al., 2001; Stevens, 2001; APGII, 2003), have not been sampled here. The relationship of Orobanchaceae to *Paulownia* had a bootstrap support value of 75% but a decay value of only three steps. Three recently erected families (members of Scrophulariaceae *sensu lato*) were also only poorly supported. These were Scrophulariaceae *sensu stricto*, Stilbaceae, and Veronicaceae, all of which also received low support in previous molecular analyses (Olmstead et al., 2001).

The two problems that have troubled Lamiales phylogeny – poorly supported interfamilial relationships, and the positions of a number of isolated small clades – persist. Total evidence analyses and maximum likelihood analysis of molecular sequences both reproduced the type of tree model previously found in Lamiales, featuring short internal branches perhaps coincident with a rapid evolutionary

radiation of families (Olmstead et al., 2001; Bremer et al., 2002). If isolated genera such as *Thomandersia* are more appropriately ranked as families, the difficulty in determining their relationships is seen to be a second aspect of the same problem. Identifying candidate sister groups for rogue taxa may enable better-targeted sampling in future analyses of larger numbers of molecular markers to reconstruct more robust phylogenetic relationships. With this in mind, the potential sister groups of Thomandersiaceae are outlined below.

6.4 The sister group of Thomandersiaceae

As recent investigations have shown, certain longstanding concepts in Lamiales classification are now considered erroneous. For example, Scrophulariaceae *sensu lato* are polyphyletic, while Lamiaceae and Verbenaceae do not form a clade (Wagstaff & Olmstead, 1997; Olmstead et al., 2001). The traditional classification of *Thomandersia* in Acanthaceae (e.g. Bentham, 1876; Baillon, 1891; Lindau, 1897; Burkill & Clarke, 1899; Heine, 1963; Long, 1970; Hedren et al., 1995; Scotland et al., 1995; Scotland & Vollesen, 2000) is another concept that has been overturned. The analyses presented here clearly place *Thomandersia* outside a monophyletic Acanthaceae. Acanthaceae may be part of a more derived Lamialean clade including Lamiaceae, Orobanchaceae and *Paulownia* (Endress, 2001). Constraint analyses, which tested the cost to parsimony when *Thomandersia* was forced into various phylogenetic positions, rejected the possibility that *Thomandersia* is a member of, or sister group to, Acanthaceae.

The branch subtending Thomandersiaceae and its sister group is one of the shortest and most difficult in Lamiales to resolve (Fig. 6.1). Until large quantities of molecular

sequence data are available, the sister group of Thomandersiaceae will not be conclusively determined. However, Thomandersiaceae is probably most closely-related to Bignoniaceae, Schlegeliaceae or Verbenaceae, although the Bignoniaceae-Schlegeliaceae-Thomandersiaceae-Verbenaceae clade has little support, and arrangements within it are unstable. For example, Thomandersiaceae was sister to Verbenaceae in the maximum likelihood phylogeny (Fig. 1.5), while in maximum parsimony analysis with rogue taxa removed, only Bignoniaceae and Schlegeliaceae were closely related to Thomandersiaceae.

The most frequently resolved sister group to Thomandersiaceae was Schlegeliaceae, for instance in the strict consensus tree from total evidence analysis (Fig. 4.12). The most likely MP tree shows Thomandersiaceae and Schlegeliaceae as sister group to a clade comprising Bignoniaceae, Verbenaceae and Pedaliaceae (Fig. 6.1). The relationship of Thomandersiaceae and Schlegeliaceae was not well supported, with a decay value of three steps and bootstrap value of 57% in total evidence analysis. This represents a marked increase to the support obtained from molecular evidence alone, either in maximum parsimony (16%; Fig. 1.4) or maximum likelihood analysis (6%; Fig. 1.5).

Schlegeliaceae comprises four genera – *Exarata, Gibsoniothamnus, Schlegelia* and *Synapsis* – including small shrubs, lianas and large canopy trees (Gentry, 1992b; Reveal, 1995; Burger & Barringer, 2000). Members of Schlegeliaceae have previously been classified in Bignoniaceae, Scrophulariaceae and Verbenaceae (e.g. Bureau & Schumann, 1896-1897; Urban, 1926; Gentry, 1982; Barringer & Gentry, 1995; Gentry, 1997), and previous molecular studies have suggested they are most

likely to be related to Bignoniaceae (Olmstead et al., 2001; Olmstead, in progress). An exception to this was the asterid phylogeny by Bremer et al. (2002), which resolved Schlegeliaceae as sister to Acanthaceae.

Schlegeliaceae displays some derived characters that parallel those of Thomandersiaceae. These include anisophyllous leaves, a persistent and accrescent calyx, single staminode, disk nectary absent, large, axile placentas (Burger & Barringer, 2000), and calycine glands in some taxa, which were reported here for the first time. Like Thomandersiaceae but unlike Acanthaceae they lack interpetiolar articulation (Burger & Barringer, 2000). Thomandersiaceae is, however, geographically and morphologically distinct from Schlegeliaceae in a number of respects. Schlegeliaceae are confined to the neotropics, have flowers with well-developed pedicels and a corolla that is radially or bilaterally symmetrical but not bilabiate, with a straight tube (Fig. 6.3) (Burger & Barringer, 2000). The fruits of Schlegeliaceae are very different to those of Thomandersiaceae, being indehiscent drupes containing numerous small seeds (Burger & Barringer, 2000).

6.5 The role of morphology

Morphology has a crucial role in the alpha-taxonomic activities of species discovery, description and delimitation (Wilson, 2000; Daly et al., 2001; Heywood, 2001; Wortley et al., 2002). However, it no longer plays a dominant role in phylogenetic analysis (Mishler, 2000; Endress, 2003; Scotland et al., 2003). As Chapters 4 and 5 showed, molecular sequence data should in future be sufficient to reconstruct a well-resolved, supported and accurate phylogeny of Lamiales. Morphological datasets,

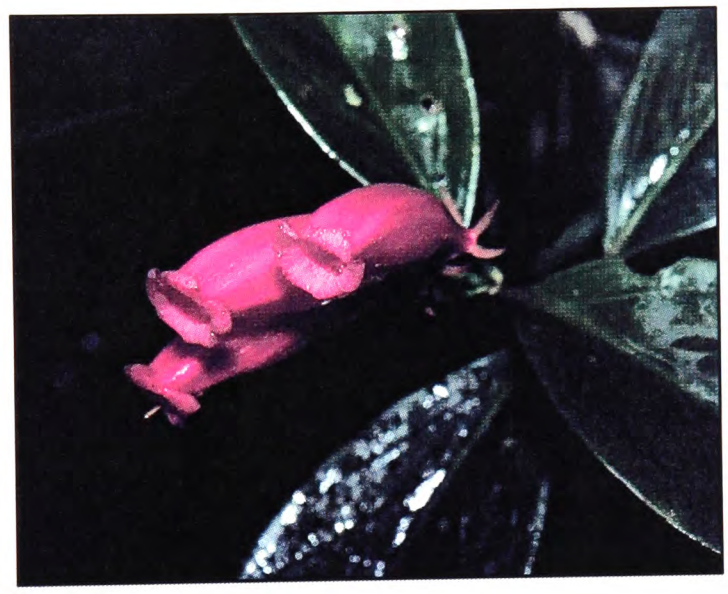


Figure 6.3 Flowers of Schlegeliaceae: *Gibsoniothamnus c.f. epiphyticus* (*Knapp & Mallet* 9233, Panama: Chiriqui; image supplied by S. Knapp).

even in combined analysis, are decreasingly comparable with molecular ones in terms of size and informativeness for inferring the phylogenetic relationships of extant groups. However, possible insights may still be gained from integrating molecular phylogenies and morphological data.

Endress (2003) reviewed the importance of studying morphological character evolution. Following molecular sequence-based, or combined molecular and morphological, phylogenetic analysis it is possible to infer the patterns of character transformation by optimisation. This can be used to guide further in-depth investigations of character evolution, integrating molecular developmental-genetic approaches, more refined analyses of morphological features and, perhaps, data from fossil taxa. In addition, morphological data can be reconsidered in the context of a molecular phylogenetic hypothesis to find potential supporting synapomorphies for clades, which might contribute to a diagnostic classification of monophyletic groups.

6.5.1 Investigating character evolution on the total evidence phylogeny. A well-resolved phylogeny of Lamiales is expected to be valuable for understanding the evolution of particular morphological traits. This is especially true for the origin and genetic basis of floral zygomorphy, for which one member of Lamiales (*Antirrhinum*) has been established as a model organism (Coen et al., 1995; Donoghue et al., 1998; Endress, 1999; Ree & Donoghue, 1999; Endress, 2001).

The reliable inference of character evolution depends on three factors: a robust phylogenetic tree, correctly-conceptualised (homologous) characters, and a method of unambiguously relating the characters to the tree, which is usually achieved by an

optimisation procedure. These criteria are not yet fulfilled in Lamiales, as discussed below.

1. The best hypothesis of Lamiales phylogeny at present is not robustly supported at interfamilial level. Inferences of character evolution are unlikely to be accurate until a more robust phylogenetic hypothesis is generated.
2. The morphological characters included in the total evidence analysis may not all be correctly-conceptualised primary homologies. Character selection was conducted at a certain level of inclusiveness, to avoid the incorporation of too many non-homologous characters in the matrix without excluding too many unambiguous and informative ones (Poe & Wiens, 2000). This means some of the former may have "slipped through the net". The smaller, more strictly selected morphological matrix (Chapter 4), may contain a higher proportion of correctly-conceptualised morphological characters, making it a better starting point for studies of character evolution in Lamiales. Both matrices, however, contain a large amount of missing data. There is a great deal of morphological and anatomical investigation outstanding before rigorous evolutionary questions can be answered; the empty cells in the matrix provide a useful guide to targeting future work. Even once the cells in this matrix are filled they may not fully represent the character variation within families of Lamiales, because an exemplar taxon sampling was employed.
3. Parsimony is the most widely-used method for mapping characters onto phylogenies (Huelsenbeck et al., 2003). In this framework there are two ways in which the optimisation of character state changes on the total evidence phylogeny is ambiguous. Firstly, there are multiple MP topologies, on which different optimisations result. Secondly, two possible optimisation algorithms

exist which make different assumptions about the relative probability of multiple parallel evolutionary events or secondary losses. Delayed transformation (DELTRAN) assumes the former is more likely, accelerated transformation (ACCTRAN) the latter (Swofford, 2002). It has been argued (De Pinna, 1991) that ACCTRAN optimisation is theoretically superior, but reliable conclusions can only be drawn for character states that display the same distribution under both optimisation procedures. When the characters of the large (105-character) morphological matrix are optimised on the total evidence tree they provide 60 unambiguous character state changes These 60 events involve only 47 different character state changes; thus 13 are repeated, parallel evolutionary events. Only eight of the 60 transformations involve characters included in the strict matrix, which should be the best conceptualised characters available.

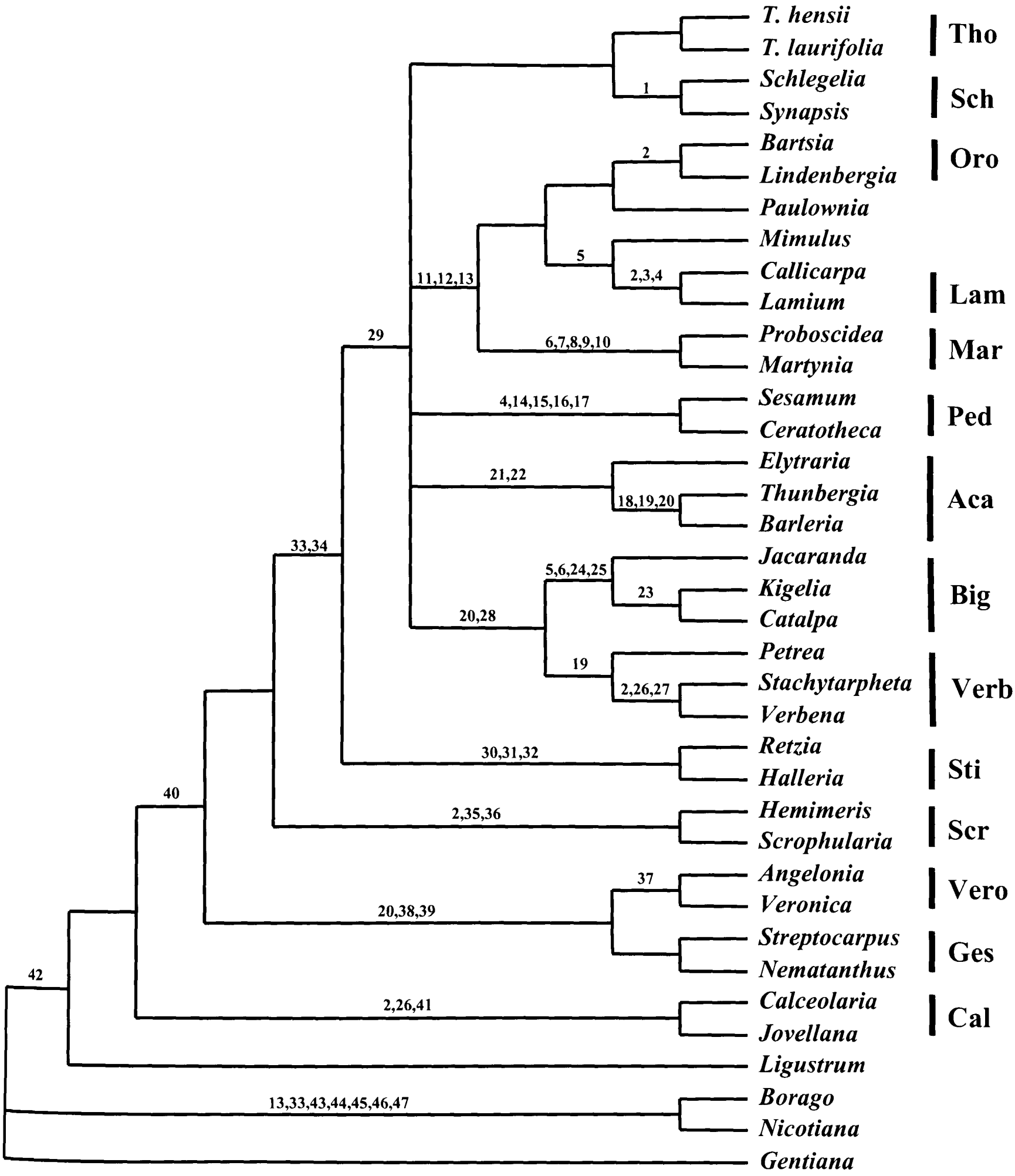
Recently, there have been a number of attempts to develop a likelihood-based method of inferring character evolution (Huelsenbeck & Rannala, 1997; Lewis, 2001; Huelsenbeck et al., 2003). This would take into account not only the topology, but also the branch lengths, of the tree (Lewis, 1998; 2001). Whether branch lengths derived from molecular data are relevant to the reconstruction of morphological character evolution is debatable (Lewis, 2001). What is certain is that these methods, like the parsimony optimisation procedures more commonly applied, require both a robust phylogeny and correctly-conceptualised characters in order to generate useful inferences about character evolution.

Therefore, however the optimisation is conducted, current understanding of Lamiales phylogeny and morphological characters fails to provide a robust framework within which to study character evolution. Any such investigation, in the absence of detailed knowledge of the traits' genetic basis, would, at best, be superficial. At worst it could lead to incorrect reconstruction of ancestral states and inferences of character evolution in Lamiales.

In future, once a robust, resolved phylogeny has been reconstructed, it could be used to investigate issues such as the evolution of morphological characters. Current indications are that the evolutionary histories of many characters, such as the retinacula of *Thomandersia* and Acanthaceae and the four-loculed gynoecium shared by Lamiaceae and Verbenaceae, are more complex than was once thought.

6.5.2 Reconstructing synapomorphic states and diagnostic characters. Inferring character evolution by optimisation is unlikely to be successful at present in Lamiales. A second application of morphological characters in the context of a phylogeny is the reconstruction of possible synapomorphies on the branches subtending clades. This pattern-based inference invokes fewer assumptions about evolutionary and genetic processes. ACCTRAN and DELTRAN optimisation algorithms may be used to identify the character state changes underlying phylogenetic relationships, which could be employed in a diagnostic, monophyletic classification. Figure 6.4 shows the distribution of the morphological character state changes that are unambiguously reconstructed on all three MP topologies and under both ACCTRAN and DELTRAN optimisation algorithms.

Figure 6.4 (next page) Strict consensus of the three MP trees obtained from total evidence analysis, showing unambiguous morphological synapomorphies reconstructed on each branch. 1, leaf margin revolute; 2, leaf margin toothed; 3, terminal inflorescence unit determinate; 4, pistil cells four; 5, calyx lobes shorter than tube; 6, calyx not persistent; 7, anthers cohering; 8, pollen inaperturate; 9, gynoecium locules one; 10, fruit fleshy; 11, anthers basifixed; 12, branched multicellular hairs present; 13, leaf epidermis mucilaginous; 14, anther filaments glabrous; 15, pollen grains three-celled when shed; 16, pollen apertures more than six; 17, gynoecium locules four; 18, anther endothecium without fibrous thickenings; 19, endothelium not differentiated; 20, endosperm absent at seed; 21, stomata diacytic; 22, polar nuclei fusion after only one fertilised; 23, glandular hairs absent; 24, anther tapetum amoeboid; 25, micropylar endosperm haustoria absent; 26, stem nodes articulated; 27, flowers sessile; 28, synergids hooked; 29, embryogeny onagrad; 30, xylem fibres mixed; 31, leaves sessile; 32, bracteoles present; 33, corolla lobes five; 34, axial parenchyma vasicentric (or scanty vasicentric); 35, stem internode square or tetragonal; 36, anther thecae one; 37, corolla lobes longer than tube; 38, annular disk nectary present; 39, septicidal dehiscence absent; 40, stomata anisocytic; 41, corolla lobes two; 42, wood semi-ring porous; 43, leaves alternate; 44, leaf hairs present; 45, multicellular hairs present; 46, calyx lobes five; 47, iridoids absent. Families taken from Olmstead et al. (2001). Aca, Acanthaceae; Big, Bignoniaceae; Cal, Calceolariaceae; Ges, Gesneriaceae; Lam, Lamiaceae; Mar, Martyniaceae; Oro, Orobanchaceae; Ped, Pedaliaceae; Sch, Schlegeliaceae; Scr, Scrophulariaceae *s.s.*; Sti, Stilbaceae; Tho, Thomandersiaceae; Verb, Verbenaceae; Vero, Veronicaceae.


T. hensii
T. laurifolia
Schlegelia
Synapsis
Bartsia
Lindenbergia
Paulownia
Mimulus
Callicarpa
Lamium
Proboscidea
Martynia
Sesamum
Ceratotheca
Elytraria
Thunbergia
Barleria
Jacaranda
Kigelia
Catalpa
Petrea
Stachytarpheta
Verbena
Retzia
Halleria
Hemimeris
Scrophularia
Angelonia
Veronica
Streptocarpus
Nematanthus
Calceolaria
Jovellana
Ligustrum
Borago
Nicotiana
Gentiana
Tho
Sch
Oro
Lam
Mar
Ped
Aca
Big
Verb
Sti
Scr
Vero
Ges
Cal
1
2
5
11,12,13
2,3,4
6,7,8,9,10
29
4,14,15,16,17
21,22
18,19,20
33,34
5,6,24,25
23
20,28
19
2,26,27
30,31,32
40
2,35,36
37
20,38,39
42
2,26,41
13,33,43,44,45,46,47

Of the 60 transformations that can be unambiguously mapped on the tree, more than half (32) lie on branches subtending existing families, and seven define groups within families. Seven transformations support a relationship between the two out-groups *Borago* and *Nicotiana*, and one lies on the branch connecting the in-group and out-group. Thus only 13 unambiguous character state changes correspond to interfamilial branches in Lamiales. Instead, the inferred morphological synapomorphies are concentrated at family level (Fig. 6.4). Almost every family, including those only recently circumscribed on the basis of molecular data (Olmstead et al., 2001), is supported by at least one unambiguous morphological synapomorphy. The exceptions are Gesneriaceae and Thomandersiaceae. Both Gesneriaceae and Thomandersiaceae do have a number of morphological synapomorphies (shown by the length of their subtending branches on Fig. 6.2) but these are not unambiguously reconstructed on the tree, reflecting the limitations of parsimony-based optimisation procedures. Lamiales as a whole are supported by a single character, semi-ring porous wood. Core Lamiales are also supported by a single character, onagrad embryogeny.

Even groups such as families, subtended by several unambiguous character state changes, are often not diagnosable because most morphological synapomorphies are homoplastic on the tree. Two characters that are potentially diagnostic are the amoeboid anther tapetum of Bignoniaceae and inaperturate pollen of Martyniaceae, but a thorough examination of a dense sample of species in these families is necessary to determine the generality of the characters. In general, prospects for a diagnostic classification of Lamiales are likely to be limited to long-established groups (mostly families) that have already been defined on the basis of morphological characters. Any new infra-ordinal clades that are discovered through advances in molecular

phylogenetics, however well-supported, are unlikely to be diagnosable using morphological characters.

6.6 Implications and generality

This thesis centred on the taxonomy and phylogenetic relationships of *Thomandersia,* in the context of Lamiales, one of the largest orders of flowering plants (Olmstead et al., 2001; Stevens, 2001; Bremer et al., 2002). Since the study began there have been continued advances in capability for molecular sequencing and computer-based phylogenetic analysis, so that the most productive approach to resolving the phylogenetic relationships of Thomandersiaceae will now be to conduct a large-scale molecular sequence-based investigation of Lamiales, using multiple genetic markers and a range of representative exemplar taxa from all potential sister groups.

The most difficult aspect of such a study may prove to be taxon sampling: material will be needed for sequencing from a wide range of correctly identified, geographically dispersed and sometimes range-restricted species, such as *Exarata* (Gentry, 1992b) and *Retzia* (Goldblatt & Keating, 1976; Dahlgren et al., 1979; Carlquist, 1986), requiring a world-wide collaborative effort. This means that, logistically and financially, the project may now lie outside the scope of a D.Phil. research programme.

A large molecular phylogenetic research programme for Lamiales would be complemented and built upon by continued generic-level morphological investigations such as those presented here, which will contribute to a better understanding of the taxonomy and morphology of smaller groups within the order. In

tandem with the developing molecular-based hypotheses of relationship, this would ultimately provide a comprehensive understanding of Lamiales phylogeny and evolution.

Such an integrated approach can be applied to the systematics of many rogue genera and other problematic orders (e.g. Chandler & Bayer, 2000; Albach et al., 2001b; Beardsley & Olmstead, 2002; Davis & Chase, 2004). Similar recent investigations enabled the placement of the enigmatic genera *Hydrostachys* within Hydrangeaceae (Albach et al., 2001b) and *Mimulus* in Phrymaceae; (Beardsley & Olmstead, 2002), and the recognition of *Emblingia* and *Biebersteinia* as monotypic families (Bakker et al., 1998; Chandler & Bayer, 2000). The phylogenetic position and status of other rogue genera remains to be confirmed. These include *Paulownia* in Lamiales (Armstrong, 1985; Reeves & Olmstead, 1998), *Hauya,* possibly in Onagraceae (Bult & Zimmer, 1993), and *Peridiscus*, of uncertain placement in Saxifragales following its exclusion from Malpighiales (Davis & Chase, 2004).

The predicted, comprehensive solution to the entire tree of life (Wheelis et al., 1992; Doolittle, 1999) is a long-term objective of molecular systematics (Sanderson et al., 2003), but an accurate, supported and resolved phylogeny of well-studied parts of the tree such as Lamiales will soon be achievable if current rates of progress and collaboration continue. Well focused taxon sampling, large-scale molecular sequencing projects and subsequent concatenation of multiple markers should provide resolution of many phylogenies with problematic tree models, such as the angiosperm orders Apiales, Cornales, Ericales, Laurales and Malpighiales (Plunkett et al., 1996; Soltis et al., 1999; Anderberg et al., 2000; Renner & Chanderbali, 2000; Xiang et al.,

2002). The quantity of molecular data available is not yet sufficient in these groups. For instance, the largest and most recent analysis of Cornales employed 6,348 aligned nucleotide sites (Fan & Xiang, 2003), where at least 10,000 bp may be required. In other plant groups, and other organisms, increasingly large multi-gene and whole-genome molecular phylogenetic investigations are rapidly becoming the norm (e.g. Graham & Olmstead, 2000; Bapteste et al., 2002; Matsuoka et al., 2002; Soltis et al., 2002; Goremykin et al., 2003; Rokas et al., 2003). The use of increasingly large concatenated datasets raises new questions, such as how to identify and deal with incongruent signals and large amounts of missing data (Sanderson & Driskell, 2003; Sanderson et al., 2003). The challenge of overcoming these obstacles to large-scale phylogenetic analysis is one of the most stimulating prospects facing systematics over the next few years.

6.7 Conclusions

This investigation into the systematics of *Thomandersia* has resulted in the recognition of Thomandersiaceae, a family of isolated phylogenetic position with a large number of molecular and morphological autapomorphies. At the same time it has highlighted the problems of resolving interfamilial relationships in Lamiales, and contributed to their future solution. The best way to investigate the systematics of rogue taxa within problematic ordinal phylogenies involves a combination of detailed generic-level investigations and comprehensive molecular phylogenetic analysis.

A new taxonomic account for *Thomandersia* has been presented, together with a detailed description of its anatomical and morphological features. The distinctive retinaculum that led to its classification in Acanthaceae has been shown to be similar

in both groups but of independent origin, a striking example of parallel morphological evolution.

The ultimate aim of phylogenetic analysis in Lamiales is a fully and accurately-resolved tree, with strongly-supported positions for all taxa. In this context, morphological characters are of limited use; large quantities of molecular data are needed to provide a strong phylogenetic signal. In the near future it will be feasible to generate sufficient molecular sequence data to resolve Lamiales phylogeny accurately, and elucidate the relationships of many isolated taxa such as Thomandersiaceae. The prospects for increasingly large-scale phylogenetic analysis, complemented by an ever more detailed understanding of morphological characters, make this an exciting time for Lamiales systematics.

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Appendices

Appendix 1 List of taxa sampled in phylogenetic analysis, with GenBank accession numbers & references for DNA sequences. Classification as Olmstead et al. (2001) authorities as Brummitt and Powell (1992).

Taxon	DNA source (previous studies) or voucher	GenBank accession numbers *rbcL*	*ndhF*
Lamiales			
Acanthaceae			
Aphelandra sinclairiana Nees	Albert et al. (1992)	L01884	-
Aphelandra squarrosa Nees	Manktelow (2001)	-	AJ249405
Avicennia marina (Forssk.) Vierh.	Bremer et al. (2002), Schwarzbach & McDade (2002)	AY008832	AJ429116
Barleria prionitis L.	Chase et al. (1993), Scotland et al. (1995)	L01886	U12653
Elytraria crenata Vahl	Scotland et al. (1995)	AF188127	U12657
Justicia americana Vahl	Olmstead et al. (1993), Scotland et al. (1995)	L14401	U12663
Ruellia graecizans Backer	Hedren et al. (1995)	L12595	-
Ruellia ciliosa Pursh	Scotland et al. (1995)	-	U12664
Thunbergia alata Bojer.	Scotland et al. (1995)	-	U12667
T. usambarica Lindau	Chase et al. (1993)	L12596	-
Bignoniaceae			
Catalpa speciosa Warder	Olmstead et al. (1992)	L11679	L36397
Jacaranda sparrei A.H.Gentry	Spangler & Olmstead (1999)	AF102647	AF102631
Kigelia africana Benth.	Spangler & Olmstead (1999)	AF102648	AF102632
Calceolariaceae			
Calceolaria mexicana Benth.	Olmstead et al. (2001)	AF123669	AF123769
Jovellana sp.	Olmstead et al. (2001)	AF123666	AF123684
Gesneriaceae			
Nematanthus hirsutus (Mart.) Wiehler	Olmstead & Reeves (1995)	L36446	L36404
Streptocarpus holstii Engl.	Olmstead et al. (2001)	L14409	L36415
Lamiaceae			
Callicarpa dichotoma (Lour.) K.Koch	Olmstead et al. (1993)	L14393	L36415
Lamium purpureum L.	Olmstead et al. (1993)	U75702	U78694
Martyniaceae			
Martynia annua L.	Olmstead & Reeves (1995)	-	AF190906
Proboscidea louisianica (Mill.) Thell.	Chase et al. (1993)	L01946	AF123690
Oleaceae			
Ligustrum vulgare L.	Olmstead et al. (1992)	L11686	AF130164
Orobanchaceae			
Bartsia alpina L.	Young et al. (1999)	AF190903	AF123678
Lindenbergia phillipensis Benth.	Young et al. (1999)	AF123664	AF123686
Paulowniaceae			
Paulownia tomentosa Steud.	Olmstead & Reeves (1995)	L36447	L36406
Pedaliaceae			
Ceratotheca triloba (Bernh.) Hook.f.	Olmstead & Reeves (1995)	Not yet submitted	Not yet submitted
Sesamum indicum L.	Olmstead et al. (1993)	L14408	L36413
Phrymaceae			
Mimulus aurantiacus Curtis	Young et al. (1999)	AF026835	AF188186
Schlegeliaceae			

Schlegelia parviflora (Oerst.) Monach.	Olmstead & Reeves (1995)	L36448	L36410
Synapsis ilicifolia Griseb.	*Ekman* 19085	Not yet submitted	Not yet submitted
Scrophulariaceae			
Hemimeris sabulosa L.	Young et al. (1999)	AF123668	AF123682
Scrophularia californica Cham. & Schltdl.	Olmstead & Reeves (1995)	L36449	L36411
Stilbaceae			
Halleria lucida L.	Olmstead et al. (2001)	AF026828	AF188185
Retzia capensis Thunb.	Bremer et al. (1994)	Z29669	AF14776
Verbenaceae			
Petrea racemosa Kunth	Olmstead & Reeves (1995), Wagstaff & Olmstead (1997)	U28879	Not yet submitted
Stachytarpheta dichotoma Vahl	Olmstead et al. (2001)	U32161	L36414
Verbena bonariensis L.	Olmstead et al. (1993)	L14412	-
V. bracteata Lag. & Rodr.	Olmstead & Reeves (1995)	-	L36418
Veronicaceae			
Angelonia pubescens Benth.	Olmstead et al. (2001)	AF123672	AF123675
Veronica catenata Pennell	Olmstead & Reeves (1995)	L36453	L36419
Unassigned to family			
Thomandersia hensii De Wild. & T.Durand	*Carroll* 1008	Not yet submitted	Not yet submitted
Thomandersia laurifolia (Benth.) Baill.	*Cable* 4012	Not yet submitted	Not yet submitted
Out-groups			
Borago officinalis L.	Olmstead et al. (1992)	L11680	L36393
Gentiana procera Holm	Olmstead et al. (1993)	L14398	L36400
Nicotiana tabacum L.	Olmstead et al. (1993)	Z00044	Z00044

Appendix 2 Outline of methods for DNA extraction, purification and sequencing.

Nucleotide sequences for *rbcL* and *ndhF* were obtained from fresh, silica-dried or herbarium-dried plant material according to the following steps, described in detail in the subsequent appendices.

1. DNA extraction (Appendix 3)
2. Check DNA extraction by electrophoresis on agarose gel (Appendix 4)
3. Amplify specific region using polymerase chain reaction (PCR; Appendix 5)
4. Check PCR products by electrophoresis on agarose gel (Appendix 4)
5. Purify PCR products (Appendix 6)
6. Sequence DNA by PCR with single primer and fluorescent dNTPs (Appendix 7)
7. Purify products of sequencing PCR (Appendix 8)
8. Run PCR product through automated sequencer (ABI Prism 377).

Appendix 3 "CTAB" micro-extraction protocol for one sample (multiple samples were extracted concurrently), modified from Doyle and Doyle (1987).

1. Preheat 1 ml CTAB buffer (see below) with 2 µl 2-mercaptoethanol at 65°C in a water-bath under a fume-hood (preheat a little extra in case some samples require dilution; 2-mercaptoethanol protects DNA from the action of quinones, disulphides, peroxidases and polyphenoxidases).
2. Harvest a small amount (e.g. 2 cm^2) of leaf material; remove and discard midrib. Place the lamina in a 1.5 ml Eppendorf tube and add one spatula of sand to aid in the grinding process.
3. With the Eppendorf lid open, hold the tube in liquid nitrogen until it stops making a bubbling sound, indicating that the sample has been chilled sufficiently. Take care not to allow any leaf material out of the tube, or any $N_{2(l)}$ into it.
4. Macerate the leaf tissue with a sterile plastic pestle until it is reduced to small fragments. The sample can be re-chilled in $N_{2(l)}$ if maceration is difficult. Do not allow the material to defrost as this will enable the DNA to degrade.
5. Transfer 200 µl preheated CTAB buffer to the tube and continue to gently grind the sample.
6. Add a further 400 µl preheated CTAB buffer, remove the pestle and add a final 400 µl of buffer and a pinch of polyvinylpolypirrolidone (PVPP; this aids the removal and reduces the effects of phenolic compounds). Close the tube and mix the contents by flicking. The solution should be thick and green, but not so thick that it cannot be poured.
7. Incubate the tube for at least 30 minutes in a heating block at 65°C.
8. Remove the sample from the heating block and allow it to cool to room temperature.
9. Add 500 µl "wet chloroform" (24:1 chloroform:isoamyl alcohol; used to precipitate proteins and carbohydrates). Ensure the lid of the tube is secure, and mix gently by shaking four or five times to obtain a momentary single-phase solution. Transfer to an orbital shaker and shake on a low speed for at least 20 minutes, checking occasionally for leakage.
10. Micro-centrifuge for 10 minutes at 13,000 rpm.
11. Transfer the supernatant to a clean 1.5 ml Eppendorf tube and repeat the chloroform extraction (steps 9 and 10). Dispose of chloroform waste in a labelled bottle.
12. Transfer the supernatant to a clean 1.5 ml Eppendorf tube. Precipitate the DNA by adding 600 µl (if started with 1 ml; otherwise ⅔ volume) freezer-cold isopropanol and rocking gently. Store the DNA in a freezer (experience with *Thomandersia* and Schlegeliaceae material from herbarium collections suggests the longer the freezing time the better, i.e. a week or more if possible).
13. Micro-centrifuge for 10 minutes at 13,000 rpm to pellet the DNA.
14. Remove the supernatant and discard. Drain the tube carefully by inverting it onto a towel, then add 1 ml wash buffer (see below). Vigorously agitate to release the pellet from the bottom of the tube and leave to wash for at least 30 minutes.
15. Micro-centrifuge for 5 minutes at 13,000 rpm. Remove the supernatant and discard. Drain the tube carefully by inverting it onto a towel. Dry the pellet in a vacuum centrifuge for 5 minutes or until the pellet is dry.

16. Dissolve the pellet in 50-75 µl of Tris-HCl:EDTA, *p*H 8.0 (TE; *p*H 8.0 is optimal for inhibiting the action of both degradative enzymes and nuclear DNases).

CTAB buffer
2% cetyltrimethylammonium bromide (CTAB; solubilises plant membranes and complexes with DNA)
20 mM EDTA (inhibits metal-dependent enzymes by chelating divalent cations)
100 mM Tris-HCl *p*H 8.0
1.4 M NaCl

Wash buffer
76% $ethanol_{(aq)}$
10 mM NH_4Ac

Appendix 4 Agarose gel electrophoresis using 100 ml gel (quantities can be adjusted for different sizes of gel).

1% agarose gel electrophoresis is the standard method of separating DNA fragments to check their quality and quantity. Fragments migrate across the gel from negative to positive according to their size, which can be estimated relative to standard-sized fragments also run on the gel.

1. Set up a gel tray and combs with an appropriate number of teeth for the samples plus standard marker. Seal the ends of the tray with masking-tape.
2. Weigh 1 g agarose in a conical flask.
3. Add 100 ml 1xTBE (see below) to the agarose and swirl gently.
4. Heat the solution in a microwave on maximum heat for 30 seconds, remove and gently swirl. Continue to heat, swirling occasionally to mix. Heat to beyond the boiling point, until large bubbles appear in the solution, and check that all the agarose has dissolved. Stand until the flask is cool enough to handle.
5. Wearing gloves and a dedicated pipette, add 1-2 µl ethidium bromide (with care: carcinogenic) and gently swirl the flask to mix. Pour the solution into the gel tray and leave to set.
6. Load the gel lanes each with 3 µl loading dye and 5 µl DNA; load the final lane with 5 µl of an appropriate control marker (this investigation used a 1 kb ladder).
7. Switch on the power supply and allow to run for 15-20 minutes at ~80 V.
8. View and photograph the gel under an ultraviolet light source.

1xTBE buffer (1L)
200 ml Tris-borate buffer (TBE)
800 ml distilled H_2O

Appendix 5 25 µl polymerase chain reaction (PCR) amplification of *ndhF* and *rbcL* DNA sequences for *n* samples. Procedure to be carried out in a laminar flow hood, using dedicated PCR pipettes and sterile distilled water. All reagents are kept frozen; all except *Taq* polymerase are to be defrosted and stored on ice before use; *Taq* polymerase is used frozen.

1. Label *n*+2 0.2 ml micro-centrifuge tubes (for samples, positive control and negative control).
2. Make up reagent solutions for the required sequences, each in a 1.5 ml Eppendorf tube, according to the table below, multiplying quantities by *n*+2. Add distilled H_2O first and *Taq* polymerase last.

Table showing volumes of reagents for 25 µl PCR reaction (µl).

Reagent	***ndhF* section A**	***ndhF* section B**	***rbcL***
10x reaction buffer	2.5	2.5	2.5
dNTPs (2 mM)	2.5	2.5	2.5
$MgCl_2$ (50 mM)	1.25	1.25	1.25
Forward primer (10 µM)*	0.75	0.75	0.75
Reverse primer (10 µM)*	0.75	0.75	0.75
Dimethyl sulphoxide (DMSO)**	1.0	1.0	1.0
Distilled H_2O	14.125	14.125	14.125
Taq polymerase (Biotaq)	0.125	0.125	0.125
Total	**23**	**23**	**23**

*Primers used: *ndhF* section A, -52F and 1218R; *ndhF* section B, 803F and 2112R; *rbcL*, 1F and 1460R.

**DMSO facilitates strand separation and prevents the formation of secondary structures and is commonly used to enhance difficult PCR amplifications (Baldwin et al., 1995).

3. Transfer 23 µl of the correct reaction mixture to each of the labelled micro-centrifuge tubes.
4. Add 2 µl DNA to each tube, and 2 µl distilled H_2O to the negative control, depending on experience. If, for example, 4 µl DNA is to be used instead of 2 µl, reduce the volume of distilled H_2O in the mix accordingly, to keep the total volume to 25 µl. The volume should be similarly maintained if DMSO is omitted.
5. Close the micro-centrifuge tubes firmly and transfer them to the PCR machine (a Perkin Elmer DNA Engine was used in this investigation). Programme the machine to perform the relevant protocol (see below).
6. Check the PCR products' quality by electrophoresis using 2 µl product and 3 µl loading dye on a 1% agarose gel (Appendix 4) before purification and sequencing.

*PCR amplification protocol (*ndhF*)*

1. 95°C, 3 minutes
2. 95°C, 1 minute (denaturation)
3. 50°C, 1 minute (re-annealing)
4. 72°C, 1 minute (extension)
5. return 29 times to step 2 (30 cycles)
6. 72°C, 7 minutes
7. 4°C, hold.

*PCR amplification protocol (*rbcL*)*

1. 94°C, 4 minutes
2. 94°C, 1 minute (denaturation)
3. 45°C, 2 minutes (re-annealing)
4. 72°C, 3 minutes (extension)
5. return 29 times to step 2 (30 cycles)
6. 72°C, 7 minutes
7. 4°C, hold.

Appendix 6 Purification of PCR products using QIAGEN QIAquick PCR purification kit and protocol, to remove un-annealed primers which may hinder the sequencing reaction.

1. Add 5 volumes buffer "PB" to 1 volume PCR product and mix by vortexing.
2. Place a QIAquick spin column in a 2 ml collection tube.
3. Apply the sample (now 6 volumes) to the QIAquick column and micro-centrifuge for 1 minute at 13,000 rpm to bind DNA to the column.
4. Discard "flow-through" and replace the QIAquick column back in the same tube.
5. Add 0.75 ml buffer "PE" (previously prepared by dilution with ethanol) to the QIAquick column; micro-centrifuge for 1 minute at 13,000 rpm to wash DNA.
6. Discard "flow-through" and replace the QIAquick column back in the same tube. Micro-centrifuge for a further 1 minute at maximum speed (residual ethanol will not be completely removed unless the "flow-through" is discarded before this additional centrifugation).
7. Place the QIAquick column in a clean 1.5 ml Eppendorf tube.
8. Add 50 µl buffer "EB" (10 mM Tris-Cl, *p*H 8.5) to the QIAquick membrane, let it stand for 1 minute, then micro-centrifuge for 1 minute at 13,000 rpm to elute DNA. Discard QIAquick column.

The c.48 µl eluent should now contain DNA ready for sequencing PCR. Check the DNA quality by electrophoresis using 2 µl product and 3 µl loading dye on a 1% agarose gel (Appendix 4) before sequencing.

Appendix 7 20 µl sequencing PCR. Procedure to be carried out in a laminar flow hood, using dedicated PCR pipettes and sterile distilled water. All reagents are kept frozen; all except *Taq* polymerase are to be defrosted and stored on ice before use; *Taq* polymerase is used frozen.

1. Label four 0.2 ml micro-centrifuge tubes (one for each primer).
2. Each in a 0.5 ml Eppendorf tube, make up reagent solutions for the required sequence primer according to the table below. Add distilled H_2O first and reagent premix (containing *Taq* polymerase) last.

Table showing volumes of reagents for 20 µl sequencing PCR reaction (µl).

Reagent	***ndhF* section A**	***ndhF* section B**	***rbcL***
Thermo Sequenase II reagent premix*	16	16	16
DNA template	8	8	12
Distilled H_2O	54	54	50
Total	**78**	**78**	**78**

*reagent premix contains pre-mixed *Taq* polymerase, dNTPs, fluorescent labelled dNTPs, buffer and $MgCl_2$

3. Transfer 19.5 µl of the correct mixture to each of the labelled micro-centrifuge tubes.
4. Add 0.5 µl of the correct primer (at 10 µM concentration) to each tube. The primers used were: for *ndhF* section A, -52F, 803F, 972R and 1218R; *ndhF* section B, 803F, 1318F, 1603R and 2112R; *rbcL*, 1F, 636F, 724R and 1460R.
5. Close the micro-centrifuge tubes firmly and transfer them to the PCR machine (a Perkin Elmer DNA Engine was used in this investigation). Programme the machine to perform the sequencing protocol (see below).
6. Transfer each 20 µl PCR product to a fresh 0.5 ml tube containing 2 µl of freezer-cold sodium acetate/EDTA buffer.

PCR sequencing protocol

1. 96°C, 10 seconds
2. 50°C, 5 seconds
3. 60°C, 4 minutes
4. return 24 times to step 1 (25 cycles)
5. 4°C, hold.

Appendix 8 Purification of sequencing PCR products.

1. Add 2.5 volumes (55 µl) 100% ethanol at -20°C to each reaction. Mix briefly with a vortex mixer and place on ice for 15-20 minutes to precipitate the DNA (precipitating for longer than 20 minutes may result in incomplete removal of labelled dNTPs).
2. Micro-centrifuge for 15 minutes at 13,000 rpm.
3. Draw off as much of the supernatant as possible.
4. Add 250 µl cold 70% ethanol$_{(aq)}$ to wash the pellet. Micro-centrifuge for 5 minutes at 13,000 rpm.
5. Draw off the supernatant and dry the pellet in a vacuum centrifuge for 2-5 minutes (do not over-dry).
6. Store the DNA in clearly-labelled tubes in a freezer.
7. Run PCR product through the ABI Prism 377 sequencer.
8. Edit and assemble the traces using Sequencher (Gene Codes Corporation) for Macintosh.

Appendix 9 Alignment matrix for the *ndhF* region (positions 24-2,109 in *Nicotiana tabacum*). Alignment gaps are indicated by "-". Missing data are indicated by "?".

```
Thomandersia hensii          NNNNNNNNNNNNNNNNNNNNNNNNNTCCAGTTCCTNTGTTAATAGGAGTGGGCCTTCTTCTTTTTCCGACAGC
Thomandersia laurifolia      GTGGATCATACCTTTCGTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Bartsia alpina               GTGGATTATACCCTTCGTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGATTTCTTCTTTTTCCGACAGC
Lindenbergia phillipensis    GTGGATTATACCTTTCGTTCTACTTCCAATTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Paulownia tomentosa          GTGGATTATACCTTTCCTTCCACTTCCAGTTCCTATGTTAATAGGACTGGGACTTCTTCTTTTTCCGACAGC
Mimulus aurantiacus          GTGGATTATACCTTTCCTTCCACTTCTAGTTACTATGTTAATAGGAGTGGGACTTCTACTTTTTCCGACAGC
Callicarpa dichotoma         ????ATTTTTCCTT???TTCCACTTCCAATCCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Lamium purpureum             GTGTATTTTACCTT???TTCCACTTATAGTTCCTATATTAATAGGAGTGGGACTTGTTATTTTTCCGACAGT
Sesamum indicum              ??????????????????????????????????????????????????AGGACTTCTTCTTTTTCCGACAGC
Ceratotheca triloba          GTGGATCATACCTTTCGTTCCACTTCCAGTTCCTGTGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Elytraria crenata            ????????????????????????????????????????????????????????????????????????
Thunbergia alata             ?TGGATCATACCTTTCGTTCCACTTCCAGTTCCTATGCTAATAGGAGTGGGACTTCTTCTTTTTCCAATAGA
Barleria prionitis           ????????????????????????????????????????????????????????????????????????
Jacaranda sparrei            GTGGATCATACCTTNCGTTNCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Kigelia africana             GTGGATCATACCTTTCCTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Catalpa speciosa             GTGGATCATACCTTTCCTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCC??CAGC
Schlegelia parviflora        GTGGATCATACCTTTCGTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Synapsis ilicifolia          GTGGATCATACCTTTCGTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Petrea racemosa              GTGGATCATACCNTTCGTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Stachytarpheta dichotoma     GTGGATCATACCTTTCGTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Verbena bracteata            GTGGATCATACCTTTTGTTCCACTTCCGGTTTCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Proboscidea louisianica      GTGGATCATACCTTTTGTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Martynia annua               GTGGATCATACCTTTTGTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Hemimeris sabulosa           GTGGATCATACCTTTCGTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Scrophularia californica     GTGGATGATACCCTTCCTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Retzia capensis              ????ATCATACCTTTCGTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Halleria lucida              GTGGATCATACCTTTTGTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Angelonia pubescens          GTGGATCATACCTTTCGTTCCCCTTCCAGTTCCTATGTTAATAGGGGTGGGACTCCTTCTTTTTCCGACAGT
Veronica catenata            GTGGATCATACCTT?CGTT?CACTTCCAGTTCCTATGTTAATAGGGGTAGGACTTCTTCTTTTTCCGACAG?
Streptocarpus holstii        ATGGATCATATCTTTCCTTCCACTTCCAGTTCCTATGTTAATAGGAATGGGACTTCTTCTTTTTCCGACAGC
Nematanthus hirsutus         GTGGATCATACCTTTCGTTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Calceolaria mexicana         GTGGATCATACCTTTTATTCCACTTCCAGTTCCTATTTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Jovellana sp.                GTGGATCATACCTTTTATTCCACTTCCAGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Ligustrum vulgare            GTGGATCATACCTTTTGTTCCACTTCCAGTTCCTATGTTAATAGGAGTCGGACTTCTTCTTTTTCCGACGGC
Borago officinalis           ?TGGATCATACCTTTGCTTCCACTTATGGTTCCTATGTTAATAGGAGTGGGACTTCTTCTTTTTCCGACAGC
Gentiana procera             GTGGATTATACCTTTCATTCCACTTCCCGTTCCTATATTCCTAGGGTTGGGTCTTCTTCTTTTTCCAAGAAC
Nicotiana tabacum            GTGGATCATACCTTTCATTCCACTTCCAGTCCCTATGTTAATAGGAGCGGGACTTTTTCTTTTTCCAACGGC

Thomandersia hensii          AACAAAAAATCTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Thomandersia laurifolia      AACAAAAAATCTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Bartsia alpina               AACAAAATATTTTCGCCGTATGTGGGCCTTTATCAGTATTTTACTGTTAAGTATAGTCATGATTTTTTCAAC
Lindenbergia phillipensis    AACAAAAAATCTTCGTCGTATTTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Paulownia tomentosa          AACAAAAAATCTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Mimulus aurantiacus          AACAAAAAGCCTTCGTCGTATGTGGGCTTTTTCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Callicarpa dichotoma         AACAAAAAAATCTTCGTCGTATGTGGGCTTTTCCAAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Lamium purpureum             AACAAAAAAATCTTCATCGTATGTGGGCTTTTCCAAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Sesamum indicum              AACAAAAAAGCTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Ceratotheca triloba          AACAAAAAAGCTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Elytraria crenata            ????????????????????????????????????????????????????????????????????????
Thunbergia alata             AACACAAAATCTTCGCCGTCTGTGGGCTTTTGTAACTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Barleria prionitis           ????????????????????????????????????????????????????????????????????????
Jacaranda sparrei            AACAAAAAAATCTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Kigelia africana             AACAAAAAAATCTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTGCAAC
Catalpa speciosa             AACAAAAAAATCTTCGTCGTATGTGGGCTTTTCTGAGTATTTTATTGTTAAGTATAGTCATGATTTTTGCAAC
Schlegelia parviflora        AACAAAAAATCTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Synapsis ilicifolia          AAAAAAAAATCTTCGTCGTATGCGGGCTTTCCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Petrea racemosa              AACAAAAAATCTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAT
Stachytarpheta dichotoma     AACAAAAAATCTTCGTCGTATGTGGGCTTTGCCGAGTATTTTATTGTTAAGTATAGTCATRATTTTTTCA--
Verbena bracteata            AACAAAAAA??TTCGTCGTATGTGGGCTTTTCCGAGTATTTTAGTGTTAAGCATAGTCATGATTTTTTCAAC
Proboscidea louisianica      AACAAAAAGTCTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Martynia annua               AACAAAAAAATCTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Hemimeris sabulosa           AACAAAAAATATTCGTCGTATGTGGGCTTTGCCAAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Scrophularia californica     AACAAAAAAAACTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAT
Retzia capensis              AACAAAAAAATCTTCGTCGTATGTGGGCTTTTGCGAATATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Halleria lucida              AACAAAAAAATCTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Angelonia pubescens          AACAAAAAAATCTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTTATGATTTTTTCAAC
Veronica catenata            ???AAAAATTTTT?GTMG?ATGT?GTCTTTTCTAAGTATTTTATTGTTAAG?ATAGTCATGATTTTTGCAAC
Streptocarpus holstii        AACAAAAGATCTTCGTCGTATGTGGGCCTTTACAAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAT
Nematanthus hirsutus         AACAAAAAAATCTTCGTCGTATGTGGGCCTTGTTGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAC
Calceolaria mexicana         AACCAAAAGTATTCGGCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTGCAAC
Jovellana sp.                AACCAAAAATATTCGGCGTATGTGGGCCTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTGCAAC
Ligustrum vulgare            AACAAAAAAATCTTCGTCGTATGTGGGCTTTTCCGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAT
Borago officinalis           AACAAAAAAATCTTCGTCGTATGTGGGCTTTTCAGAGTATTTTATTGTTAAGTATAGTCATGATTTTTTCAAT
Gentiana procera             AACAATCAAATTTCGTCGTATGTGGGCCTTTCAGAGTGTTTTATTGTTAAGTATAATCGTGGTTTTTTCAAC
Nicotiana tabacum            AACAAAAAGTTTTCGCCGTATGTGGGCTTTTCAGAGTGTTTTATTGTTAAGCATAGTCATGGTTTTTTCAAT
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Thomandersia hensii	TAATCTGTCTATTCAGCAAATAAATAGGAGTTCTACCTATCAATATGTATGGTCTTGGGCCCTCGATAATGA
Thomandersia laurifolia	TAATCTGTCTATTCAGCAAATAAATAGGAGTTCTACCTATCAATATGTATGGTCTTGGGCCCTCGATAATGA
Bartsia alpina	TAATCTGTCTATTCAGCAAATAAATAGCAGGTCCATCGATCAATATGTATGGTCTTGGACCCTCGATAATGC
Lindenbergia phillipensis	TAATCTGTCTATTCAGCAAATAAATAGAAGTTCTATCTATCAACATGTATGGTCTTGGACCCTTGATAATGA
Paulownia tomentosa	AAATCTGTCTATTCAGCAAATAAATAGGAGTTCTATCTATCAATATGTATGGTCTTGGACCCTCGAAAATGA
Mimulus aurantiacus	TAATCTGTCTATTCAGCAAATAAATAGCAGTTCTATCTATCAATATGTATGGTCTTGGACCCTCGATAATGA
Callicarpa dichotoma	TAATCTGTCTATTCAGCAAATAAATAGCAGTTCTATCTATCAATATGTATGGTCTTGGACACTCGATAATGA
Lamium purpureum	TAATCTGTCTATTCAGCAAATAAATAGCAGTTATATCTATCAATATGTATGGTCTTGG????TCGATAATGA
Sesamum indicum	TAA?CTGTCTATTCAGCAAATAAATAGCAGTTCTATCTATCAATATGTATGGTCTTGGACC?TCGATAATGA
Ceratotheca triloba	TAATCTGTCTATTCAGCAAATAAATAGCAGTTCTATCTATCAATATGTATGGTCTTGGACCCTCGATAATGA
Elytraria crenata	??
Thunbergia alata	CAATGTGTCTATTGAACAAATAAATAGCAGTTCTATATATCAATATGTATGGTCTTGGACGCTCAATAATGA
Barleria prionitis	??
Jacaranda sparrei	TAATCTGTCTATTCAGCAAATAAATAGCAGTTCTATATATCAATATGTATGNTCTTGGACCCTCGATAATGA
Kigelia africana	TAATCTGTCTATTCAGCAAATAAATAACAGTTCTATCTATCAATATGTATGGTCTTGGACCCTCGATAATGA
Catalpa speciosa	TAATCTGTCTATTCAGCAAATAAATACGAGTTCTATCTATCAATATTTATGGTCTTGGACCCTCGATAATGA
Schlegelia parviflora	TAATCTGTCTATTCAGCAAATAAATAGCAGTTCTATCTATCAATATGTATGGTCTTGGACCCTCGATAATGA
Synapsis ilicifolia	TAATCTGTCTATTCAGCAAATAAATAGCAGTTCTATCTATCAATATGTATGGTCTTGGACTCTCGATAATGA
Petrea racemosa	TAATCTGTCTATTCAGCAAATAAATAGCAGTTCTATCTATCAATATGTATGGTCTTGGACCCTCGATAATGA
Stachytarpheta dichotoma	-AATCTGTCTATT???????????TAGCAGTTCTATCTATCAATATGTAGGGTCTTGCACTCTCGATAATGM
Verbena bracteata	TAACCTTTCTATTCTGCAAATAAATAGCAGTTCTATCTATCAATATGTATGGTCTTGGACCCTCGATAATGA
Proboscidea louisianica	TAATCTGTCTATTCAACAAATAGATAGCAGTTCTATCTATCAATATGTATGGTCTTGGACCCTCGATAATGA
Martynia annua	TAATCTGTCTATTCAACAAATAAATAGCAGTTCTATCTATCAATATGTATGGTCTTGGACCCTCGATAATGA
Hemimeris sabulosa	AAATCTGTCTATTCTACAAATAAATAGTAGTTCTATTTATCAATATATATGGTCTTGGACCCTCGATAATGA
Scrophularia californica	TAATCTGTCTATTCAGCAAATAAATAGCAATTTTATCTATCAATATGTATGGTCTTGGACGCTCGATAATGA
Retzia capensis	TAATCTGTCTATTCAGCAAATAAATAGCAGTTCTATCTATCAATATGTATGGTCTTGGACCCTCGATAATGA
Halleria lucida	TAATCTGTCTATTCAGCAAATAAATAGCAGTTCTATCTATCAATATGTATGGTCTTGGACCCTCGATAATGA
Angelonia pubescens	AAATCTGTCTATTCAGCAAATAAATAGCAATTCTATCTATCAATACGTATGGTCTTGGACCCTCGATAATGA
Veronica catenata	TAATCTGTCTATTGAGCAAATAAATAGCAATTCTATCTATCAATATGTCTGGTCTTGGGCTCTCAATAATGA
Streptocarpus holstii	TAATCTGTCTATTCAGTTAATAAATAGCAGTTCTATGTATCAATATATATGGTCTTGGACTCTCGATAATGA
Nematanthus hirsutus	AAACCTGTCTATTCAGCTAATAAATAGCAGTTCCATCTATCAATATGTATGGTCTTGGACCCTTGATAATGA
Calceolaria mexicana	TAACCTGTCTATTCAGTTAATAAATAGCAGTTCTATCTATCAATATGTATGGTCGTGGACACTCAATAATGA
Jovellana sp.	TAACCTGTCTATTCAGTTAATAAATAGCAGTTCTATCTATCAATATGTATGGTCTTGGACACTCAATAATGA
Ligustrum vulgare	TAATCTGTCTATTCAGCAAATAGATAGTAGTTGTATCTACCAATATGTATGGTCTTGGACCATCAATAATGA
Borago officinalis	CAATTTGTCTATTCAACAAATAAATAGGAGTTCTATCTACCAATATCTATGGTCTTGGACCATTAATAATGA
Gentiana procera	CAATCTGTCTATTCAACAAATAAATAGCAACTCTATTTATCAATATGTATGGTCTTGGCTCATTATTAACGA
Nicotiana tabacum	CTACCTGTCTATTCAGCAAATAAATAGCAGTTCTTTTTATCAATATGTATGGTCTTGGATCATCAATAATGA

Thomandersia hensii	TTTTTCTTTAGAATTCGGATACTTGATCGATCCACTTACTTCTATTATGTCAATGTTAATCACTACTGTTGG
Thomandersia laurifolia	TTTTTCTTTAGAATTCGGATACTTGATCGATCCACTTACTTCTATTATGTCAATGTTAATCACTACTGTTGG
Bartsia alpina	TTTTTCTTTCGAATTCGGCTACTTCATTGATCCACTTACTTCTATTATGTTACTGTTAATCAGTACTGTTGG
Lindenbergia phillipensis	TTTTTCTTTAGAATTCGGCTACTTTCTTGATCCACTTACTTCTATTATGTTACTGTTAATCACTACTGTTGG
Paulownia tomentosa	TTTTTCTTTAGAATTTGGCTACTTGATCGATCCACTTACTTCTATTATGTTAATGTTAATCACTACTGTTGG
Mimulus aurantiacus	TTTTTCTTTAGAATTTGGCTACTTGATCGATCCACTTACTTCTATTATGTTAATGTTAATCACTACTGTTGG
Callicarpa dichotoma	TTTTTCTTTAGAATTCGGCTACTTGATCGATCCACTTACTTCTATTATGTTAATGTTAATCACTACTGTTGG
Lamium purpureum	TTTTTCTTTAGAATTTGGCTGCTTAATTGATCCGCTTACTTCTATTATGTTGATGTTAATCACTACTGTTGG
Sesamum indicum	TTTTTCTTTAGAATTCGGCTACTTGATCGATCCACTTACTTCTATTATGTCAATGTTAA?CACTACTGTTGG
Ceratotheca triloba	TTTTTCTTTAGAATTTGGCTACTTGATCGATCCACTTACTTCTATTATGTTAATGTTAATCACTACTGTTGG
Elytraria crenata	??CACTACCGTTGG
Thunbergia alata	TTTTTCTTTAGAATTCGGTTACTTGATTGATTCGCTTACTTCTATTATGTCAATGTTAATCACTACTGTTGG
Barleria prionitis	??CACTACGGTTGG
Jacaranda sparrei	TTTTTCTTTAGAATTCGNNTACTTGATCGATCCACTTACTTCTATTATGTCAATGTTAATCACTACTGTTGG
Kigelia africana	TTTTTCTTTAGAATTTGGCTACTTGATTGATCCACTTACTTCTATTATGTCAATGTTAATCACTACTGTTGG
Catalpa speciosa	TTTTTCTTTAGAATTTGGCTACTTGATTGATCCACTTTCTTCTATTATGTTAATCTTAATCACTACTGTTGG
Schlegelia parviflora	TTTTTCTTTAGAATTCGGCTACTTGATCGATCCACTTACTTCTATTATGTCAATGTTAATAACTACTGTTGG
Synapsis ilicifolia	TTTTTCTTTAGAATTCGGCTACTTGATCGATCCACTTACTTCTATTATGTCAATGTTAATCACTACTGTTGG
Petrea racemosa	CTTTTCTTTAGAATTTGGCTATTTGATCGATCCACTTACTTCTATTATGTCAATGTTAATCACTACTGTTGG
Stachytarpheta dichotoma	TTTTTCTTTAGAATTCGGCTACTTGATCGATCCACTTACTTCTATTATGTCAATGCTAATCACTACTGTTGK
Verbena bracteata	TTTTTCTTTAG???
Proboscidea louisianica	TTTTTCTTTAGAATTCGGCTACTTGATCGATCCACTTACTTCTATTATGTTAATGTTAATCACTACTGTTGG
Martynia annua	TTTTTCTTTAGAATTCGGCTACTTGATCGATCCACTTACTTCTATTATGTTAATGTTAATCACTACGGTTGG
Hemimeris sabulosa	TTTTTCATTAGAATTCGGCTACTTGATGGATCCACTTACTTCTATTATGTCAATGTTAATCACTACTGTTGG
Scrophularia californica	TTTTTCTTTAGAATTCGGCTACTTGATAGATCCGCTTACTTCTATTATGTTAATGTTAATCACTACTGTTGG
Retzia capensis	TTTTTCTTTAGAATTCGGCTACTTGATCGATCCGCTTACTTCTATTATGTTAATGTTAATCACTACTGTTGG
Halleria lucida	TTTTTCTTTAGAATTCGGCTACTTGATCGATCCACTTACTTCTATTATGTTAATGTTAATCACTACTGTTGG
Angelonia pubescens	TTTTTCTTTCGAATTCGGCTACTTGATCGATCCGCTTACTTCTATTATGTCAATGTTAATCACTACTGTTGG
Veronica catenata	TTTTTCTTTAGAATTCGGATACTTGATTGATCCACTTACTTCTATTATGTCAATGTTAATCACTACTGTTGG
Streptocarpus holstii	TTTTTGTTTAGAATTTGGGTACTTTATTGATCCACTTACTTCTATTATGTCAATGTTAATCACCACCGTTGG
Nematanthus hirsutus	TTTTTCTTTAGACTTTGGTTACTTGATCGATCCACTTACTTCTATTATGTCAAT?TTAATCACTACTGTTGG
Calceolaria mexicana	TTTTTCTTTAGAATTTGGCTACTTGATTGATCCACTTACTTCTATTATGTCAATATTAATAACTACTGTTGG
Jovellana sp.	TTTTTCTTTAGAATTTGGCTACTTGATCGATCCACTTACTTCTATTATGTCAATATTAATCACTACTGTTGG
Ligustrum vulgare	TTTTTCTTTAGAATTCGGATACTTGATCGATCCACTTACTTCTATTATGTCAATGTTAATCACTACTGTTGG
Borago officinalis	TTTTTCTTTAGAATTCG???ACTTGATCGACCCACTTACTTCTATTATGTCAATATTAATCACTACTGTTGG
Gentiana procera	TTTTTCTTTAGAGTTGGGTTATTTGCTAG??CCCCTTACGTCTATTATGTCAATATTAATCACTACCGTTGG
Nicotiana tabacum	TTTTTCTTTAGACTTCGGATACTTGATCGACCCACTTACTTCTATTATGTCAATATTAATCACTACGGTTGG

Thomandersia hensii	AATTTTGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Thomandersia laurifolia	AATTTTGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Bartsia alpina	GATTATGGTTCTTATTTATAGTGATAATTATATGGCCCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Lindenbergia phillipensis	GATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Paulownia tomentosa	AATTATGGTTATTATTTATAGTGATAATTATATGACTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Mimulus aurantiacus	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTAAGATTTTTTGCTTATAT
Callicarpa dichotoma	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Lamium purpureum	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGGTTTTTTGCTTATAT
Sesamum indicum	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGATATTTTTTGCTTATAT
Ceratotheca triloba	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Elytraria crenata	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Thunbergia alata	AATTATGGTTCTGATTTATAGTGATAATTATATGGCTCATGATCAAGGATACTTGAGATTTTTTGCTTATAT
Barleria prionitis	AATTACGGTTCTTCTTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTCTAT
Jacaranda sparrei	NNTTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Kigelia africana	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Catalpa speciosa	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Schlegelia parviflora	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Synapsis ilicifolia	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTAAGATTTTTTGCTTATAT
Petrea racemosa	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Stachytarpheta dichotoma	W?TT?TGGTTCTT?TTTWT??????????ATATGGCTTACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Verbena bracteata	??????????????????????????????ATTATATGGCTCACTATCAAGGATACTTGAGATTTTTTGCTTATCT
Proboscidea louisianica	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Martynia annua	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Hemimeris sabulosa	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCATGATCAAGGATACTTGAGGTTTTTTTCTTATAT
Scrophularia californica	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Retzia capensis	AATTATGGTTATTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Halleria lucida	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGMTTATAT
Angelonia pubescens	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTTCTTATAT
Veronica catenata	AATTTTGGTTCTTATTTATAGTGATAATTATATGGCTCATGATCAAGGCTACTTGAGATTTTTTTCTTATAT
Streptocarpus holstii	AATTATGGTTCTTCTTTATAGTGATAATTATATGGCTTACGATCAAGGATATTTGAGATTTTTTGCTTATAT
Nematanthus hirsutus	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTTACGATCAAGGATATTTGAGATTTTTTTCTTATAT
Calceolaria mexicana	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Jovellana sp.	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCACGATCAAGGATACTTGAGATTTTTTGCTTATAT
Ligustrum vulgare	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCATGATCAAGGATACTTGAGATTTTTTGCTTCTAT
Borago officinalis	AATTCTGGTTCTTATTTATAGTGATAATTATATGTCTCATGATCAAGGATATTTGAGATTTTTTGCTTATAT
Gentiana procera	AATTACGGTTCTTTTTTATAGTGATAATTATATGGCTCACGATCAATCCTATTTGAAATTTTTCACTTATAT
Nicotiana tabacum	AATTATGGTTCTTATTTATAGTGATAATTATATGGCTCATGATCAAGGCTATTTGAGATTTTTTGCTTATAT

Thomandersia hensii	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Thomandersia laurifolia	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Bartsia alpina	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCAAATTTGATACAAATTTATATTTTTTTGGGA
Lindenbergia phillipensis	GAGTTTTTTTCAGTACTTCGATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Paulownia tomentosa	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Mimulus aurantiacus	GAGTTTTTTTCAATACTTCCATGTTGGGATTAATTACTAGTTCAAATTTGATACAAATTTATATTTTTTTGGGA
Callicarpa dichotoma	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCAAATTTGATACAAATTTATATTTTTTTGGGA
Lamium purpureum	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCAAATTTGATACAAATTTATATTTTTTTGGGA
Sesamum indicum	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Ceratotheca triloba	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Elytraria crenata	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Thunbergia alata	GAGTTTTTTTTAGTACTTCCATGTTAGGATTAGTTACTAGTTCCAATTTGATACAAATTTATATTTTTTTGGGA
Barleria prionitis	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCCAATTTGATCCAAATTTATATTTTTTTGGGA
Jacaranda sparrei	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Kigelia africana	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Catalpa speciosa	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Schlegelia parviflora	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Synapsis ilicifolia	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Petrea racemosa	GAGTTTTTTTCAGTACTTCTATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Stachytarpheta dichotoma	GAGTTTTTTTCAGTACTTCTATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Verbena bracteata	GAGTTTTTTT?AGTACTTCTATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Proboscidea louisianica	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Martynia annua	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Hemimeris sabulosa	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTAACTAGTTCAAATTTGATACAAATTTATATTTTTTTGGGA
Scrophularia californica	GAGTTTTTTTTAGTACTTCCATGTTAGGATTAGTTACTAGTTCTAATTTGATACAAATTTATATTTTTTTGGGA
Retzia capensis	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Halleria lucida	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Angelonia pubescens	GAGTTTTTTTCAGTACTTCGATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Veronica catenata	GAGTTTTTTTCAGTACTTCAATGTTAGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Streptocarpus holstii	GAGTTTTTTTCAGTATTTCTATGTTGGGATTAGTTACTAGTTCGAATTTGATCCAAATTTATATTTTTTTGGGA
Nematanthus hirsutus	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA
Calceolaria mexicana	GAGTTTTTTTCAGTACTTCTATGTTAGGATTAGTTACTAGTTCAAATTTGATACAAATTTATATTTTTTTGGGA
Jovellana sp.	GAGTTTTTTTCAGTACTTCTATGTTAGGATTAGTTACTAGTTCAAATTTGATACAAATTTATATTTTTTTGGGA
Ligustrum vulgare	GAGTTTTTTTCAGTACTTCCATGTTAGGATTAGTTACTAGCTCCAATTTGATACAAATTTATATTTTTTTGGGA
Borago officinalis	GAGTTTTTTTCAGTACGTCCATGTTAGGATTAGTTACTAGTTCTAATTTGATACAAATTTATATTTTTTTGGGA
Gentiana procera	GAGTTTTTTTCAGTACTTCAATGCTAGGATTAGTTACTAGTGCTAATTTGATACAAATTTATATTTTTTTGGGA
Nicotiana tabacum	GAGTTTTTTTCAGTACTTCCATGTTGGGATTAGTTACTAGTTCGAATTTGATACAAATTTATATTTTTTTGGGA

Thomandersia hensii	ATTGGTTGGAATGTGTTCATATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Thomandersia laurifolia	ATTGGTTGGAATGTGTTCATATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Bartsia alpina	ATTGGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Lindenbergia phillipensis	ATTGGTTGGAATGTGTTCCTATCTATTAATAGGATTTTGGTTCACACGACCTCCTGCGACAAATGCTTGTCA
Paulownia tomentosa	ATTGGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Mimulus aurantiacus	ACTCGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTCCGGCGGCAAATGCTTGTCA
Callicarpa dichotoma	ATTGGTTGGAATGTGTTCCTATTTATTAATAGGGTTTTGGTTCACAAGACCTCTTGCAGCAAATGCTTGTCA
Lamium purpureum	GTTGGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGCCA
Sesamum indicum	ATTAGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTTCTGCGGCAAATGCTTGTCA
Ceratotheca triloba	ATTAGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Elytraria crenata	ATTGGTTGGAATGTGTTCCTATCTATTAATAGGATTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Thunbergia alata	ATTGGTTGGAATATGTTCCTATCTATTAATAGGATTTTGGTTCACACGACTTCCTGCAGCAAATGCTTGTCA
Barleria prionitis	ATTGGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTCTTGCAGCAAATGCTTGTCA
Jacaranda sparrei	ATTGGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Kigelia africana	ATTAGTTGGAATGTGTTCCTATCTATTAATAGGCTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Catalpa speciosa	ATTGGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGSAAATGCTTGTCA
Schlegelia parviflora	ATTGGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Synapsis ilicifolia	ATTGGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Petrea racemosa	ATTGGTTGGAATGTGTTCCTATTTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Stachytarpheta dichotoma	ATTAGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTACTGCAGCAAATGCTTGTCA
Verbena bracteata	ATTGGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAACGCTTGTCA
Proboscidea louisianica	ATTGGTTGGAATGTGTTCTTATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Martynia annua	ATTGGTTGGAATGTGTTCTTATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Hemimeris sabulosa	GTTGGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTACTGCAGCAAATGCTTGTCA
Scrophularia californica	ATTGGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCAGCAAATGCTTGCCA
Retzia capensis	ATTGGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Halleria lucida	ATTGGTTGGAATGTGTTCCTATCTATTAATAGGGTTTTGGTTCACACGACCTCCTGCGGCAAATGCTTGTCA
Angelonia pubescens	ATTGGTTGGAATGTGTTCTTATCTATTAATAGGGTTTTGGTTTACACGACCTCTTGCGGCAAATGCTTGTCA
Veronica catenata	ATTGGTTGGAGTCTGTTCCTATTTATTAATAGGTTTTTGGTTTACAAGACCTCTTGCAGCAAATGCTTGTCA
Streptocarpus holstii	ATTGGTTGGAATGTGTTCCTATCTATTAATAGGCTTTTGGTTTACACGACCTTTTGCGGCAAATGCTTGTCA
Nematanthus hirsutus	ATTGGTTGGAATGTGTTCCTATATATTAATAGGATTTTGGTTCACACGACCTCTTGCGGCAAATGCTTGTCA
Calceolaria mexicana	ATTGGTTGGAATGTGTTCCTATCTACTAATAGGGATTTTGGTTCACACGACCTCTTGCGGCAAATGCTTGCCA
Jovellana sp.	ATTGGTTGGAATGTGTTCCTATCTACTAATAGGGTTTTGGTTTACACGACCTCTTGCGGCAAATGCTTGTCA
Ligustrum vulgare	ATTGGTTGGGATGTGTTCCTATTTATTAATAGGGTTTTGGTTCACACGACCTCTTGCGGCAAATGCTTGTCA
Borago officinalis	ATTGGTTGGAATGTGCTCCTATCTATTAATAGGATTTTGGTTTGCACGACCTGCTGCGGCAAATGCTTGTCA
Gentiana procera	ATTGGTTGGGATGTCTTCTTATCTATTAATTGGCTTTTGGTTCACACGACCTCTTGCGGCAAATGCTTGCCA
Nicotiana tabacum	ATTGGTTGGGCTGTGTTCCTATCTATTAATAGGATTTTGGTTTACACGACCTGTTGCGGCAAATGCTTGTCA

Thomandersia hensii	AAAAGCATNNNNNNNNNNNNNNNNNNNNNNTTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Thomandersia laurifolia	AAAAGCATTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Bartsia alpina	AAAGGCGTTTGTAACTAATCGTATAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Lindenbergia phillipensis	AAAAGCGTTTGTAACTAATCGCGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Paulownia tomentosa	AAAGGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Mimulus aurantiacus	AAAAGCCTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTCGGTTTTTTATTGGATAAC
Callicarpa dichotoma	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Lamium purpureum	AAAAGCGTTTGTAACTAATCGTGTAGGAGATTTTGGTTTGTTATTAGGAATTTTGGGTTTTTTATTGGATAAC
Sesamum indicum	AAAAGCCTTTGTAACTAATCGTGTAGGAGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Ceratotheca triloba	AAAAGCCTTTGTAACTAATCGTGTAGGAGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Elytraria crenata	AAAAGCATTTGTAACTAATCGTGTAGGAGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Thunbergia alata	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTTTTGGATAAC
Barleria prionitis	AAAAGCGTTTGTAACTAATCGTGTAGGGGACTTTGGTTTATTATTAGGAATTTTTGGTTTTTTTTTGGATAAC
Jacaranda sparrei	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Kigelia africana	AAAAGCGTTTGTAACTAATCGTG??GGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Catalpa speciosa	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Schlegelia parviflora	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Synapsis ilicifolia	AAAAGCGTTTATAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Petrea racemosa	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Stachytarpheta dichotoma	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Verbena bracteata	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Proboscidea louisianica	AAAAGCCTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Martynia annua	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Hemimeris sabulosa	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Scrophularia californica	AAAGGCATTTGTAACTAATCGTGTAGGGGATTTTGGTTTCTTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Retzia capensis	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTGGGTTTTTTATTGGATAAC
Halleria lucida	AAAGGCATTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Angelonia pubescens	AAAAGCGTTTGTAACTAATCGTGTAGGAGA????????????????AGGAATTTTAGGCTTTTATTGGATAAC
Veronica catenata	AAAGGCATTTGTAACAAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTATAGGTTTTTTATTGGGTAAC
Streptocarpus holstii	AAAAGCATTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTTGGTTTTTTATTGGATAAC
Nematanthus hirsutus	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTACTAGGAATTTTAGGTTTTTTATTGGATAAC
Calceolaria mexicana	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTCGGTTTTTTATTGGATAAC
Jovellana sp.	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTCGGTTTTTTATTGGATAAC
Ligustrum vulgare	AAAGGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Borago officinalis	AAAAGCGTTTGTAACTAATCGTTTAGGCGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC
Gentiana procera	AAAAGCTTTTGTAACTAATCGGGTAGGAGATTTTGGTTTATTATTAGGAATTTTAGGGTTTTTATTGGATAAC
Nicotiana tabacum	AAAAGCGTTTGTAACTAATCGTGTAGGGGATTTTGGTTTATTATTAGGAATTTTAGGTTTTTTATTGGATAAC

Thomandersia hensii	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTCAATAACTTGATTTATAATAAT------GAAGTCAA
Thomandersia laurifolia	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTCAATAACTTGATTTATAATAAT------GAAGTCAA
Bartsia alpina	AGGTAGTTTTGAATTCCGGGATTTATTTGAAATATTCAATCACTTGATTTATAATCAT------GAAGTCAA
Lindenbergia phillipensis	AGGTAGTTTTGAATTTCGGGATTTATTCCAAATCTTCAATAACTTGATTTATAATCAT------GAAGTCAA
Paulownia tomentosa	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTGAATAACTTGATTCATAATAAT------GAAGTCAA
Mimulus aurantiacus	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATAGTCAATAGCTTGATTTATAATAAT------GAAGTTAA
Callicarpa dichotoma	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATACTCAATAACTTGATTTATAATAAT------GAAGTCAA
Lamium purpureum	AGGTAGTTTTGAATTTCGAGATTTATTCGAAATACTTAATAACTTGATTTATAATAAT------GAAGTCAA
Sesamum indicum	CGGTAGTTTTGAATTTCAGGATTTATTCGAAATATTCAATAACTTGATTTATAATAAT------CAAGTTAA
Ceratotheca triloba	AGGTAGTTTTGAATTTCAGGATTTATTCGAAATATTCAATAACTTGATTTATAATAAT------GAAGTTAA
Elytraria crenata	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTCAATAACTTGGTTTATAAT---------GAAGTCAA
Thunbergia alata	AGGTAGTTTTGAATTTCGGGATGTATTCCAAATATTAAATAACTTGGTTGACAATAAT------GAAGTAAA
Barleria prionitis	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTCAATAACTTGGTTTACAATAAT------GAAGTCAA
Jacaranda sparrei	AGGTAGTTTTGAATTGCGGGATTTATTCGAAATATTCAATAACTTGATTTATAATAAT------GAAGTAAA
Kigelia africana	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTCAATAACTTGATTTCTAATAAT------GAAGTAAA
Catalpa speciosa	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTCAATAATTTGATTTCTAATAAT------GAAGTAAA
Schlegelia parviflora	AGGGAGTTTTGAATTTCGGGATTTATTCGAAATATTCAATAACTTGATTTATAATAAT------GAAGTAAA
Synapsis ilicifolia	AGGGAGTTTTGAATTTCGAGATTTATTCGAAATATTCAATAACTTGATTTATAATAAT------GAAGTAAA
Petrea racemosa	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTCAATAACTTGCTTTATAATAAT------GAAGTAAA
Stachytarpheta dichotoma	GGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTCAATAACATGATTTATAGTAAT------GAAGTAAA
Verbena bracteata	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTGAATAACTTGATTTATAATAAT------GAAGTCAA
Proboscidea louisianica	AGGTAGTTTTGAATTTCGGGATTTATTTGAAATATTCAATAACTTGATTTATAATAAT------GAAGGAAA
Martynia annua	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTCAATAACTTGATTTATAATAAT------GAAGTAAA
Hemimeris sabulosa	GGGTAGTTTTGAATTTCAGGATTTATTCGAAATCTTCAATAACTTGATTTCGAATAAT------GAAGTAAA
Scrophularia californica	TGGGAGTTTTGAATTTCGGGATTTATTCGAAATATTCAATAACTTGATTTATAATAAT------GAAGTAAA
Retzia capensis	AGGTAGTTTTGAATTTCAGGATTTATTCGAAATATTCAATAACTTGATTTATAATAAT------GAAGTCAA
Halleria lucida	GGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTCAATAACTTGATTTATAATAAT------GAAGTCAA
Angelonia pubescens	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTCAATAACTTGATTTATAATAAT------GAAGTCAA
Veronica catenata	TGGTAGTTTTGAATTTAGGGATTTATTTGAAATATTCAATAACTTGGTTTATAACAAC------CAAGTAAA
Streptocarpus holstii	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTCAATAACTTGATTTATAATAAT------GAAGTCAA
Nematanthus hirsutus	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATATTCACTGACTTGATTTATAATAATAATAATGAAGTCAA
Calceolaria mexicana	GGGTAGTTTTGAATTTCGGGATTTATTTGAAATCTTCAATAACTTGATTTTAAATAAT------CAAATAAA
Jovellana sp.	AGGTAGTTTTGAATTTCGGGATTTATTCGAAATCTTCAATAACTTGATTTCGAATAAT------GAAATAAA
Ligustrum vulgare	AGGTAGTTTCGAATTTAGGGATTTATTCGAAATATTAAATAACTTTATTTCTAAAAAT------GAAGTCAA
Borago officinalis	AGGTAGTTTCGAATTTCTAGATTTATTTGAAATATTCAAAAATTTGATTTCAAATAAT------GAGGTTAA
Gentiana procera	AGGTAGTTTCGAATTTGAGGATTTATTCGAAATAGTGAATAACTTGATTTTTAATAAT------GAAGTAAA
Nicotiana tabacum	AGGGAGTTTCGAATTTAGGGATTTATTCGAAATATTCAATAACTTGATTTATAATAAT------GAAGTCGA

Thomandersia hensii	TTCTCCATTTGTTACTTTGTGCGCTGCTCTACTATTTGCGGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Thomandersia laurifolia	TTCTCCATTTGTTACTTTGTGCGCTGCTCTACTATTTGCGGGTGCAGTTGCCAAATCTGCACAATTTCCCCT
Bartsia alpina	TTCTCCTTTTGTTACTTTGTGTGCTGCTCTAGTATTTGCCGGCGCAGTGGCTAAATCTGCACAATTTCCCCT
Lindenbergia phillipensis	TTCTCCCTTTGTTACTTTGTGTGCTGCTCTATTATTTACCGGTGCAGTCGCAAAATCTGCACAATTTCCCCT
Paulownia tomentosa	TTCTCTTTTTGTTACTTTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Mimulus aurantiacus	TCCCGCTTTTGTTACTTTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCGCAATTTCCCCT
Callicarpa dichotoma	TTTTCCATTTGTTACTTTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Lamium purpureum	TTTTGCACTTGTTATTTTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Sesamum indicum	TTCTCCATTTGTTACTTTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Ceratotheca triloba	TTCTCCATTTGTTACTTTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Elytraria crenata	TTCTCCATTTGTTACTTTGTGTGCTCTTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Thunbergia alata	TCCTCCATTTGTAAATTTGTGTGCTGTTCTTTTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Barleria prionitis	TTCTCTATTTGTTACTTTGTGTGCTGTTCTATTATTTGCCGGTGCAGTTGCTAAATCCGCACAATTTCCGCT
Jacaranda sparrei	TTCTCCATTTGTTATTTTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Kigelia africana	TTTTCCATTTATTACTTCCTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCAGCACAATTTCCCCT
Catalpa speciosa	TTTTCCATTTGGTACTTTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCGGCACAATTTCCCCT
Schlegelia parviflora	TTCTCCATTTGTTACTTTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Synapsis ilicifolia	TTCTCCATTTGTTACTTTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Petrea racemosa	TTCTCCATTTGTTACTTTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Stachytarpheta dichotoma	TTCTCCATTTGTTACTTTGTGTGCTGTTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCCCAATTTCCCCT
Verbena bracteata	TTTTCCATTTGTTACTTTATGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Proboscidea louisianica	TTCTACATTTGTTTCTTTGTGTGCTCTTCTCTTATTTGCCGGTGCAGTTGCTAAATCCGCACAATTTCCCCT
Martynia annua	TTCTACATTTGTTTCTTTGTGTGCTCTTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Hemimeris sabulosa	TTCTCTATTTGTTACTTTGTGTGCTGCTCTATTATTTGCTGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Scrophularia californica	TTCTCCATTTGTTACTTTGTGTGCGGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Retzia capensis	TTCTCCATTTGTTATTTTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCCCAATTTCCCCT
Halleria lucida	TTCTCCATTTGTTACTTTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Angelonia pubescens	TTTTCTATTTGTTACTTTGTGTGCTGCGCTATTATTTGTTGGTGCAGTTGCTAAATCTGCGCAATTTCCCCT
Veronica catenata	TTATCCCTTTGTTACATTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCTGCACAATTTCCTCT
Streptocarpus holstii	TTCTCCATTTGTTACGTTGTGTGCTGCTCTATTATTTTCCGGTGCAGTTGCTAAATCTGCACAATTTCCTCT
Nematanthus hirsutus	TTTTCCGTTTGTTACTTTGTGTGCTGCTCTATTATTTGCCGGTGCAGTTGCTAAATCCGCACAATTTCCCCT
Calceolaria mexicana	TTATCCAATTGCTACTTTGTGTGCTGCTCTATTGTTTGCTGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Jovellana sp.	TTATCCATTTGCTACTTTGTGCGCTGCTCTATTGTTTGCTGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Ligustrum vulgare	TTCTTCATTTGGTACTTTGTGTGCTGCTCTATTATTTACCGGTGCAGTTGCTAAATCTGCACAATTTCCCCT
Borago officinalis	TTTGATATTTGCTACTTTATGTGCCGTTCTATTATTTACCGGTGCAATTGCAAAATCTGCACAATTTCCACT
Gentiana procera	TCCTTTATTTCTTACTTTGTGTGCTGTTCTATTATTTGTTGGTTCTGTTGCTAAATCTGCACAATTTCCCCT
Nicotiana tabacum	TTTTTTATTTGTTACTTTATGTGCTGTTCTCTTATTTGCCGGTGCAGTTGCTAAATCCGCCCAATTCCCCCT

Thomandersia hensii	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACATGCCGCTACTATGGT
Thomandersia laurifolia	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACATGCCGCTACTATGGT
Bartsia alpina	TCATGTATGGTTACCCGATGCTATGGAGGGACCTACTCCTATTTCCGCTCTGATACATGCTGCTACCATGGT
Lindenbergia phillipensis	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTCATACATGCCGCTACGATGGT
Paulownia tomentosa	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACATGCTGCTACTATGGT
Mimulus aurantiacus	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACATGCCGCTACTATGGT
Callicarpa dichotoma	TCATGTGTGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACTTGCTGCTACTATGGT
Lamium purpureum	TCATGTGTGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACATGCTGCTACTATGGT
Sesamum indicum	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCCGCTCTTATACATGCTGCTACTATGGT
Ceratotheca triloba	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCCGCTCTTATACATGCTGCTACTATGGT
Elytraria crenata	TCATGTATGGTTACCCGATGCTATGGAGGGGCCCACTCCTATTTCGGCTCTTATACATGCTGCTACTATGGT
Thunbergia alata	TCATGTATGGTTACCAGATGCTATGGAAGGACCCACTCCGATTTCAGCTCTTATCCATGCTGCTACTATGG?
Barleria prionitis	TCATGTATGGTTACCTGATGCTATGGAGGGGCCCACCCCTATTTCGGCTCTTATCCATGCCGCTACTATGGT
Jacaranda sparrei	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACATGCTGCTACTATGGT
Kigelia africana	TCATGTATGGTTACCTGATGCTATGGAGGGACCGACTCCTATTTCGGCTCTTATACATGCTGCTACTATGGT
Catalpa speciosa	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACATGCTGCTACCATGGT
Schlegelia parviflora	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACATGCTGCTACCATGGT
Synapsis ilicifolia	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACATGCTGCTACGATGGT
Petrea racemosa	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACTTGCTGCTACTATGGT
Stachytarpheta dichotoma	TCATGTATGGTTACCCGATGCTATGGAGGGACCCACTCCCATTTCGGCTCTTATACGTGCTGCTACTA?GGT
Verbena bracteata	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCAGCTCTTATACATGCTGCTACTATGG?
Proboscidea louisianica	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACATGCTGCTACTATGGT
Martynia annua	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACATGCTGCTACTATGGT
Hemimeris sabulosa	TCATGTATGGTTACCTGATGCTATGGAAGGGCCCACTCCTATTTCGGCTCTTATACATGCCGCTACTATGGT
Scrophularia californica	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACA??CTGCTACTATGGT
Retzia capensis	TCATGTATGGTTACCTGATGCTATGGAGGGACCTACTCCTATTTCGGCTCTTATACATGCTGCTACTATGGT
Halleria lucida	TCATGTATGGTTACCTGATGCTATGGAGGGACCCACTCCGATTTCGGCTCTTATACATGCTGCTACTATGGT
Angelonia pubescens	TCATGTATGGTTACCCGATGCTATGGAGGGACCCACTCCTATTTCGGCTCTTATACATGCTGCTACTATGGT
Veronica catenata	TCATATATGGTTACCCGATGCTATGGAAGGACCCACTCCCATTTCAGCTCTTATAC?TGCTGCCACTATGGT
Streptocarpus holstii	TCATGTATGGTTACCTGATGCTATGGAGGGACCTACTCCTATTTCAGCTCTCATACATGCTGCTACTATGGT
Nematanthus hirsutus	TCATGTATGGTTACCTGATGCTATGGAGGGACCTACTCCTATTTCGGCTCTTATACATGCTGCTACTATGGT
Calceolaria mexicana	TCATGTATGGTTACCTGATGCTATGGAGGGACCTACTCCTATTTCGGCTCTTATACATGCTGCTACTATGGT
Jovellana sp.	TCATGTATGGTTACCTGATGCTATGGAGGGACCTACTCCTATTTCGGCTCTTATACATGCTGCTACTATGGT
Ligustrum vulgare	TCATGTATGGTTACCTGATGCTATGGAGGGACCTACTCCTATTTCGGCTCTTATACATGCTGCTACTATGGT
Borago officinalis	TCATGTATGGTTACCTGATGCCATGGAGGGACCTACCCCTATTTCGGCTCTTATCCAT?CTGCTACTATGGT
Gentiana procera	TCATGTCTGGTTGCCTGATGCTATGGAGGGGCCCACTCCTATTTCTGCTCTTATACATGCTGCCACTATGGT
Nicotiana tabacum	TCATGTATGGTTACCTGATGCCATGGAGGGGCCTACTCCCATTTCGGCTCTTATACATGCTGCTACTATGGT

Thomandersia hensii	AGCAGCGGGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATCAATTTGAT
Thomandersia laurifolia	AGCAGCGGGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAATTATACCTTATATAATGAATTTGAT
Bartsia alpina	AGCGGCGGGGGTTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCCTATATAATGAATTTTAT
Lindenbergia phillipensis	AGCGGCGGGGATTTTTCTTGTAGCTCGACTTCTTCCTCTTTTCATAGTTATACCCTATATAATGAATTTTAT
Paulownia tomentosa	AGCAGAGGGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTAAT
Mimulus aurantiacus	AGCAGCGGGGATTTTTCTTGTAGCTCGTCTTCTTCCTCTTTTTATAGTTATACCTTATATAATGAAATTAAT
Callicarpa dichotoma	CGCAGCAGGGATTTTTCTCGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTAAT
Lamium purpureum	AGCRGCGGGGRTTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCCTAGTTATACCTTATATAATGAATTTAAT
Sesamum indicum	AGCAGCGGGGATTTTTCTTGTAGCTCGTCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTTAT
Ceratotheca triloba	AGCAGCGGGGATTTTTCTTGTAGCTCGTCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTTAT
Elytraria crenata	AGCAGCGGGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCGTAGTTATACCTTCTACAATGAATTTCAT
Thunbergia alata	??????????ATTTTTCTTGTARCTCGACTTTTTCCTCTTTTTATAGTTATACCTTATATAATGAATTTCAT
Barleria prionitis	AGCAGCAGGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTCAT
Jacaranda sparrei	AGCAGCGGGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCGTAGTTATACCTTATATAATGAATTTAAT
Kigelia africana	AGCAGCGGGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTTAT
Catalpa speciosa	AGCAGCGGGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTAAT
Schlegelia parviflora	AGCAGCGGGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTAAT
Synapsis ilicifolia	AGCAGCGGGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTAAT
Petrea racemosa	AGCAGCGGGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTAAT
Stachytarpheta dichotoma	A???GCAGGTATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTAAT
Verbena bracteata	AGCA?CGGGGATTTTTCTTGTAGCTCGTCTTCTTCCTCTTTTCATACTTATACCTTATATAATGAATTTGAT
Proboscidea louisianica	AGCAGCGGGGATTTTTCTTGTAGCCCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTTAT
Martynia annua	AGCAGCGGGGATTTTTCTTGTAGTCCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTTAT
Hemimeris sabulosa	AGCGGCGGGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTTATACTTATACCTTATATAATGAATTTAAT
Scrophularia californica	????????GGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTCTATAATGAATTTAAT
Retzia capensis	???????GGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTAAT
Halleria lucida	AGCAGCGGGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTAAT
Angelonia pubescens	AGCAGCTGGTATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTAAT
Veronica catenata	GGCWGYGGGGATTTTTCTTGTAGCTCGCCTTCTTCCCCTTTTCGTAGTTATACCTTATATAATGAATATAAT
Streptocarpus holstii	AGCAGCGGGTATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTTAT
Nematanthus hirsutus	AGCAG??GGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATACCTATACCTTATATAATGAATTTTAT
Calceolaria mexicana	AGCAGCAGGGATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTCAT
Jovellana sp.	AGCAGCGGGGATTTTTCTTGTAGCTCGCCTTCTTCCGCTTTTCATAGTTATACCTTATATAATGAATTTCAT
Ligustrum vulgare	AGCAGCGGGAATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTTATAGTTATACCTTACATAATGAATTTCAT
Borago officinalis	A???GCGGGAATTTTTCTTGTAGCTCGCCTTCTTCCTCTTTTCATAGTTATACCTTATATAATGAATTTTAT
Gentiana procera	AGCAGCGGGAATTTTTCTTGTAGCTCGGCTTCTTCCTCTTTTCATAGTTATACCTCCCATAATGAATTTAAT
Nicotiana tabacum	AGCGGCGGGAATTTTTCTTGTAGCTCGGCTTCTTCCTCTTTTCAGAGTTATACCTTACATAATGTATTTGAT

Thomandersia hensii	CTCGTTGATAGGCATAATCACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGT
Thomandersia laurifolia	CTCGTTGATAGGCATAATCACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Bartsia alpina	CTCCTTGATAGGAATAATCACAGTATTATTAGGAGCGACTTTAGCTGTTGCGCAAAAAGACATTAAAAGGGG
Lindenbergia phillipensis	CTCGTTGATAGGAATAATCACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAAAAGGG
Paulownia tomentosa	CTCGTTGATAGGCATAATCACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGATATTAAAAGGGG
Mimulus aurantiacus	CTCGTCGATAGGCATAATTACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Callicarpa dichotoma	CTCGTTGATAGGTATAATCACATTATTATTAGGAGCTACTTTAGCTCTTACTCAAAAAGATATTAAGAGGGG
Lamium purpureum	TGCGCTGATCGGCCTAATTACACTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGAG
Sesamum indicum	CTCGTTGATAGGCATAATCACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Ceratotheca triloba	CTCGTTGATAGGCATAATCACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Elytraria crenata	TTCGTTGATAGGCATAATCACAGTATTCTTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Thunbergia alata	CTCGTTGATAGGCATAATCACAGTATTATTAGGAGCTACTTTAGCTATTGCTCAAAAAGACATTAAAAGAGG
Barleria prionitis	CTCCTTGATAGGCATAATTACGGTATTATTAGGAGCTACTTTAGCTCTTTCTCAAAAAGACATTAAAAGGGG
Jacaranda sparrei	CTCGTTGATAGGCATAATCACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Kigelia africana	CTCCTTGATAGGCCTAATCACGGTCTTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Catalpa speciosa	CTCCTTGATAGGCCTAATCACAGTCTTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Schlegelia parviflora	CTCGTTGATAGGCATAATCACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Synapsis ilicifolia	CTCGTTGATAGGCATAATTACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Petrea racemosa	CTCGTTGATAGGCATAATAACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Stachytarpheta dichotoma	CTCGTTGATAGGCATAATCACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Verbena bracteata	TTCGTTGATAGGCATAATCACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAAGGC
Proboscidea louisianica	CTCGTTGATAGGAATAATCACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Martynia annua	CTCGTTGATAGGAATAATCACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Hemimeris sabulosa	TTCGTTGATAGGTATAATTACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Scrophularia californica	CTCGTTGATAGGCATAATCACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Retzia capensis	CTCGTTGATAGGTATAATCACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Halleria lucida	CTCGTTGAAAGGTATAAACACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Angelonia pubescens	CTCGTTGATAGGCATAATAACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Veronica catenata	TTCGTTGATAGGTATAATAACACTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGAGG
Streptocarpus holstii	CTCGTTGATAGGCATAACAACAGTATTTTTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGAGG
Nematanthus hirsutus	CTCGTTGATAGGCATAATAACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Calceolaria mexicana	CGCGTTGATAGGTATAATAACCGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Jovellana sp.	CGCGTTGATAGGTATAATAACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Ligustrum vulgare	CTCGTTCATAGGAATAATAACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGGGG
Borago officinalis	CGCATTGATAGGAATAATAACAGTATTATTAGGAGCGACTTTAGCTATTGCTCAAAAAGACATTAAAAGGGG
Gentiana procera	CTCGTTGATAGCAATAATAACAGTCTTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGAGG
Nicotiana tabacum	CTCGGTTATAGGAATAATAACAGTATTATTAGGAGCTACTTTAGCTCTTGCTCAAAAAGACATTAAGAGAGG

Thomandersia hensii	TTTAGCCTATTCGACGATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Thomandersia laurifolia	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Bartsia alpina	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTGGGAATGGGGTCTTATCGAAGTGC
Lindenbergia phillipensis	TTTAGCCTATTCGACAATGTCTCAGTTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Paulownia tomentosa	TTTAGCCTATTCGACAA??TCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Mimulus aurantiacus	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Callicarpa dichotoma	TTTAGCTTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAATGC
Lamium purpureum	TTTAGCTTATTCGACAATGTCTCAATTGGGTTATATGATGTTCGCTCTAGGAATGGGGTCTTATCGAAGTGC
Sesamum indicum	TTTAGCCTATTCGACAATGTCTCAATTGG?TTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Ceratotheca triloba	TTTGGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Elytraria crenata	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Thunbergia alata	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCCCTAGGAATAGGATCTTATCGAAGTGC
Barleria prionitis	TTTAGCCTATTCAACAATGTCTCAATTGGGTTATATGATGCTAGCTCTAGGAATGGGGTCTTTTCGAAGTGC
Jacaranda sparrei	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCAAAGTGC
Kigelia africana	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCACTAGGAATGGGTTCTTATCGAATTGC
Catalpa speciosa	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCACTAGGAATGGGTTCTTATCGAAGTGC
Schlegelia parviflora	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGATCTTATCGAAGTGC
Synapsis ilicifolia	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGATCTTATCGAAGTGC
Petrea racemosa	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTACCGAAGTGC
Stachytarpheta dichotoma	TTTAGCCTATTCGACAATGTCTCAACTGGGTTATATGATGTTAGCTCTAGGAATGGGATCTTATCGAAGTGC
Verbena bracteata	TTT---CTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Proboscidea louisianica	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Martynia annua	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Hemimeris sabulosa	TTTAGCCTATTCAACAATGTCTCAGTTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Scrophularia californica	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGATCTTATAGAAGTGC
Retzia capensis	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Halleria lucida	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGGAGTGC
Angelonia pubescens	TTTAGCCTATTCAACAATGTCTCAGCTGGGTTATATGATGTTAGCTCTCGGAATGGGGTCTTATCGAAGTGC
Veronica catenata	TTTAGCCTATTCGACCATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAGGTGC
Streptocarpus holstii	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Nematanthus hirsutus	TTTAGCCTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCAAAGTGC
Calceolaria mexicana	TTTAGCCTATTCAACAATGTCTCAATTGGGTTATATGATGTTAGCTTTAGGAATGGGGTCTTATCAAAGTGC
Jovellana sp.	TTTAGCTTATTCGACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Ligustrum vulgare	TTTAGCCTATTCGACCATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGAATGGGGTCTTATCGAAGTGC
Borago officinalis	CTTAGCCTATTCCACAATGTCTCAATTGGGTTATATGATGTTAGCTCTAGGTATGGGGTCTTATCAAAATGC
Gentiana procera	TTTAGCATATTCCACAATGTCACAATTGGGTTATATGATGTTAGCTCTTGGTATGGGATCTTATCGAAATGC
Nicotiana tabacum	TTTAGCCTATTCCACAATGTCTCAATTGGGTTATATGATGTTAGCTCTTGGTATGGGGTCTTATCGAAGCGC

Thomandersia hensii TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGATCTGGATCCGTTATTCATTC
Thomandersia laurifolia TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCCGTTATTCATTC
Bartsia alpina TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGGTCTGTTATTCATTC
Lindenbergia phillipensis TTTATTTCATTTGATTACTCATGCTTATTCTAAAGCATTATTATTTTTAGGGTCTGGATCCGTTATCCATTC
Paulownia tomentosa TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCCGTTATTTATTC
Mimulus aurantiacus TTTATTTCATTTGATTACTCATGCTTATTCTAAAGCATTATTATTTTTAGGGTCTGGATCCGTTATTCATTC
Callicarpa dichotoma TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCCGTTATTCATTC
Lamium purpureum TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTGGGTTCCGGATCCGTTATCCATTC
Sesamum indicum TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCCGGATCCGTTATTCATTC
Ceratotheca triloba TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCCGGATCCGTTATTCATTC
Elytraria crenata TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGGTCCGTTATTCATTC
Thunbergia alata TTTATTTCATTTGATTACTCAYGCTTATTCCAAAGCATTATTATTTTTAGCATCTGGATCCGTTATTCATTC
Barleria prionitis TTTATTTCATTTGATTACTCATGCTTATTCAAAAGCATTATTATTTTTAGGGTCTGGGTCTGTTATTCATTC
Jacaranda sparrei TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCCGTTATTCATTC
Kigelia africana TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCTGTTATTCATTC
Catalpa speciosa TTTATTTCATTTGATTACTCATGCCTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCCGTTATTCATTC
Schlegelia parviflora TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCCGTTATTCATTC
Synapsis ilicifolia TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCCGTTATTCATTC
Petrea racemosa TTTATTTCATTTGATTACTCNTGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCCGTTATTCATTC
Stachytarpheta dichotoma TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCTGTTATTCATTC
Verbena bracteata TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTGTTCTTAGGGTCCGGATCTGTTATTCATTC
Proboscidea louisianica TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCTGTTATTCATTC
Martynia annua TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCTGTTATTCATTC
Hemimeris sabulosa TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCGGGATCCGTTATTCATTC
Scrophularia californica TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCCGTTATTCATTC
Retzia capensis TTTATTTCATTTGATTACTCATGCTTATTCAAAAGCATTATTATTTTTAGGGTCTGGATCCGTTATTCATTC
Halleria lucida TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCCGTTATTCATTC
Angelonia pubescens TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGGTCCGTTATTCATTC
Veronica catenata TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGATCCGGATCTGTTATTCATTC
Streptocarpus holstii TTTATTTCATTTAATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGATCTGGATCCGTTATTCATTC
Nematanthus hirsutus TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCCTTATTATTTTTTAGGGTCTGGATCCGTTATTCATTC
Calceolaria mexicana TTTATTTCATTTGATTACTCATGCTTATTCGAAAGCATTATTATTTTTTAGGGTCTGGATCTGTTATTCATTC
Jovellana sp. TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCTGTTATTCATTC
Ligustrum vulgare TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGGTCTGGATCTATTATTCATTC
Borago officinalis TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGATCCGGATCTGTTATTCATTC
Gentiana procera TTTATTCCACTTGATAACTCATGCCTATTCCAAAGCATTATTATTTTTAGGATCAGGATCCATTATTCATTC
Nicotiana tabacum TTTATTTCATTTGATTACTCATGCTTATTCCAAAGCATTATTATTTTTAGGATCCGGATCCATTATTCATTC

Thomandersia hensii AATGGAAACTATTATTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTCTTATGGGCGGTTTAACAAAACA
Thomandersia laurifolia AATGGAAACTATTATTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTCTTATGGGCGGTTTAACAAAACA
Bartsia alpina AATGGAAACTGTTGTTGGCTATTCTCCGGATAAAAGCCAGAATCTGGTTTTTATGGGCGGTTTAAGAAAGTA
Lindenbergia phillipensis AATGGAAACTATTGTTGGTTATTCTCCGGATAACAGTCAGAATATGGTTTTTATGGGTGGTTTAACAAAGTA
Paulownia tomentosa AATGGAAACTATTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTCTTATGGGCGGTTTAACAAAACA
Mimulus aurantiacus AATGGAAACTATTGTTGGCTATTCTCCAGATAAAAGTCAGAATATGGTTCTTATGGGTGGTTTAACAAAACA
Callicarpa dichotoma AATGGAAACTATTGTTGGTTATTCTCCGGATAAAAGTCAGAATATGGTTCTTATGGGTGGTTTAACAAAACA
Lamium purpureum AATGGAAACTCTTGTTGGTTATTCTCCGGATAAAAGTCAGAATATGGTTCTTATGGGTGGTTTAACAAAACA
Sesamum indicum AATGGAAACTATTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTCTTATGGGTGGTTTAAGAAAACA
Ceratotheca triloba AATGGAAACTATTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTCTTATGGGTGGTTTAACAAAACA
Elytraria crenata AATGGAAACTATTGTTGGCTATTCCCCGGATAAAAGTCAGAATATGGTTCTTATGGGTGGTTTAACAAAACA
Thunbergia alata AATGGAAACTATTGTTGGCTATTCGCCGGATAAAAGYCAGAATATGGTTCTGATGGGTGGTTTAACAAAACA
Barleria prionitis AATGGAAAGTATTGTTGGCTATTCCCCTGATAAAAATCAGAATATGGTTCTTATGGGTGGTTTAAGAAAACA
Jacaranda sparrei AATGGAAACTATTGTTGGCTATTCGCCGGATAAAAGTCAGAATATGGTTCTTATGGGCGGTTTAACAAAACA
Kigelia africana AATGGAAACTATTGTTGGCTATTCTCCGCATAAAAGTCAGAATATGGTTCTTATGGGCGGTTTAAAAAAACA
Catalpa speciosa AATGGAAACTATTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTCTTATGGGTGGTTTAACAAAACA
Schlegelia parviflora AATGGAAACTATTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTCTTATGGGCGGTTTAACAAAACA
Synapsis ilicifolia AATGGAAACTATTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTCTTATGGGCGGTTTAACAAAACA
Petrea racemosa AATGGAAACTATTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTCTTATGGGCGGTTTAACAAAACA
Stachytarpheta dichotoma AATGGAGACTATTGTTGGCTATTCTCCGGATAAAAGTCAGGATATGGTTCTTATGGGCGGTTTAACAAAACA
Verbena bracteata AATGGAAACTCTTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTCTTATGGGTGGTTTAACAAAACA
Proboscidea louisianica AATGGAAACTATTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGCTCTTATGGGCGGTTTAACAAAACA
Martynia annua AATGGAAACTATTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGCTCTTATGGGCGGTTTAACAAAACA
Hemimeris sabulosa AATGGAAACTATTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTTTTATGGGCGGTTTAACAAAACA
Scrophularia californica AATGGAAACTGTTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTCTGATGGGCGGTTTAACAAAACA
Retzia capensis AATGGAAACTATTGTTGGGTATTCTCCGGATAAAAGTCAGAATATGGTTCTCATGGGTGGTTTAACAAAACA
Halleria lucida AATGGAAACTATTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTCTGATGGGTGGTTTAACAAAACA
Angelonia pubescens AATGGAAACTATTGTTGGTTATTCTCCGGATAAAAGTCAAAATATGGTTCTTATGGGCGGTTTAACAAAACA
Veronica catenata AATGGAAACTGTTGTTGGTTATTCTCCGGATAAAAGTCAGAATATGGCTCTTATGGGTGGTTTAACAAAATA
Streptocarpus holstii CATGGAAACTCTTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTATTATGGGTGGTTTAACAAAACA
Nematanthus hirsutus CATGGAAACTATTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTCCTTATGGGCGGTTTAACAAAACA
Calceolaria mexicana CATGGAAACTGTTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTCTTATGGGCGGTTTAACAAAACA
Jovellana sp. CATGGAAACTATTGTTGGCTATTCTCCGGATAAAAGTCAGAATATGGTTCTTATGGGCGGTTTAACAAAACA
Ligustrum vulgare AATGGAAACTATTGTTGGCTATTCTCCAGATAAAAGTCAGAATATGGTTCTTATGGGTGGTTTAAGAAAACA
Borago officinalis AATGGAAACTATTGTTGGTTATTCTCCAGATAAAAGTCAGAATATGGTTCTTATGGGAGGTTTAACAAAACA
Gentiana procera AATGGAAAGGCTTGTTGGCTATTCACCCTATAAAAGTCAAAATATGGTTCTTATGGGAGGGTTAGGAAAACA
Nicotiana tabacum AATGGAAACTATTGTTGGATATTCTCCAGCTAAAAGTCAGAATATGGGTCTTATGGGAGGTTTAAGAAAACA

Thomandersia hensii	TGTACCGATTACCAAAACCTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Thomandersia laurifolia	TGTACCGATTACCAAAACCTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Bartsia alpina	TGTACCAATTACCAAAACCTCGTTTTTTATTAGGTACACTTTCCCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Lindenbergia phillipensis	TGTACCAATTACCAAAACCTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCACCTCTTGCTTGTTT
Paulownia tomentosa	TGTGCCAATTACCAAAACCTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCACCTCTTGCTTGTTT
Mimulus aurantiacus	TGTACCTATTACCAAAACTTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Callicarpa dichotoma	TGTACCGATTACCAAAACTTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCACCTCTTGCTTGTTT
Lamium purpureum	TGTACCGATTACCAAAACCTCTTTTTTATTAGGTACACTGTCTCTTTGTGGTATTCCGCCGCTTGCTTGTTT
Sesamum indicum	TGTACCGATTACCAAAACCTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTGGCTTGTTT
Ceratotheca triloba	TGTACCTATTACCAAAACCTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTGGCTTGTTT
Elytraria crenata	TGTACCGATTACCAAAACTGCTTTTCTATTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Thunbergia alata	TRTACCGATTACCAAAACCTCYTTTCTATTAGGTACACTCTCTCTTTGTGGTATTCCGCCYCTTGCTTGTTT
Barleria prionitis	TGTACCGATTACCAAAACCTCTTTTTTATTAGGTACATTTTCTCTTTGTGGTATTCCACCTCTTGCTTGTTT
Jacaranda sparrei	TGTACCGATTACCAAAACCTCGTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Kigelia africana	TGTACCGATTACCAAAACCTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCCCCTCTTGCTTGTTT
Catalpa speciosa	TGTACCGATTACCAAAACCTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Schlegelia parviflora	TGTACCGATTACCAAAACCTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Synapsis ilicifolia	TGTACCGATTACCAAAACCTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Petrea racemosa	TGTACCGATTACCAAAACCTCTTTTTTTTTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Stachytarpheta dichotoma	TGTACCGATTACCAAAACCTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Verbena bracteata	TGTACCGATTACCAAAACTTCTTTTTTATTAGGTACACTTTCGCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Proboscidea louisianica	TGTACCGATTACCAAAACCTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Martynia annua	TGTACCGATTACCAAAACCTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Hemimeris sabulosa	CGTACCGATTACCAAAATCTCCTTTTTAGTAGGTACACTTTCTCTTTGTGGTATTCCACCTCTTGCTTGTTT
Scrophularia californica	TGTACCAATTACCCAAATTTCTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Retzia capensis	TGTACCAATTACCAAAACCTCTTTTTTATTAGGTACACTTTCGCTTTGTGGTATTCCCCCTCTTGCTTGTTT
Halleria lucida	CGTACCGATTACTAAAACCTCTTTTTTTCTTAGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Angelonia pubescens	TGTACCGATTACCAAAACTTCTTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCACCTCTTGCTTGTTT
Veronica catenata	TGTACCGATTACCAAAACCTCTTTTTTTAGTCGGTACACTTTCTCTTTGTGGTATTCCACCCCTTGCTTGTTT
Streptocarpus holstii	TGTACCGATTACCAAGATCTCTTTTTTTATTCGGTACACTTTCTCTTTGTGGTATTCCCCCTCTTGCGTGTTT
Nematanthus hirsutus	TGTACCCATTACCAAAATCTCTTTTTTTATTCGGTACACTTTCTCTTTGTGGTATTCCTCCTCTTGCTTGTTT
Calceolaria mexicana	TGTACCAATTACCAAAATATCTTTTTTTAATTGGTACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Jovellana sp.	TGTACCAATTACCAAAATCTCTTTTTTTATTTGGWACACTTTCTCTTTGTGGTATTCCGCCTCTTGCTTGTTT
Ligustrum vulgare	TGTACCCATTACCCAAATCTCTTTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCACCTCTTGCTTGTTT
Borago officinalis	TGTACCAATTACCAAAACGTCTTTTTTTATTAGGTACACTTTCTCTTTCTGGTATTCCACCTCTTGCGTGTTT
Gentiana procera	TATACCAATTACAAAAACGTCTTTTTTTTTTAGGTACACTTTCTCTTTCTGGTATTCCACCTTTAGCCTGCTT
Nicotiana tabacum	TGTACCAATTAGCAAAATCACATTTTTTATTAGGTACACTTTCTCTTTGTGGTATTCCACCTCTTGCTTGTTT

Thomandersia hensii	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTCGCAATAATAGCTGGGGCCGC
Thomandersia laurifolia	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTCGCAATAATAGCTTGGGTCAC
Bartsia alpina	TTGGTCAAAAGACGAAATTCTTAATGATAGTTGGTTATATTCGCCCATTTTCGCAATACTAGCTTGCGTCAC
Lindenbergia phillipensis	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTCGCAATAATAGCTTGGGCGAC
Paulownia tomentosa	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCACCGATTTTCGCAATAATAGCTTGGGCCAC
Mimulus aurantiacus	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTCGCAATAATAGCTTGGGCCAC
Callicarpa dichotoma	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTCGCAATAATAGCTTGGGCCAC
Lamium purpureum	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTAGCAATAATAGCTTGGGCCAC
Sesamum indicum	TTGGTCAAAAGATGAAATCCTTAATGAGAGTTGGTTGTATTCGCCGATTTTCGCAATAATAGCTTGGGCCAC
Ceratotheca triloba	TTGGTCAAAAGATGAAATTCTTAATGAGAGTTGGTTGTATTCGCCGATTTTCGCAATAATAGCTTGGGCCAC
Elytraria crenata	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTCGTATTCGCCGATTTTTGCAATAATAGCTTGGGCCAC
Thunbergia alata	TTGGTCGAAAGATGAAATTCTGAATGATAGTTGGTTGTATTCGCCKAGTTTCGCAATAATAGCTTGGGCCAC
Barleria prionitis	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCTATTTTCGCAATAATAGCTTGGATCAC
Jacaranda sparrei	TTGGTCAAAAGATGGAATTCTTAATGATAGTTGGTTGTATTCGCCAATTTTCGCAATAATAGCTTGGGCCAC
Kigelia africana	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTCGCAATAATAGCTTGGGCCAC
Catalpa speciosa	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCTATTTTCGCAATAATAGCTTGGGCCAC
Schlegelia parviflora	TTGGTCAAAAGAAGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTCGCAATAATCGCTTGGGCCAC
Synapsis ilicifolia	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTCGCAATAATAGCTTGGGCCAC
Petrea racemosa	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTATATTCGCCGATTTTCGCAATAATAGCTTGGGCCAC
Stachytarpheta dichotoma	TTGGTCAAAAGATGAAATTCTTAACGATAGTTGGTCGTATTCGCCGATTTTCGCAATAATAGCTTGGGCCAC
Verbena bracteata	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCAATTTTCGCAATAATCGCTTGGGGTAC
Proboscidea louisianica	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTCGCAATAATAGCTTGGGCCAC
Martynia annua	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTCGCAATAATAGCTTGGGCCAC
Hemimeris sabulosa	TTGGTCAAAAGATGAAATTCTTAATGACAGTTGGTTATATTCGCCGATTTTCGCAATCATAGCTTGGGCCAC
Scrophularia californica	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTTGCAATAATAGCTTGGGCGAC
Retzia capensis	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTCGCAATAATAGCTTGGGCCAC
Halleria lucida	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTCGCAATAATAGCTTGGGCCAC
Angelonia pubescens	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCTATTTTTGCAGTAATAGCTTGGTCCAC
Veronica catenata	TTGGTCCAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCTATTTTTGCAATAATCGCTTGGACCAC
Streptocarpus holstii	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCTCCGATTTTCGCAATAATAGCTTGGTCCAC
Nematanthus hirsutus	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCCATTTTCGCAATAATAGCTTGGGCCAC
Calceolaria mexicana	TTGGTCAAAAGAGGAGATTCTTAATGATAGTTGGTTGTATTCGCCAATTTTCGCAATAATAGCTTGGGCGAC
Jovellana sp.	TTGGTCAAAAGACGAAATTCTTAATGATAGTTGGTTGTATTCGCCAATTTTCGCAATAATAGCTTGGGCCAC
Ligustrum vulgare	TTGGTCAAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCGATTTTCGCAATAATAGCTTGGAGTAC
Borago officinalis	TTGGTCCAAAGATGCAATTCTTAATGATAGTTGGTTATATTCACCGCTTTTTGCAATAATAGCTTGGTCAAC
Gentiana procera	TTGGTCTAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCAGCCATTTTTGCACTAATAGCTTGGTGTAC
Nicotiana tabacum	TTGGTCCAAAGATGAAATTCTTAATGATAGTTGGTTGTATTCGCCAATTTTCGCAATAATAGCTTGGGCCAC

Thomandersia hensii	AGCANNNNNNNNNNNNNNNNNNNNNNNNNNNGATCTATTTACTTACTTTTGAAGGGCATTTNAACGTTCATTT
Thomandersia laurifolia	AGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAAGGGCATTTAAACGTTCATTT
Bartsia alpina	AGCAGGATTAACTGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGGCATTTAAACGTTCATTT
Lindenbergia phillipensis	AGCAGGGTTAACTGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGGCATTTAAACGTTCATTT
Paulownia tomentosa	AGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGGCATTTAAACGTTCATTT
Mimulus aurantiacus	AGCAGGATTAACCGCATTTTATATGTTTCGGATTTATTTACTTACTTTTGAAGGGCATTTAAACGTTCATTT
Callicarpa dichotoma	AGCAGGATTAACCGCATTTTATATGTTTCGCATCTATTTACTTACTTTTGAGGGGCATTTAAACGTTCATTT
Lamium purpureum	GG??GGATTAACAGCATTTTAT??????????????????????????????????ATTTAAATATTCATTT
Sesamum indicum	AGCAGGATTAACTGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGGCATTTAAACGTTCATTT
Ceratotheca triloba	AGCAGGATTAACTGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGGCATTTAAACGTTCATTT
Elytraria crenata	AGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGGCATTTAAACGTTCATTT
Thunbergia alata	GGMGGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACCTTTGAGGGGCATTTAAACGTGCATTT
Barleria prionitis	GGCGGGATTAACTGCATTTTATATGTTTCGGATCTATTTACTTACGTTTGAAGGGCATTTAAACGTTCATTT
Jacaranda sparrei	AGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGGCATTTAAACGTTCATTT
Kigelia africana	AGCAGGGTTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAAGGGCATTTAAACGTTAATTT
Catalpa speciosa	AGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGGCATTTAAACGTTCATTT
Schlegelia parviflora	AGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGGCATTTAAACGTTCATTT
Synapsis ilicifolia	AGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGG?CATTTAAACGTTCATTT
Petrea racemosa	AGCGGGATTAACCGCATTTTATATGTTTCGGATCTATTTATTGACTTTTGAAGGGCATTTAAACGTTCATTT
Stachytarpheta dichotoma	GGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAAGGGCATTTAAACGTTCATTT
Verbena bracteata	AGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAAGGGCATTTAAACGTTCATTT
Proboscidea louisianica	AGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGGCATTTAAACGTTCATTT
Martynia annua	AGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGGCATTTAAACGTTCATTT
Hemimeris sabulosa	AGCAGGATTAACCGCGTTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGGCATTTAAACGCTCATTT
Scrophularia californica	GGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGG?ATTTAAACGTTAATTT
Retzia capensis	AGCAGGATTAACCGCATTTTATATGTTTCGTATCTATTTACTTACGTTTGAGGGGCATTTAAACGTTCATTT
Halleria lucida	AGCAGGATTCACCGCATTTTATATGTTTAGGATCTATTTACTTACTTTTGAGGGGCATTTAAACATTCATTT
Angelonia pubescens	AGCAGGATTAACTGCATTTTATATGTTTCGGATTTATTTACTTACTTTTGAGGGGCATTTAAATATTTATTT
Veronica catenata	AGCAGCACTAACCGCATTTTATATGTTTCGAATCTATTTACTTACTTTTGAAGGGCACTTTAACAGTAATTT
Streptocarpus holstii	AGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAAGGGCCTTTAAATATTCATTT
Nematanthus hirsutus	AGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGACATTTAAACGTTCATTT
Calceolaria mexicana	AGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGCATTTAAACGTTCATTT
Jovellana sp.	AGCAGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAGGGCATTTAAACGTTCATTT
Ligustrum vulgare	AGCAGGATTAACAGCATTTTATATGTTTCGTATCTATTTACTTACTTTTGAGGGACATTTAAATGTTCATTT
Borago officinalis	TGCGGGATTAAC?GCATTTTATATGTTTCGTATCTATTTTATTACTTTTGAGGGACATTTAAATGTTTATTT
Gentiana procera	GGCGGGATTAACCGCATTTTATATGTTTCGGATCTATTTACTTACTTTTGAAGGGCATTTAAACGCTCATTT
Nicotiana tabacum	

Thomandersia hensii	TCAAAACTACAGTAGCAACCAAAATACCTCTTTATATTCAATATCTCTATGGGGTAAAGGGTGT---TCAAA
Thomandersia laurifolia	TCAAAACTACAGTAGCAACCAAAATACCTCTTTATATTCAATATCTCTATGGGGTAAAGGGTGT---TCAAA
Bartsia alpina	TCAAAATTACACTGGAAACAAAAATATTTGTTTATATTCAATATCTTTATGGGGTAAGGGGTGT---TCGAA
Lindenbergia phillipensis	TCAAAACTACACCGGAAGCAAAAATATCTCTTTATATTCCATATCTCTATGGGGCAAGGGGTGT---TCGAA
Paulownia tomentosa	TCAAAATTACACTGGAAATCAAAATACCTCTTTATATTTAATATCTCTATGGGGTAAAGGGTGT---TCAAA
Mimulus aurantiacus	TCAAAATTACAGTGGAAACCAAAATACCTCTTTATATTCAATATCTCTGTGGGGTAAAGGGTGT---TCAAA
Callicarpa dichotoma	TCAAAATTACAGTGGCAACCAAAATACTTCCTTCTATTCCATATCTCTATGGGGAAAAGGGTGT---TCAAA
Lamium purpureum	CCAAAATTATAGTGGCAA???GAATACCTCTTTCTATTCCATATCCATATGGGGTAAAGGATAT---TCAAA
Sesamum indicum	TCAAAATTACAGTGGCAACCAAAATGCCTCTTTATATTCAATATCTCTATGGGGTAAGGGGTGT---TCAAA
Ceratotheca triloba	TCAAAATTACAATGGCAACCAAAATACTTCTTTATATTCAATATCTCTATGGGGTAAGGGGTGT---TCAAA
Elytraria crenata	TCAAAATTACAGCGGCAACCAAAAGAACTTTTTATATTCAATATCTCTATGGGGTAAAGGGTAC---TCAAA
Thunbergia alata	TCAAAATTACAGCGGCAGCCAAAATGCCTCTTTATATTCAATATCTCTATGGGGCAAGGGGCGT---TCACA
Barleria prionitis	TCAAAATTACAGTGGCAATCAAAATATCTTTTTCTATTCGATATCTCTATGGGGAAAAGGACGT---TCAAA
Jacaranda sparrei	TCAAAATTACAGTGGCAGCCAAAATACTTCTTTCTATTCAATATCTCTATGGGGTAAAGGGTGT---TCAAA
Kigelia africana	TCAAAATTACAGCGGCAGTCAAAATACCTCTTTATATTCAATATCTCTATGGGGTAAGGGGTGT---TCACA
Catalpa speciosa	TCAAAATTACAGTGGCAGCCAAAATACCTCTTTATATTCAATATCTCTATGGGGGAAGGGGTGT---TCACA
Schlegelia parviflora	TCAAAATTTCAGTGGTAACCAAAATACCTCCTTATATTCAATATCTCTATGGGGTAAAAGGTGT---TCAAA
Synapsis ilicifolia	GCAAAATTTCAGTGGCAACCAAAATACCTCCTTATATTCAATATCTCTATGGGGTAAAAGGTGT---TCAAA
Petrea racemosa	TCAAAATTACAGTGGCAACCAAAATACCTCTTTATATTCAATATCTCTATGGGGTAAAAGGTGT---TCAAA
Stachytarpheta dichotoma	TCAAAATTACAGCGGCAACCAAAATACCTCTTTATATTCAATATCTCTATGGGGTAAAGGGTGT---TCAAA
Verbena bracteata	TCAAAATTACAGTGGAAAGCAAAATACCTCTTTCTATTCAATATCTCTATGGGGTAAGGGGTGT---TCAAA
Proboscidea louisianica	TACAAATTACAGTGGAAACCAAAATACCTCTTTATATTCAATATCTCTATGGGGTAAAGGGTGT---TCCAA
Martynia annua	TACAAATTACAGCGGAAACCAAAATACCTCTTTATATTCAATCTCTCTATGGGGTAAAGGGTGT---TCCAA
Hemimeris sabulosa	TCAAAATTACAGCGACGGTCAAAATCTCCCTTTATATTCAATATCTCTATGGGGTAAAGGGTGT---TCAAA
Scrophularia californica	TCAAAATTACAGTGGCAGTCAAAATATCCCCTTCTATTCAATATCTCTATGGGGTAAAGGGTGT---TCGAA
Retzia capensis	TCAAAATTACAGCGGCAACAAAAATACCTCTTTATATTCAATATCTTTATGGGGTAAAGGGTGT---TCCGA
Halleria lucida	TCAAAATTACAGTGGCAACAAAAATATTCCTTTATACTCAATATCTCTATGGGGTAAAGGGTGT---TCAGA
Angelonia pubescens	TCAAAATTACAGCGGCAACAAAAATATTCCCTTATATTCAATATCTCTATGGGGTAAGGGGTGT---TCAAA
Veronica catenata	TCAAAATTACAGTGGCAAAAAAAATACAGCTTTGTATTCAATATCTATATGGGGAAAAGGGTGT---TCAAA
Streptocarpus holstii	TCAAACTTACTGTACAAACAAAAACATCCTTTTATATTCAATATCTTTATGGGGTAAAGAAGGGTCGTCAAA
Nematanthus hirsutus	TCAAAATTACCGTGGAAACAAAAAAAGCCCCATATATTCAATATCTATATGGGGTAAAGGGTCT---TCAAA
Calceolaria mexicana	TAAAAATTACAGTGGTAACACAAATACTTCTTTATATTCAATATCTCTATGGGGTAAGGAATCT---TCAAA
Jovellana sp.	TCAAAATTACAGTGGTAACAAAAATACTTCTTTATATTCAATATCTCTATGGGGTAAGGAGTCT---TCAAA
Ligustrum vulgare	TCAAAATTACAGTGGCAAACAAAATACCCCTTTTTATTCAATATCTCTATGGGGAAAAGGGGAT---TCGAA
Borago officinalis	TCAAAACTACAGTGGTAAACAAAATACTCTCTCCTATTCCCTATCTCTATGGGGTAAAAAGGGT---TCGAA
Gentiana procera	TCAAAATTATAGTGGTAAACAAAAAATCCCCTTCTATTCAATATCTTTGTGGGGTAAGGGGATT---TCAAA
Nicotiana tabacum	TCCAAATTATGGTGGGAAACAAAAAACCCCCTTCTATTCAATATCTCTATGGGGTAAA----------AA

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Thomandersia hensii          AAGAATTAACAAAAATTTTCGTTTA------TTAAGAATG------------AATAATAATGAAAGTTCTTC
Thomandersia laurifolia      AAGAATTAACAAAAATTTTCGTTTA------TTAAGAATG------------AATAATAATGAAAGTTCTTC
Bartsia alpina               CCGAGTTAACAAAAATTTTTGTTTA------TTACGAATG------------ACTAATAATGAAAGTTCTTC
Lindenbergia phillipensis    ACGAATTAACAAAAACTTTCATTTA------TTAAAACTA------------AATAATAATGAAAGTTCTTC
Paulownia tomentosa          AAGAATTAACAAAAATTTTCGTTTA------TTAAGAATG------------AATAATAATGAAAGTTCTTC
Mimulus aurantiacus          AAGAATTAACAAAAATTTTCGTTTA------TTAAAAATG------------AATAATAATGAAAGTTATTC
Callicarpa dichotoma         AAGAATTAACAAAAATTTTCGTTTA------TTAAGAATG------------AATAATAATAAAAGTTCTTC
Lamium purpureum             AAGAATTAACCCCCATTTTGGTTTA------TTAAGA--------------------AATGAAAGTCTTTC
Sesamum indicum              AAGAATTAACAAAAATTTTCTTTTA------TTAAAAATG------------AATAATAATGAAAGTTCGTC
Ceratotheca triloba          AAGAATTAACAAAAATTTTCTTTTA------TTAAAAATG------------AATAATAATGAAAGTTCGTC
Elytraria crenata            AAGAATTAACAAAAGTTTTCGTTTA------TTAAGAGTG------------AATAATAATGAAAGTTATTC
Thunbergia alata             AARAATTAACAAAAATTTTCGTTTA------TTMARAATG------------AATAATAATCAAAGTTCTTC
Barleria prionitis           AAGAATTAACAAAAATTTTCGTTTA------TTAAGAATG------------AATAATAATGAAAGT-----
Jacaranda sparrei            AAGAATTAACAAAAATTTTCGTTTA------TTAAGAATG------------AATAATAATGAAAGTTCTTC
Kigelia africana             AAGAATTAACAAAAATTTTCGTTTA------TTAAGAATG------------AATAATAATGAAAGTTTTTC
Catalpa speciosa             AAGAATTAACACAAATTTTCGTTTA------TTAAGAATG------------AATAATAATGAAAGTTCTTC
Schlegelia parviflora        AAAAATTAACAAAAATTTTCGTTTA------TTAAGAATG------------AATAATAATGAAAGTTCTTC
Synapsis ilicifolia          AAGAATTAACAAAAATTTTCGTTTA------TTAAGAATG------------AATAATAATGAAAGTTCTTC
Petrea racemosa              AAGAATTAACAACAATTTTCGTTTA------TTAAGAATG------------AATAATAATSAAAGTTCTTC
Stachytarpheta dichotoma     AAGAATTAACAACAATTTTCGTTTA------TTAAGAATGAAATTAAGAATGAATAATAATAAAAGTTCTTC
Verbena bracteata            AAGAATTAACAAAACTTTTCGTTTA------CTAACAATG------------AATGATAATGAAAGTTTTTC
Proboscidea louisianica      AAGAATTAACAAAAATTTTCGTTTA------TTAAGAATG------------AATAATAATGAAAGTTCTTC
Martynia annua               AAGAATTAACAAAAATTTTCGTTTA------TTAAGAATG------------AATAATAATGAAAGTTCTTC
Hemimeris sabulosa           AAGAATTAACAAGACTTTTTGTTTA------TTAACAATC------------AATAATAGTGAAAGTTCTTC
Scrophularia californica     AAGAATTAATAAAAATTTGCGTTTA------TTAAGAATG------------AATAAGAATGAAAGTTCTTC
Retzia capensis              AAGAATTAACAAAAATTTTCGTTTA------TTAAGAATG------------AATAATAATGAAAGTTCTTC
Halleria lucida              AAGAATTAACAAAAATTTTCGTTTA------TTAAGAATG------------AATAATAATGAAAGTTCTTC
Angelonia pubescens          AAAAATTAACAAAAATTTTCGTTTA------TTAAGAATC------------AATAATAACGAAAGTTCTTC
Veronica catenata            AATAATTAACAAAAATTTTAGTTTA------TTAAAAATT------------CATAAT---------TCTTC
Streptocarpus holstii        AAGTATTAACAAAAATTTTCATTTAGATTTAGTAAGAATG------------ACTAATAATCAAAGTTTTTC
Nematanthus hirsutus         AAGCATTAAGAAAAATTTTCGTTTA------TTAAAAATG------------AATAATAATGAAAGTTTTTC
Calceolaria mexicana         CAGTATTAAGAAATGTTTTCGTTTA------TTAAAAATA------------AATCATAATGAGAATTCGTC
Jovellana sp.                AAGCATTAAGAAATATTTTCGTTTA------TTAAAAATA------------AATAATAATGAGAATTCATC
Ligustrum vulgare            AAGAATTAACAAAAATTTTCGTTTA------TTAAAAATG------------AATAATAGTAAAAGTTCTTC
Borago officinalis           AAAAATTAACCAAAATTTTTGTTTG----------------------------AAGAATAATGAAAATGCTTC
Gentiana procera             AAGAATTAGCAAAAATTTTTGTTTA------TTAACTAGT------------------------GGAAGTTC
Nicotiana tabacum            CGGAGTTAAGAAAAACTCTTGTTTA------TTAACTATG------------AATAATAATGAAAGTACTTA

Thomandersia hensii          TTTTTTTTCGAAAAAGACATATCGAAGTGATGAGAATGTAAGAAAAAGAAATAGGGGACGGCCTTTTATTAA
Thomandersia laurifolia      TTTTTTTTCGAAAAAGACATATCGAAGTGATGAGAATGTAAGAAAAAGAAATAGGGGACGGCCTTTTATTAA
Bartsia alpina               TTTATTTTTGAAAAAGGCAGATCGAAGTGATGAGAATGTACGAAAAAATAACGGGCGCCGCCCTTTTATTAA
Lindenbergia phillipensis    TTTATTTTCGAAAAAGATATATCGAAGTGATGAGAATGTACGAAAAAATAATGGGCAGCGGCCTTTTATTAA
Paulownia tomentosa          TTTTTTTTCGAAAAAGACATATCGAAGTGATGAGAATGTAAGAAAAAGAAATGGGGGACGGCCTTTTATTAA
Mimulus aurantiacus          TTTTTTTTCGAAAAAGCCATATCGAAGTGATGAGAATGTAAGAAAAAGAAATGGGGCACGGCCCTTTATTAA
Callicarpa dichotoma         TTTTTTTTCGAAAAAGACATGTCGAAGTGATGAGAATGTAAGAAAAAGAAACGGGGGACGACCTTTTATTAA
Lamium purpureum             TTTTTTTTTGGAAAAAGACATRTCGAAGTGATGAGAATGTAAGAAAAAAAAACGGCGAACGGACCCTTATTAA
Sesamum indicum              TTTTTTTTCGAAAAACACATACCGAAGTGATGAGAATGTAAGAAAAAGAAATAGGGGACGTCCTTTTATTAA
Ceratotheca triloba          TTTTTTTTCGAAAAACACATACCGAAGTGATGAGAATGTAAGAAAAAGAAATAGGGGACGTCCTTTTATTAA
Elytraria crenata            TTTTTTTGCGAAAAAAACATATCGAAGTGATGAGAATGTAGGAAAAAGAACTAGGAGACGGCCTTTTATTAA

Thunbergia alata             TTTTTTTWCGAAAAAGACATATCAAACTGATGAGACTGTAAGAAAACCAAATAGGGGACGGCCCTTTATTCA
Barleria prionitis           -CTTTTTTCGAAAAAGATGTATCAAGGTGATGAAAATGGAAAAAAAAGAAATAAGGGACAGCCTTTTATTAA
Jacaranda sparrei            TTTTTTTTCGAAAAAGACATATCGAAGTGATGAGAATGTAAGAAAAAGAACTAGGGGACGGCCTTTTATTAA
Kigelia africana             TTTTTTTTCGAAAAAGACATATCGAAGTGATGAGACTGTAAGAAAAACAAATAAGGGACAGCCTTTTATTAA
Catalpa speciosa             TTTTTTTTCGAAAAGGACATATCGAAGTGATGAGACTGTAAGAAAAAAAAAATGGGGGACGGCCTTTTATTAA
Schlegelia parviflora        TTTTTTTTCGAAAAAAACATATCGAAGTGATGAGAATGTAAGAAAAAAAAAATAGGGAACGGCCTTTTATTAA
Synapsis ilicifolia          TTTTTTTTCGAAAAAAACATATCGAAGTGATGAGAATGTAAGAAAAAGAAATAGGGAACGGCCTTTTATTAA
Petrea racemosa              TTTTTTTTCGAAAAAGACATATCGAACTGATGAGAATGTAAGAAAAAGAAATAGGGGACGTCCTTTTATTAA
Stachytarpheta dichotoma     TTTTTTTTCGAAAAAGACATATCGAAATGATGAGAATGTAAGAAAAAGAGATAGGGGACGGCCTTTTATTAA
Verbena bracteata            CTTTGTTTCCAAAAAGACATATCAA---------AACATAAGAAAAAGAAATAGGGGACGGCCTTTTATGAA
Proboscidea louisianica      TTTTTTTTCGAAAAAGACATATCGAAGTGATGAGAATGTAAGAAAAAGAAATAGGGGACGGCCTTTTATTAA
Martynia annua               TTTTTTTTCGAAAAAGACATATCGAAGTGATGAGAATGTAAGAAAAAGAAATAGGGGACGGCCTTTTATTAA
Hemimeris sabulosa           TTTTTTTTCAACAAAGACCTATCAAAGTGATGAGAATGTAAGAAAAAGAAAGAGAGGATCGCCTTTTATTAA
Scrophularia californica     TTTTTTTTCGAAAACGACCTATCGAAGTGATGGCAATGTAAGAAAAAAAAAATAGGGAACGCCCCTTTATTAA
Retzia capensis              TTTTTTTTCGAAAAAGACATATCAAAGTGATGAGAATGTAAGAAAAAAAAAATAGGGAACGCCCTTTTATTAA
Halleria lucida              TTTTTTTTCGAAAAAGACATATCGAAGTGATGAGAATGTAAGAAAAAGAAATAGGGGACGGCCTTTTATTAA
Angelonia pubescens          TTTTTTTTCAAAAAAAACATATCCAAGTGGCGATAATGTAAGAAACCGAAATAGGGTACGACCTTTTTTTAA
Veronica catenata            TTTTTTTTCGAAAAAAACATATCGAAGTGATGAGAATCTAAAAAAAAATAAATAGAGGACAACCTTTTATCAA
Streptocarpus holstii        TTTTTTTTCGAAAAAAGCATATCGAAATGATGAGGATGTAAAAAAAAAGAATTCATGGACGACCTGTTGCT--
Nematanthus hirsutus         TTTTTTTTCGAAAAAAACATATCGAAGTGATGGGAATGGAAGAAAAAGAAATGGGGGACGACCTTTTATTAA
Calceolaria mexicana         TTTTTTCAAAAAAAAAGACATATCGAAGTAATGAGAATGTAAGAAAAAAAAGATAGTGGACAACCTTGTATTAA
Jovellana sp.                TTTTTTGAAAAAAAAAGACATATCGAAGTGATGAGAATGTAAGAAAAAAAAAATAGTGGACAACCTTGTATTAA
Ligustrum vulgare            TTTTTTTTCAAAAAAGCCATATCGAAGTGGTAAAAATGTAAGAAA------CAGGGTAGGACCTTTTCTTAC
Borago officinalis           GTTTTTTCCACAAAAAATATATAAAAGTGATGAAAATGTAACAAG------CATGAGAGGACCTTTTATTAG
Gentiana procera             C------TCAAAAAAGAAATATTGGATTGATCATAATTTTCTAAC------TAGGATACGACCCTTTCTTGC
Nicotiana tabacum            TTTTTTTGCAAAAACTAAATATCCAATTGATAAAAATGGAAGAAA------GATGACACGACCTTTTATGAC
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Thomandersia hensii	TATT------GTT------CATTTTGATAATAAAAATAAAAACCCCTTTCCCTATNNNNNNNNNNNNNNNNN
Thomandersia laurifolia	TATT------GTT------CATTTTGATAATAAAAA------CCCCTTTTCCTATCCTTATGAATCAGACAA
Bartsia alpina	TATT------GTT------CATAAGGATAATAAAAA------GCTTTTTTCCTATCCTTATGAATCGGACAA
Lindenbergia phillipensis	TATT------GTT------CATAAAGATAATAAAAA------AACTTTTTCCTATCCTTATGAATCGGACAA
Paulownia tomentosa	TATT------GTT------CATTTTGATAATAAAAA------GCCTTTCTCCTATCCTTCTGAATCGGACAA
Mimulus aurantiacus	TATT------GTT------CATTTTGATAATCAAAA------GACTTTTTCCTATCCTTATGAATCGGACAA
Callicarpa dichotoma	TATT------CTT------CATTTTGATAATCAAAA------GCCCTTTTCCTATCCTTATGAATCGGATAA
Lamium purpureum	TATT------CTT------CATTTTGATAATCAAAA------GTCTTTTTCCTATCCTTATGAATCGGACAA
Sesamum indicum	TATT------GTG------GATTTTTATAATCAAAA------GTCTTTTTCCTATCCTTATGAATCGGATAA
Ceratotheca triloba	TATT------GTG------GATTGTTATAATCAAAA------GTCTTTTTCCTATCCTTATGAATCGGATAA
Elytraria crenata	TATT------GTT------CGTTTTGATAATAAAAA------GTCTTTTTCCTATCCTTATGAATCGGACAA
Thunbergia alata	TATT------GCT------?CTTTTGATACTAAAAA------GTACTTTTCATATCCCTATGAATCAGACAG
Barleria prionitis	TATT------GTT------CCTTTTGAGAATAAAAA------GATTTTTTCCTACCCCTATGAATCAGAAAA
Jacaranda sparrei	TATT------GTT------CATTTTTATAATAAAAA------GCCTTTTTCCTATCCTTATGAATCGGACAA
Kigelia africana	TATT------GTT------GATTTTGATACTAAAAA------GCCTTTTTCCTATCCTTATGAATCCGACAA
Catalpa speciosa	TATT------GTT------CATTTTGATACTAAAAA------GCCTTTTTCCTATCCTTATGAATCCGACAA
Schlegelia parviflora	TATT------ATT------CATAAGGATAATAAAAA------GCCTTTTTCCTATCCTTATGAATCGGACAA
Synapsis ilicifolia	TATT------ATT------CATTTTGATAATAAAAA------GCCTTTTTCCTATCCTTATGAATCAGACAA
Petrea racemosa	TACT------GTT------CATTTTGATAATACAAA------GCCTTTTTCCTATCCTTATGAATCGGACAA
Stachytarpheta dichotoma	TATT------GCT------CATTTTTATAATAAAAA------GCCTTTTTCCTATCCTTATGAATCGGACAA
Verbena bracteata	TATT------GTT------CATTTTGATAATAAAAA------GCCTTTTTCCTATCCTTATGAATCGGACAA
Proboscidea louisianica	TATTAATGTTGTT------GCTTTTTATAATAAAAA------GCCTTTTTCCTATCCTTATGAATCGGACAA
Martynia annua	TATTAATGTTGTT------GGTTTTTATAATAAAAA------GCCTTTTTCCTATCCTTATGAATCGGACAA
Hemimeris sabulosa	TATT------GTT------CATTTTGATAATAAAAA------GTCTTATTCCTATCCTTATGAATCGGACAA
Scrophularia californica	TATT------CTT------CATTTTGATAATAAAAA------GCCTTATTCCTATCCCTATGAATCAGACAA
Retzia capensis	TATT------GTT------CATTTTGATAATAAAAA------GCCTTATTCCTGTCCTTATGAATCGGACAA
Halleria lucida	TATT------GTT------CATTTTGATAATAAAAA------GCCTTGTTCCTATCCTTATGAATCGGACAA
Angelonia pubescens	TATT------TTT------CATTTTGATAATAAAAA------GTCTTTTTCTTATCCTTACGAATCAGATAA
Veronica catenata	TATT------ATT------CATTTTGAAAATAAAAC------AGTTCATCTCTACCCGCACGAATCGGACAA
Streptocarpus holstii	-ATT------CTT------CATGTTGATAATAAAAA------GTCTTATTCCTATCCTTATGAATCG??GAA
Nematanthus hirsutus	TATT------GTT------CATTTTGATAAAAAAAA------GCCTTATTCCTATCCTTATGAATCGGACAA
Calceolaria mexicana	TATT------GTT------CATTTTGAGAATCAAAA------GCCTTATTCCTATCCTTATGAATCGGACAA
Jovellana sp.	TATT------GTT------CATTTTGAGAATCAAAA------GCCTTATTCCTATCCTTATGAATCGGACAA
Ligustrum vulgare	TATT------GTT------CATTTTGAGAATCAAAA------GTCTTATTCCTATCCTTATGAATCCGACAA
Borago officinalis	TATT------GTT------CAGTTTAAGAATAAAAA------GACTTATTCCTATCCTTATGAATCGGACAA
Gentiana procera	TAGT------ATGAGGAGTCCTTTTGAGAATAAAAA------ACTTTATCCYTWTCCTTGTGAATCGRACAA
Nicotiana tabacum	TATT------GCT------CATTTTGAGCATAAAGC------GGTTTATTCCTATCCTTATGAATCGGACAA

Thomandersia hensii	NNNNNNNNNNNNNNNNNNNNNNTGTATTAGTCCTATTTACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAA
Thomandersia laurifolia	TACTATGTTATTTTCCTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAA
Bartsia alpina	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACGTTGTTTGTTGGATCTATCGGAATTCCTTTTAA
Lindenbergia phillipensis	CACWAWGTTATTTCCTTWACTTGTATTAGTCCTATTTACCTTGTTTGTTGGATCTATAGGAATTCCTTTTAA
Paulownia tomentosa	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAA
Mimulus aurantiacus	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTATTGGATCTCTAGGCATTCCTTTTAA
Callicarpa dichotoma	TACGATGTTATTTCCTTTACTTCTATTAGTCCTATTTACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAA
Lamium purpureum	TACGATGTTATTTCCTTTACTTCTATTAGTCCTATTTACTTTGTTTGTCGGATCTCTAGGAATTYCTTTTAA
Sesamum indicum	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGAATTCTAGGAATTCCTTTTAA
Ceratotheca triloba	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGAATTCTAGGAATTCCTTTTAA
Elytraria crenata	TACTATGTTATTTCCTTTACTTGTATTAGTCCTCTTTACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAA
Thunbergia alata	TACCATGTTGTTTTCTTTACTTGTATTACTGCTCTTTACTTTGGTTGTTGAATTCATAGGAATTCCCTTTAA
Barleria prionitis	TACTATGTTATTTTCGTTACTTGTATTAGTATTCTTTACTTTCTTTGTTGGATCGATAGGAATTCCTTTTAA
Jacaranda sparrei	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGATCTCTAGGAATTTCTTTTAA
Kigelia africana	TACGATGTTGTTTCCTTTACTTGTATTA?TCCTATTTACTTTGTTTGTTGGATCCCTA?GAATTCCTTTTAA
Catalpa speciosa	TATGATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAA
Schlegelia parviflora	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGATCTATAGGAATTCCTTTTAA
Synapsis ilicifolia	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAA
Petrea racemosa	TACTACGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGAATTCTAGGAATTCCTTTTAA
Stachytarpheta dichotoma	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAA
Verbena bracteata	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGCTCTCTAGGAATTCCTTTTAA
Proboscidea louisianica	TACWATGTTATTTCCTTTACTTTTATTAGTCCTATTTACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAT
Martynia annua	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAA
Hemimeris sabulosa	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGATCTCTAGGCATTCCTTTTAA
Scrophularia californica	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGAATTTTGGGAATTCCTTTTAA
Retzia capensis	TACTATGTTATTTCCTTTACTTGTATTAGTACTATTTACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAA
Halleria lucida	TACTATGTTATTTCCTTTACTTGTATTAGTACTATTTACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAA
Angelonia pubescens	TACTATGTTATTTCCTTTACTTGTATTAGTCTTATTCACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAA
Veronica catenata	TACTATGTTATTTCCTTTACTTGTATTGGTCCTATTTACTTTGTTTGTTGGAGCTCTAGGAATTCCTTTTAA
Streptocarpus holstii	TACTATGTTATTTCCTTTACTTGTATTAGTTTTATTTACTTTGTTTGTTGGATTTCTAGGAATTCCTTTTAA
Nematanthus hirsutus	TACTATGTTATTTCCGTTACTTGTATTAGTTTTATTTACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAA
Calceolaria mexicana	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTGTTTGTTGGATCTCTAGGAATTCCTTTTAA
Jovellana sp.	TACTATGTTATTTCCTTTACTTGTATTAGTCCTATTTACTTTTTTTGTTGGATCTCTAGGAATTCCTTTAAA
Ligustrum vulgare	TACTATGTTATTTCCTTTACTTGTATTGGGCATCTTAACTTTGTTCGTTGGATCTCTAGGAATTCCTTTCAA
Borago officinalis	TACTATGTTACTTCCCCTAATTATATTAGGTCTGTTGACTTTGTTTGTTGGATGTTTAGGAATTCCTTTCAA
Gentiana procera	TACTAT?GTATTGCCTCTACT?GTATTGACCTTATTTACTTTGTTCATTGGATCTATAGGAATTCCTGTCAA
Nicotiana tabacum	TACTATGCTATTCCCAATATTTGTATTAGGACTCTTTACTTTGTTTGTTGGATCTATAGGAATTCCTTTCAA

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Thomandersia hensii          T---------CAA---------GAGGGA------ACAGATTTTGATATATTATCAAAATGGTTAGTTCCCTC
Thomandersia laurifolia      T---------CAA---------GAAGGA------ACAGATTTTGATATATTATCAAAATGGTTAGTTCCTTC
Bartsia alpina               T---------CAA---------GAAAGA------ACCGGTTTGGATATATTATCCAAATGGTTAACTCCGTC
Lindenbergia phillipensis    T---TTTAATCAA---------GAAGGA------ACGGGTTTGGATATATTATCCAAATGGTTAGCTCCGTC
Paulownia tomentosa          T---------CAA---------GAAGGA------ACGGATTTGGATATATTATCCAAATGGTTAGCTCCGTC
Mimulus aurantiacus          T---------CAA---------GAAGGA------GGAGATTTGGATATATTATCCAAATGGTTAGCTCCGTC
Callicarpa dichotoma         T---------CAA---------AAAGGA------CAAGATTTGGATATATTATCCAAATGGTTAGCTCCGTC
Lamium purpureum             T---------CAA---------AAAGGA------ACAGATTTAGATCTATTATCCAAATGGTTAGCTCCGTC
Sesamum indicum              T---------CAA---------GAAGGA------AGGGATTTAGATATATTATCCAAATGGTTAGCTCCGTC
Ceratotheca triloba          T---------CAA---------GAAGGA------AGGGATTTAGATATATTATCCAAATGGTTAGCTCCGTC
Elytraria crenata            T---------CAA---------GAAGGA------ACGGATTTGGATATATTATCCAAATGGTTAGCTCCGTC
Thunbergia alata             T---------CAA---------GAAGGA------ACAGATTTGGATATATTATCAAAATGGTTAGCTTCGTC
Barleria prionitis           T---------CAA---------GAAGGA------CAAGAT------ATATTATCCAAATGGTTAGCTCCTTC
Jacaranda sparrei            T---------CAA---------GAAGGA------GGAGATTTGGATATATTATCCAAATGGTTAGCTCCGTC
Kigelia africana             T---------CAA---------GAAGGA------ACAGATTTGGATATATTATCAAAATGGTTAGCTCCGTC
Catalpa speciosa             T---------CAA---------GAAGGA------GCAGATTTGGATATATTATCCAAATGGTTAGCTCCGTC
Schlegelia parviflora        T---------CAA---------GAAGGA------ACAGATTTTGATATATTATCAAAATGGTTAGCTCCGTC
Synapsis ilicifolia          T---------CAA---------GAAGGA------ACAGATTTGGATATATTATCAAAATGGGTAGCTCCGTC
Petrea racemosa              T---------CAA---GATCAAGAAGGA------ACAGATTTGGATATATTATCCAAATGGTTAGCTCCGTC
Stachytarpheta dichotoma     T---------CAA---------GAAGGA------ACAGATTTTGATATATTATCAAAATGGTTAGCCCCGTC
Verbena bracteata            T---------CAA---------CAAGGA------??AGATTTGGATATATTATCCAAATGGTTAGCTCCGTC
Proboscidea louisianica      T---------CAA---------CAAGGAGCT---AACCATTTGGATATATTATCCAAATGGTTAGCTCCGTA
Martynia annua               T---------CAA---------CAAGGA------ACAGATTTGGATATATTATCCAAATGGTTAGCTCCGTC
Hemimeris sabulosa           T---------CAAGTTAATCAAGAAGGA------ACAGATTTGGACATATTATCTAAATGGTTAGTTCCTTC
Scrophularia californica     T---------CAA---------GAAGGA------ACAGATTTGGACATATTATCCAAATGGTTAGCTCCGTC
Retzia capensis              T---------CAA---------GAAGGA------AGAGATTTGGATATATTATCGAAATGGTTAGCTCCGTC
Halleria lucida              T---------CAA---------GAAGGA------ACAGATTTTGATATATTATCAAAATGGTTAGCTCCGTC
Angelonia pubescens          T---------CAA---------GAAGGA------ACGGATTTGGATATATTATCCAAATGGTTAGCTCCTTC
Veronica catenata            T---------CAA---------GACGGA------AGAAATATCGATATATTATCGAAATGGTTAGCCCCATC
Streptocarpus holstii        T---------CAA---------GAACGA------AGAGATTTCGATATTTTAACCAAATGGTTATCTCCATC
Nematanthus hirsutus         TAAAGTTAATCAA---------GAACCA------ACAGATTTGGATATATTAACTAAATGGTTAGCCCCGTC
Calceolaria mexicana         T---------CAA---------GAAAGA------ACAGATTTGGATATATTAACAAAATGGTTAGCTCCGTC
Jovellana sp.                T---------CAA---------GAAAGA------AGAGATTTGGATATATTATCCAAATGGTTAGCTCCGTC
Ligustrum vulgare            T---------CAA---------GAA----------------TTGGATATATTAACCAAATGGTTAACTCCGTC
Borago officinalis           T---------CAC---------GAGAGA------------GTTGCTATATTAACCAAATGGTTAGCTCCATC
Gentiana procera             T---------CAA---------G?GCAAGGGGGGATGGA??CGGATATATTATCTAAATCCTTAGCTCCATC
Nicotiana tabacum            C---------CAA---------GAGGGA------GGGAATTTGGATATATTATCGAAATGGTTAGCTCCATC

Thomandersia hensii          TATTAACCTTTTACATCAAAAGTCAAAGCACTCGACAGATTGGTATGAATTCTTGAAAGATGCAATTTTTTC
Thomandersia laurifolia      TATTAACCTTTTACATCAAAAGTCAAAGGACTTGACAGATCGGTATGAATTCTTGAAAGATGCAATTTTTTC
Bartsia alpina               TATTAACCTTTTACATGAAAAGTTAAGGGATTCCACAGATTGGTATGAGTTTTTGAAAGATGCGACTTTTTC
Lindenbergia phillipensis    TATTAATCTTTTACATCAAAACTTAAAGGATTCTAAAGATTCGTATGAGTTTTTGAAAGATGCAATTTTTTC
Paulownia tomentosa          TATTAACCTTTTACATCAAAAGTCAAAGGATTCAACAGATTGGTATGAATTTTTGAAAGATGCAATTTCTTC
Mimulus aurantiacus          TATTAACCTTTTACACCAAAACTCAAAGGATTCAACAGATTGGTATGAATTTTTGAAAGATGCAACTTTTTC
Callicarpa dichotoma         T??????????????????????????????????????????????????????????????TTTTTC
Lamium purpureum             TATTAACCTTTTACATCAAAAGTCAAAGGATTCAGCAAGTTGGTATGAATTTTGGAAAGATGCCCTTTTTTC
Sesamum indicum              TATTAACCTTTTACATCAAAAGTCAGAGGATTCGACAGATTGGTATGAATTTTTGAAAAATGCAATTTTTTC
Ceratotheca triloba          TATTAACCTTTTACATCAAAAGTCAGAGGATTCGACAGATTGGTATGAATTTTTGAAAAATGCAATTTTTTC
Elytraria crenata            TATTAACCTTTTACATCAAAAGGCAAATGATTCGACAGGTTGGTATGAATTTTTAAAAGATGCAATTTTCTC
Thunbergia alata             TATTAACCTTTTACATCAAAAGTCAAARGATTCGACRGATTGGTATGAATTTTTGAAAGATGCAATTTTTTC
Barleria prionitis           TATTAATCTTCTACATCAAAAGTCAAAGGATTCGACGTATTGGTATGAATTTTTGAAAGATGCAATTTTTTC
Jacaranda sparrei            TATTAACCTTTTACATCAAAAGTCAAAGGATTCGACAGATTGGTATGAATTTTTGAAAGATGCAATTTTTTC
Kigelia africana             TATTAACCTTTTACATCAAAAGTCAAAGGATTCGACAGATTGGTATGAATTTTTGAAAGATGCAATTTTTTC
Catalpa speciosa             TATTAACCTTTTACATCAACAGTCAAAGGATTCGACAAATTGGTATGAATTTTTGAAAAATGCAATTTTTTC
Schlegelia parviflora        TATTAACCTTTTACATCAAAAGTCAAAGGATTCGACAGATTGGTATGAATTTTTGAAAGATGCCATTTTTTC
Synapsis ilicifolia          TATTAACCTTTTACATCAAAAGTCAAAGGATTCGACAGATTGGTATGAATTTTTGAAAGATGCAATTTTGTC
Petrea racemosa              TATTAATCTTTTACATCAAAAGTCAAAGGATTCGACAGATTGGTATGAATTTTTGAAAGATGCAATTTTTTC
Stachytarpheta dichotoma     TATTAACCTTTTACATCAAAAGTCCACGGATTCGACGCATTGGGATGAATTTTTGAAAGATGCAATTTTTTC
Verbena bracteata            TATTAACCTTTTACATCAAAACTCAAAGGAGTTGACGGGTTGGTCTGAATTTTTGAAAGATGCAATTTTTTC
Proboscidea louisianica      TATTAACGTTTTACATCAAAACTCAAAGGATTCGACGGATTACTATGAATTTTTCACAGATGCAATTTTTTC
Martynia annua               TATTAACCTTTTACATCAAAAGTCAAAGGATTCGACGGATTGGTATGAATTTTTGACAGATGCAATTTTTTC
Hemimeris sabulosa           TATCAACCTTTTACACCAAAAGTCAAAGGATTCGACAGATTGGTATGAATTTTTGAAGGATGCAAGTTTTTC
Scrophularia californica     TATCAACCTTTTACATCAAAAGTCGAAGGATTCAGCAGATTGGTATGAATTTTTTAAGGATGCCGTTTTTTC
Retzia capensis              TATCAACCTTTTACATCAAAAGTCAAAGGATTCGACAGATTGGTATGAATTTTTGAAAGATGCAATTTTTTC
Halleria lucida              TATCAACCTTTTACATCAAAAGTCAAAGGATTCGACAGATTGGTATGAATTTTTAAAAGATGCAATTTTTTC
Angelonia pubescens          TATCAACCTTTTACATCAAGAGTCAAAAGATTCGACAGATTGGTATGAATTTTTCAAAAATGCAATTTTTTC
Veronica catenata            TATTAATCTTTTACATCCAAATTCAAAGGATTTAACGGATTGGCATGAATTTTTGAAAGATGCAATTTCTTC
Streptocarpus holstii        TATCAACCTTTTACATCAAAAGACAAACGATTCAATAAATTGGTATGAATTTTTGAAAGATGCAATCTTTTC
Nematanthus hirsutus         TATCAACCTTTTACATCAAAAGTCAAATGATTCAATAGATTGGTATGAATTTTTGAAAGATGCAATTTTGTC
Calceolaria mexicana         TATAAACTTTTTACATCAAAAGCCAAATGATTCAACAGATTGGTATACATTTTTAAAAGATGCACTTTTTTC
Jovellana sp.                TATAAACTTTTTACATCAAAAGTCAAATGATTCGACAGATTGGTATACATTTTTAAAAGATGCACTTTTTTC
Ligustrum vulgare            TATAAACCTTTTACATCAAAAGTGGAATGATTCGATTGATTGGTATGAATTTTGGAAAGATGCAAGTTTTTC
Borago officinalis           TATAAACCTTTTGAATCAAGAATTAGAAAATTCAATGGATTGGTATGAATTTATAAAAGATGCTTTTTTTTC
Gentiana procera             TATAAACCTTTTACATCAAAAATCAAAGAATTACACAGAATGGTATGAGTTTGTGAAAGATGCAGTATTTTC
Nicotiana tabacum            TATAAATCTTTTGCATCAAAAGTCGAATAATTCGATGGATTGGAATGAATTTTTAAAGGATGCAGTTCTTTC
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Thomandersia hensii          GGTCAGTATAGCTTATTTCGGAATATTTCTAGCGTCCTTTTTTATATAAACCCATTTATTCCTCTTTCAAAAA
Thomandersia laurifolia      GGTCAGTATAGCTTATTTCGGAATATTTCTAGCGTCCTTTTTTATATAAACCCATTTATTCCTCTTTCAAAAA
Bartsia alpina               AGTAAGTATAGCTTATTTTGGAATATTTTCGGCGTCCTTTTTTATATAAACCTACTTATTCTTCTTTCAAAAA
Lindenbergia phillipensis    AGTCAGTATAGCTTATTTTGGAATATTTTCAGCGTCCTTTTTTATATAAACCCATTTATTCTTCTTTCAAAAA
Paulownia tomentosa          AGTCAGTATAGCTTATTTCGGAATATTTCTAGCTTCCTTTTTTGTATAAACCCATTTATTCCTCTTTCAAAAA
Mimulus aurantiacus          AGTCAGTATAGCTTATTTCGGAATATTTATAGCGTCCTTTTTTATATAAACCCATCTATTCCTCTTTTAAAAA
Callicarpa dichotoma         AGTCAGTATAGCTTATTTCGGAATATTTCTAGCTTCCTTTTTTATATAAACCCATTTATTCCTCTTTCAAAAA
Lamium purpureum             AGTTAGTATCGCTTATTCCGGAATATTTCTAGCGTCCTTTTTTATATAAACCTATTTATTCATCTTTCCAAAA
Sesamum indicum              AGTCAGTATAGCTTCTTTTGGAATATTTCTAGCGTCCTT?TTATATAAACCTATTTATTCCTCTTTCAAAAA
Ceratotheca triloba          AGTCAGTATAGCTTCTTTTGGAATATTTCTAGCGTCCTTTTTTATATAAACCTATTTATTCCTCTTTCAAAAA
Elytraria crenata            AGTTAGTATAGCATATTTAGGAATATGTCTAGCGTCTTTTTTTATATAAACCCATTTATTCCTCTTTCAAAAA
Thunbergia alata             AGTCAGTATAGCTTATTTCGGAATATTTCTAGCGTCCTTTTTTATATAAACCCATTTATTCCTCTTTCAAAAA
Barleria prionitis           AGTCAGTCTAGCTTATTTTGGAATATTTTTCGCCTCCTTTTTTATATAAACCAATTTATTCATCTTTCAAAAA
Jacaranda sparrei            AGTCAGTATAGCTTCTTTCGGAATATTTCTAGCGTCCTTTTTTATATAAACCCATTTATTCCTCTGTCAAAAA
Kigelia africana             AGTCAGTATAGCTTATTTCGCAATATTTCTAGCGTCCTTTTTTATATAAACCCATTTATTCCTCTTTCAAAAA
Catalpa speciosa             AGTCAGTATAGCTTATTTTGGAATATTTCTAGCATCCTTTTTTATATAAACCCATTTATTCCTCTTTCAAAAA
Schlegelia parviflora        AGTCAGTATAGCTTATTTCGGAATATTTCTAGCGTCCTTTTTTATATAAACCCATTTATTCCTCTTTCAAAAA
Synapsis ilicifolia          AGTCAGTATAGCTTATTTCGGAATATTTCTAGCGTCCTTTTTTATATAAACCCATTTATTCCTCTTTCAAAAA
Petrea racemosa              AGTCAGTATAGCTTATTTCGGAATATTTCTAGCGTCCTTTTTTATATAAACCCATTTATTCCTCTTTCAAAAA
Stachytarpheta dichotoma     AGTCAGCATAGCGTATTTTGGCATATTTCTAGCGTCCTTTTTTATATAAACCCA?TTATTCCTCTTTCAAAAA
Verbena bracteata            AGTCAGCATAGCTTATTTTGGAATAGTTATAGCGTCCTTTTTTATATAAGCCCATTTATTCCTCGTTCAAAAA
Proboscidea louisianica      AGTCAGCATATCCTATTTCGGAATCTTTTTTTCGTTTTTGTTATATAACCCCATTTATTCCTCTTTCAAAAA
Martynia annua               AGTCAGTATAGCTTATTTCGGAATATTTCTAGCGTCTTTTTTATATAAACCCATTTATTCCTCTTTCAAAAA
Hemimeris sabulosa           AGTTAGTCTAGCTTCTTTCGGAATATTTCTAGCGTCCTTTTTTATATAAACCTATTTATTCCTCTTTCAAAAA
Scrophularia californica     AGTCAGTATAGCTTATTTTGGAATATTTATAGCGTCCTTTTTTATATAAACCCATTTATTCTTCTTTTAAAAA
Retzia capensis              AGTCAGTATAGCTTATTTCGGAATATTTCTAGCGTCCTTTTTTATATAAACCCATTTATTCCTCTTTCAAAAA
Halleria lucida              AGTCAGTATAGCTTATTTCGGAATATTTCTAGCATCCTTTTTTATATAAACCCATTTATTCCTCTTTCAAAAA
Angelonia pubescens          AGTCAGTATAGCTTATTTTGGAATACTTCTAGCATCTTTTTTTATATAAACCCATTTATTCTTCTTTCAAAAA
Veronica catenata            CGTTAGTATATCTTATTTAGGAATACTTTTAGCGTCTTTTTTTATATAAACCCATTTTTTCTTCTTTCAAGAA
Streptocarpus holstii        AGTCAGTATAGCTTATTTCGGACTATTTCT?GCATCTTTTTTTATATAAACCCATTTATTCCTCTTTCAAAAA
Nematanthus hirsutus         AGTCAGTATAGCTTATTTCGGAATATTTATAGCGTCCTTTTTTATATAAACCCATTTATTCTTCTTTCAAAAA
Calceolaria mexicana         AGTTAGTATAGCTTATTTCGGAATATTTCTAGCGTCCTTTTTTATATAAACCCATTTATTCTTCTTTCAAAAA
Jovellana sp.                AGTCAGTATAGCTTATTTCGGAATATTTCTAGCGTCCTTTTTTATATAAACCCATTTATTCTTCTTTCAAAAA
Ligustrum vulgare            AGTGAGTATAGCTTATTTCGGAATATTTATAGCGTCCTTTTTTATATAAACCCATTTATTCCTCTTTCCCAAA
Borago officinalis           AGTCAGCATAGCTTATTTCGGAATAGTTATAGCCTCTTTTTTTATATAAACCTATTTATTCTTCTTTACAAAA
Gentiana procera             CATTAATCTATCTTATTTCGGAATAATTATAGCGTCCTTTTTTATATAAAGTCCCTTATTCCTCTTCAATAAA
Nicotiana tabacum            AGTCAGTATAGCTTATTTCGGAATATTTATAGCATCCTTTTTTATATAAACCCATTTATTCTTCTTTAAAAAA

Thomandersia hensii          TTTCGACTTAATTAATTCATTTCTTAAAACAGGTTCGAAGAGAAGCCGT------TGGGACAAACTTCTAAA
Thomandersia laurifolia      TTTCGACTTAATTAATTCATTTCTTAAAACAGGTCCGAAGAGAAGCCGT------TGGGACAAACTTCTAAA
Bartsia alpina               TTTCTACTTAATTAATTCATTTCTTAAAATACGTCCGAAGTGGAGCTCT------TGGGACAAAATTCTAAA
Lindenbergia phillipensis    TTTCTACTTAATTAACTCATTTCTTAAAATAGGTCCGAAGAGAAACTCT------TGGGACAAAATTCTAAA
Paulownia tomentosa          TTTCTACTTAATTAATTCATTTGTAAAAATAGGTCCGAAAAGAAGCCGT------TGGGACAAAATACTAAA
Mimulus aurantiacus          ATTCTACTTAATTAATTCATTTGTTAAAATAGGTCCAAAGAGGAGCCGT------TGGGACAAAATTGTAAA
Callicarpa dichotoma         TTTCGACTTAATAAATTCATTTGTTAAGCTAGGTCCGAAAAGAAAACGG------TGGGATAAAATTATAAA
Lamium purpureum             TTTCAACTTAATAAATTCATTTGGGAAGTTAGGTCCGAAAAGAAAGCGT------TTGGATAAAATTCTAAA
Sesamum indicum              TTTCGACTTAATTAATTTATTTGTTAAAACAGGTCCGAAGAGAAGTCGT------TGGGACAAAATTATAAA
Ceratotheca triloba          TTTCGACTTAATTAATTTATTTGTTAAAACAGGTCCGAAGAGAAGTCGT------TGGGACAAAATTATAAA
Elytraria crenata            TTTCGACTTAATTAATTCATTTGTTAAAACAGGTCCGAAGAGAAGATAT------TGGGACAAAACGATAAA
Thunbergia alata             TTTCGACTTAATTAATTTATTTGTTAAAACAGGCCCAAAGAGGAGCCGT------TGGGACAAAATTATAAA
Barleria prionitis           TTTTGACTTAATTAATTCATTTGTTAAAACAGGTCCGAAGAGAAGCCGT------TGGGACAAAATTCTAAA
Jacaranda sparrei            TTTCGACTTAATTAATTCATTTGTTAAAAGAGGTCCGAAGAGAAGCCGT------TGGGACAAAATTCTAAA
Kigelia africana             TTTCGACTTAATTAATTTATTTGTTAAAACAGGGCCGAAGAGGAGTCGT------TGGGACAAAATTATAAA
Catalpa speciosa             TTTCGACTTTATTAATTCATTTGTTAGAACAGGGCCGAGGAGGAGCCGT------TGGGATAAAATTCTAAA
Schlegelia parviflora        TTTCGACTTAATTAATTCATTTGTTAAAACAGGTCCGAAGAGAAGCCTT------TGGGACAAAATTCTAAA
Synapsis ilicifolia          TTTCGACTTAATTAATTCATTTGTTAAAACAGGTCCGAAGAGAAGCTTT------TGGGACAAAATTCTAAA
Petrea racemosa              TTTCGACTTAATTAATTCATTTGTTAAAACAGGTCCGAAGAGAAGCCGT------TGGGACAAAATGATAAA
Stachytarpheta dichotoma     TTTTGACTTAATTAATTCATTTGTTAAAAGAGGTCCGAAGAGAAGCCGT------TGGGACAAAACKCTAAA
Verbena bracteata            TTTCGACTTAATTAATTCATTTGTTAAAACAGGT??????????????????????????????????
Proboscidea louisianica      TTTCGACTTAATTAATTCATTTGTTAAAAAAGGTCCGAACAGAAGTCGT------TGGGACAAAATTCTAAA
Martynia annua               TTTCGACTTAATTAATTCATTTGTTAAAACAGGTCCGAAGAGAAGTCGT------TGGGACAAAATTCTAAA
Hemimeris sabulosa           TTTTGACTTAATTAATTTATTTGTTAAAACGGGTCCAAAGAGAAGCCGT------TGGGACAAAATTATAAA
Scrophularia californica     TTTTGACT?AATTAATTTATTTGTTAAAACAGGTCCGAAGAGAAGACAT------TGGGACAAACTTCTAAA
Retzia capensis              TTTCGATTTAATTAATTTATTTGTTAAAACAGGTCCGAAGAGAAGCCGT------TGGGACAAAATTCTAAA
Halleria lucida              TTTCGACTTAATTAATTCATTTGTTAAAACAGGTCCGAAGAGAAGTCAT------TGGGACAAAATTATAAA
Angelonia pubescens          TTTATACTTAATTAATTCATTTGTTAAAACAGGTCCGAAGAGAAGCGAT------TGGGACAAAATTTTAAA
Veronica catenata            TTTTGACTTAATTAATTCATTTATTAAAATGGGTCCCACAAGAA?CCGT------TGGGACTCATTTTTAAA
Streptocarpus holstii        TTTCGATTTAATCAATTCATTTGTTAAATCAGGTGCGAAGCAAAATCGT?ATCTATGGGACAAAA?TCTAAA
Nematanthus hirsutus         TTTCGACTTAATTAATTCATTTGTTAAAACAGGTCCCAAGAGAAGTTAT------TGGGACAAAATTATAAA
Calceolaria mexicana         TTTCCACTTAATTAATTCATTTGTTAGAACAGGTCCCAAGAGAGGTGGT------TGGGATAAAATCCTAAA
Jovellana sp.                TTTTGACTTAATTAATTCATTTGTTAGAACAGGTCCCAAGAGAAGTGGT------TGGGATAAAATTATAAA
Ligustrum vulgare            TTTTTTCTTAATCAATTTATTTGTTAAAACAGGTCCTAAGAGAAGTCTT------TGGGACAAAATTCTAAA
Borago officinalis           TTTTAACTTTATAAATTCATTTGTTAAAACAGGTCCCAAGAGAATTCTT------TTGGACAAAATTCTATA
Gentiana procera             TTTGGATTTTATCAATTCAGTTGTTAAAAGAGGTCCTAATAGAATGTTT------TGGGAAAAATGGATAAA
Nicotiana tabacum            TTTTGAGTTAATTAATTCTTTTGTTAAAAAGGGTCCTAAGAGAATTCTG------TGGGACAAAATTATAAA
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Thomandersia hensii CGTCCTATATGATTGGTCATATAATCGTGCTTATATAGATGTTTTTTATACAATATCCTTTACTGGGTCGAT
Thomandersia laurifolia CGTCCTATATGATTGGTCATATAATCGTGCTTATATAGATGTTTTTTATACAATATCCTTTACTGGGTCGAT
Bartsia alpina CACCCTATACGATTGGTCACATAATCGTGCTTATATAGATGCTTTTTATACAATAGCTTTTACTGGAACGAT
Lindenbergia phillipensis TGTCCTATATGATTGGTCATATAATCGTGCTTATATAGATGCTTTTTATAAGATATCTTTTACTGGAACGAT
Paulownia tomentosa CGCCCTATATGATTGGTCATATAATCGTGCTTATATAGATACTTTTTATACAACATCCTTTACTATGGCCAT
Mimulus aurantiacus CGCCCTATATGATTGGTCATATAATCGTGCTTATATAGATGCTTTTTATACAACCTCCTTTACTGGGGCGAT
Callicarpa dichotoma CGCCCTATATGATTGGTCATATAATCGCGCTTATATCGATGCTTTTTATACAACATCC?????????????
Lamium purpureum TGTCCTATATGATTGGTCATATAATCGGGCTTATATTGATTCTTTTTATACAATGTTCTTTGCCAGGGGGGT
Sesamum indicum CATCCTATAT?ATTGGTCATATAA?CG?GCTTATATAGATACTTTTTATACAACATCCTT?ACTGGGGCGAT
Ceratotheca triloba CATCCTATATAATTGGTCATATAATCGTGCTTATATAGATACTTTTTATACAACATCCTTT?CTGGGGCGAT
Elytraria crenata CGTCCTATATGATTGGTCATATAATCGTGCTTATATAGATGCTTTTTATACAACATCTTGTACTGGGGTGAT
Thunbergia alata CGTCCTATATAATTGGTCATATAATCGTGCTTATATAGATGCTTTTTATACAACATCCTTTACTGAGRCCAT
Barleria prionitis CGCCCTATATGATTGGTCATATAATCGCGCTTATATAGATGCTTTTTATACAACATCCTTTATTGCATTGAT
Jacaranda sparrei CGTCTTATATGATTGGTCATATAATCGTGCTTATATAGATGCTTTTTATACAACATCCTTTACTGGGTCGAT
Kigelia africana CGTCCTATATAATTGGTCATATAATCGTGCTTATATAGATGATTTTTATACAACATCCTTTACTGGGGCGAT
Catalpa speciosa TGTCC??
Schlegelia parviflora CGTCCTATATGATTGGTCATATAATCGTGCTTATATAGATGCTTTTTATACAACATGCTTTACTGGGGCGAT
Synapsis ilicifolia CGTCCTATATGATTGGTCATATAATCGTGCTTATATAGATGCTTTTTATACAACATGCTTTACTGGGGCGAT
Petrea racemosa CGTCCTATATGATTGGTCATATAATGGTGCTTATATAGATACTTTTTATGCAACATC?TTTACTAGGGCGAT
Stachytarpheta dichotoma CGTCCTATATGATTGGTCATATAA?CGCGCTTATATAGATGCTTTTTATGCAACATTCTTWACTGGGGCGAT
Verbena bracteata ??AGTCTTTACTGG?TCGAT
Proboscidea louisianica CGTCCTATATGATTGGTCATATAATCGTGCTTATATAGATGCTTTTTATACAACATCCTTTATTAGGGCAAT
Martynia annua CGTCCTATATGATTGGTCATATAATCGTGCTTATATAGATGCTTTTTATACAACATCCTTTATTGGGGCAAT
Hemimeris sabulosa TGTCCTATATGATTGGTCATATAATCGTGCTTATATAGATGCTTTTTATAAAACATCCTTCACTAGGGCTAT
Scrophularia californica ??????ATATGATTGGTCATA??
Retzia capensis TGGCCTATATGATTGGTCATATAATCGTGCTTATATAGATTCTTTTTATACAACATCCTTCACTGTGGCGAT
Halleria lucida TGTTCTATATGATTGGTCATATAATCGTGCTTATATAGATTCTTTTTATACAATATCTTTCACTGGAGCAAT
Angelonia pubescens TGTTGTATATGATTGGTCATATAATCGTGCTTATATAGATGTTTTTTATACATCATCCTTCACCGGGACAAT
Veronica catenata TGTCTTGTATAACTGGTCGTATAATCGTGCCTATTTAGATGTTTTATATACAAGAATTTTGACCGGTTCAAT
Streptocarpus holstii ?ATTATATATGATTGGTCATATAATCGTGCTTATATAGATACTTTTTATACAACATCCTTCACTGGGGCAAT
Nematanthus hirsutus TTTTCTATATGATTGGTCATATAATCGTGCTTATATAGATACTTTTTATACAACATCCTTCACTGGGGCGAT
Calceolaria mexicana TGGTCTATATGATTGGTCCTATAATCGTGCTTATATAGATGCTTTTTATACAACATCCTTCACTGGCACGAT
Jovellana sp. TGTTCTATATGATTGGTCATATAATCGTGCTTATATAGATGCTTTTTATACAACATCCTTCACTGGCACGAT
Ligustrum vulgare TGGTCTATATAATTGGTCATATAATCGTGCTTACATAGATGCTTTTTATACAACATCCTTAACTGGGGCGGT
Borago officinalis TGGTATATATGATTGGTCATATAA??GTGCTTACATAGAT?CTTTTTATAAAAGATCTT?TAACGGGGGGGT
Gentiana procera TTATATTTATGATTGGTCAGATAATCGTGCTTACGTAGATTCTTTTTATACAACAACCATAACTAGAGGAAT
Nicotiana tabacum TGGCATATATGATTGGTCATATAATCGTGCTTACATAGATGCTTTTTATACAAGATTCTTAGTTGGTGGGAT

Thomandersia hensii AAGAGGATTGGCTCAATTAACTCATTTTTTTGATAGACAAGTAATTGATGGAATTACGAATGGGGTTGGTGT
Thomandersia laurifolia AAGAGGATTGGCTCAATTAACTCATTTTTTTGATAGACAAGTAATTGATGGAATTACGAATGGGGTTGGTGT
Bartsia alpina AAGACGGTTGGC??
Lindenbergia phillipensis AAGAGGATTGGCTCAATTAACTCATTTTTTTGATAGACAAATAATTGATGGAATTGCGAATGGGGTTGGTGT
Paulownia tomentosa AAGAGGATTCGCTCAATTAACTTATTTTTTTGATAGACGAGTAATTGATGGAATTGCGAATGGGGTTGGTGT
Mimulus aurantiacus ACGAGGATTAGCTCAATTAACTCATTTTTTTGATAGACGAGTAATTGATGGAATCACGAATGGGGTTGGTGT
Callicarpa dichotoma ??
Lamium purpureum CGGGAAATTGGCCCAATTAACGCATTTTTTTGATAGACGAGTAATTGATGGAATTACGAATGGGGTTGGTGT
Sesamum indicum AAGAGGATTAGCTCAATTAACTGATTTTTTTGATAGACGAGTAATTGATGGAATTACGAATGGGGTTGGTGT
Ceratotheca triloba AAGAGGATTAGCCCAATTAACTGATTTTTTTGATAGACGAGTAATTGATGGAATTACGAATGGGGTTGGTGT
Elytraria crenata AAGAGGATTGGCTGAATTAACTCATTTTTTTGATAGACAAGTAATTGATGGAATTACAAACGGGATTGGTGT
Thunbergia alata AAGAGGATTGGCGGAATTAACTCATTTTTTTGATAGACGAGTAATTGATGGAATTACAAATGGGGTTGGTWT
Barleria prionitis AAGAGGATTGGGAAAATTAACTAATTTTTTTGATCGACGAGTAATTGATGGAATTACAAATGGGGTTGGTGT
Jacaranda sparrei AAGAGGATTGGCTCAATTAACTCATTTTTTTTGATAGACGATTAATTGATGGAATTACGAATGGGGTTGGGGT
Kigelia africana AAGAGGATTGGCGCAATTAACTCATTTTTTTGATAGACGAGTAATTGATGGAATTACAAATGGAGTTGCTAT
Catalpa speciosa ??
Schlegelia parviflora AAGAGGATTGGCTCAATTAACTCATTTTTTTGATAGACAAGTAATTGATGGAATTACTC?TGGGG?CGGCGT
Synapsis ilicifolia AAGAGGATTGGCTCAATTAACTCATTTTTTTGATAGACGAGTAATTGATGGAATTACTAATGGGGGTTG???
Petrea racemosa AAGAGGGTTGGCCCAATTAACTCATTTTTTTGATAGACGTATAATTGATGGAATTACNNACGGAGTTGGTGT
Stachytarpheta dichotoma AAGAGTATCGGCTCAATTAACGT??????????ATAGACGAATAAT????????????????????????????
Verbena bracteata AACAGCATTCGGAAAATTTTCAAATTTTATAGATCGGTACATAATTGACGGAATTTCTAACTCGGTCGGTGT
Proboscidea louisianica AAGAGGATTGTCTGAATTAACTAATTTTTTTGATAGAGGAATAATTGATGGAATTACCAATGGWATTGGGGT
Martynia annua AAGAGGATTGTCTCAATTAACTAATTTTTTTGATAGACGAATAATTGATGGAATTACCAATGGAATTGGTGT
Hemimeris sabulosa AAGGGGGTTAGCTCAATTAACTAATTTTTTTAGATAGACAAGTAATTGATGGAATTACGAATGGGGTTGGTGT
Scrophularia californica ???????GTGGCTCAATTAACTCATTTTTTTGATAGACAAGTAATTGATGGAATTACGAATGGGGTTGGTGT
Retzia capensis AAGAGGATTGGCTCAATTAACTCATTTTTTTGATAGACGAGTAATTGATGGAATTACGAATGGGGTTGGTGT
Halleria lucida AAGAGGATTGGCTCAATTAACTCATTTTTTTGATAGACGAGTAATTGATGGAATTACGAATGGGGTTGGTGT
Angelonia pubescens CAGAGGATTGGCTCAATTAACTCATTTTTTTGATAGACGGGTAATTGATGGAATTACAAATGGGGTTGGTGT
Veronica catenata AAGAGGATTGTCTCAATTAACTCATTTTTTTT?ATA????????????????????????????????????
Streptocarpus holstii AAGAGGATTGACACAATTAACTCATTTTTTTTGATAAACGAATAATTGATGGAATTACGAACGGGATTGGTGT
Nematanthus hirsutus AAGAGGATTGGCTCAAGTAACTCAGTTTTTTGATAGACGAGTGATTGATGCGCTTACGAATGGGGTTGGTGT
Calceolaria mexicana AAGAAGATTAGCTCAATTAACTAATTTTTTTTGATAGACGAATAATTGATGGAATTACCAATGGGGTTGGTGT
Jovellana sp. AAGAAGATTGGCTCAATTAACTCATTTTTTTTGATAGACGGGTAATTGATGGAATTACGAATGGGGTTGGTGT
Ligustrum vulgare AAGAGGCTTAGCCCAAGTAACTCATTTTTTTTGATCGACGAGTAATTGATGGAATTACGAATGGGGTTGGTGT
Borago officinalis AAGAAGATTAGCCGAATTAACTCATTTTTTTTGATCGACGGGTAATTGATGGAATTATAAATAGCGTTGGGGT
Gentiana procera AAGAGGATTAGCTGAATTAACTCATTTTTTTTGATCGATGTCTAATTGATGGAATTACCAATGGAGTTGGTTT
Nicotiana tabacum AAGAGGATTAGCTGAATTTACTCATTTTTTTTGATCGACGAGTAATTGATGGGATGACTAACGGGGTTGGGGT

Thomandersia hensii	TATGAGTTTC??????	[2,176]
Thomandersia laurifolia	TATAAGTTTCTTTGTC	[2,176]
Bartsia alpina	????????????????	[2,176]
Lindenbergia phillipensis	CATAAGTTTCTTTGTA	[2,176]
Paulownia tomentosa	CATCAGTTTCTTTGTA	[2,176]
Mimulus aurantiacus	TATGAGTTTCTTTGTA	[2,176]
Callicarpa dichotoma	????????????????	[2,176]
Lamium purpureum	CATGAGTTTTT?????	[2,176]
Sesamum indicum	TATGAGTTTCTTTGTA	[2,176]
Ceratotheca triloba	TATGAGTTTCTTTGTA	[2,176]
Elytraria crenata	TATCAGTTTCTTTGTA	[2,176]
Thunbergia alata	TATGAGTTTCTTTRTA	[2,176]
Barleria prionitis	TATCAGTTTCTTTGTA	[2,176]
Jacaranda sparrei	TATGAGTTTC??????	[2,176]
Kigelia africana	TTTGAGTTTCTTTGTA	[2,176]
Catalpa speciosa	????????????????	[2,176]
Schlegelia parviflora	TATGAGTTTCTTTGTA	[2,176]
Synapsis ilicifolia	????????????????	[2,176]
Petrea racemosa	TATGAGTTTCTTTGTN	[2,176]
Stachytarpheta dichotoma	????????????????	[2,176]
Verbena bracteata	TATGATTTTCCTTCTA	[2,176]
Proboscidea louisianica	TATGAGTTGTTTGGTA	[2,176]
Martynia annua	TATGAGTT????????	[2,176]
Hemimeris sabulosa	TATGAGTTTCTTTGTA	[2,176]
Scrophularia californica	TATGAGTTTCTTTGTA	[2,176]
Retzia capensis	TATGAGTTTCTTTGTA	[2,176]
Halleria lucida	TATGAGTTTCTTTGTA	[2,176]
Angelonia pubescens	TCTGAGTTTCTTTGTC	[2,176]
Veronica catenata	????????????????	[2,176]
Streptocarpus holstii	TATAAGTTTTTTTG??	[2,176]
Nematanthus hirsutus	TATGAGTTTCTTTGTA	[2,176]
Calceolaria mexicana	TATGAGTTTCTTTGTA	[2,176]
Jovellana sp.	TATGAGTTTCTTTGTA	[2,176]
Ligustrum vulgare	TATGAGTTTCTTTGTA	[2,176]
Borago officinalis	TCTGAGTTTTTTTGTA	[2,176]
Gentiana procera	TTTCA?TTTCTTTG??	[2,176]
Nicotiana tabacum	TATTAGTTTCATTGTA	[2,176]

Appendix 10 Alignment matrix for the *rbcL* region (positions 27-1,428 in *Nicotiana tabacum*). Alignment gaps are indicated by "-". Missing data are indicated by "?".

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Thomandersia hensii          AAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTCAAAATGGACTTATTATACTCCTGAATACGAAACCAAAGA
Thomandersia laurifolia      AAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Bartsia alpina               AAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Lindenbergia phillipensis    ???TGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACAAAAGA
Paulownia tomentosa          ???TGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Mimulus aurantiacus          ??????????????????????????????????????????????????????????????????????
Callicarpa dichotoma         AAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Lamium purpureum             ??????????????????????????????????????????????????????????????????????
Sesamum indicum              AGGTGTTGGATTCAAAGCGGGTGTTAAAGAGTATAAATTGACTTATTATACCCCTGAATACGAAACCAAAGA
Ceratotheca triloba          ??????????????????????????????????????????????????????????????????????
Elytraria crenata            TTTTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Thunbergia usambarica        ??????????TTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Barleria prionitis           ??GTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTAACTTATTATACTCCTGAATACGAAACCAAAGA
Jacaranda sparrei            ??GTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAGGA
Kigelia africana             AAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Catalpa speciosa             AAGTGTTGGATTCAAAGCAGGCGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAGGA
Schlegelia parviflora        ??????????????????????????????????????????????????????????????????????
Synapsis ilicifolia          AAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACAAAACCAAAGA
Petrea racemosa              AAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACAAAACCAAAGA
Stachytarpheta dichotoma     AAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Verbena bonariensis          AAGTGTTGGATTCAAAGCGGGTGTAAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Proboscidea louisianica      ??????????????????????????????????????????????????????????????????????
Hemimeris sabulosa           ??GTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Scrophularia californica     AGGTGTTGGATTCAAAGCCGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Retzia capensis              A?GTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Halleria lucida              AAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Angelonia pubescens          AAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Veronica catenata            TTTTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Streptocarpus holstii        AGGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Nematanthus hirsutus         ??????????????????????????????????????????????????????????????????????
Calceolaria mexicana         AAGTGTTGGATTCAA?GCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Jovellana sp.                AAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATATGAAACCAAAGA
Ligustrum vulgare            AAGTGTTGGATTCAAAGCGGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAATACGAAACCAAAGA
Borago officinalis           ??????????????????????????????????????????????????????????????????????
Gentiana procera             ??????????????????????????????????????????????????????????????????????
Nicotiana tabacum            ??????????????????????????????????????????????????????????????????????
                             AAGTGTTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCCTGAGTACCAAACCAAGGA

Thomandersia hensii          TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCGGT
Thomandersia laurifolia      TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCGGCGGT
Bartsia alpina               TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCTGCAGT
Lindenbergia phillipensis    TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCTGCGGT
Paulownia tomentosa          ????GATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCAGT
Mimulus aurantiacus          TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCGGT
Callicarpa dichotoma         ????GATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCGGT
Lamium purpureum             TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCCGAAGAAGCAGTGGCCGCGGT
Sesamum indicum              ????GATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGSSSCGGT
Ceratotheca triloba          TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCGGT
Elytraria crenata            TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCGGT
Thunbergia usambarica        TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCTGCAGT
Barleria prionitis           TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAAGAAGCAGGGGCAGCGGT
Jacaranda sparrei            TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCGGT
Kigelia africana             TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAAGAAGCAGGGGCAGCGGT
Catalpa speciosa             ????GATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGG??CGCGGT
Schlegelia parviflora        TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCGGT
Synapsis ilicifolia          TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCGGT
Petrea racemosa              TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCAGT
Stachytarpheta dichotoma     TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCAGT
Verbena bonariensis          ????GATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCTGCGGT
Proboscidea louisianica      TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCTGCGGT
Hemimeris sabulosa           TACTGATATCTTAGCAGCATTTCGAGTAACTCCTCAACCTGGAGTTCCACCTGAAGAAGCAGGGGCCGCGGT
Scrophularia californica     TACTGATATCTTAGCAGCATTCCGAGTAAGTCCTCAACCCGGAGTTCCGCCTGAAGAAGCAGGAGCCGCGGT
Retzia capensis              TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGG???AGCGGT
Halleria lucida              TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCGGT
Angelonia pubescens          TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGCGGT
Veronica catenata            TACTGATATCTTAGCAGCATTCCGAGTAAGTCCTCAACCGGGAGTTCCACCTGAAGAAGCAG????????GT
Streptocarpus holstii        ????GATATCTTGGCGGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCGGCTGT
Nematanthus hirsutus         TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCGGCGGT
Calceolaria mexicana         TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCGGCGGT
Jovellana sp.                TACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCGGCGGT
Ligustrum vulgare            ????GATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAGGCAGGGGCTGCGGT
Borago officinalis           ????GATATCTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCCTCCTGAAGAAGCAGGGGCCGCAGT
Gentiana procera             ????GATATCTTGGCAGCATTCCGAGTAACCCCTCAACCCGGAGTTCCACCCGAAGAAGCAGGGGCCGCGGT
Nicotiana tabacum            TACTGATATATTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCACCTGAAGAAGCAGGGGCCGCGGT
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Thomandersia hensii	AGCGGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Thomandersia laurifolia	AGCGGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Bartsia alpina	CGCTGCCGAATCTTCGACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGCCTTGATCGTTACAA
Lindenbergia phillipensis	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Paulownia tomentosa	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Mimulus aurantiacus	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Callicarpa dichotoma	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Lamium purpureum	AGCTGCCGAATCTTCGACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Sesamum indicum	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGTCTTGATCGTTACAA
Ceratotheca triloba	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGTCTTGATCGTTACAA
Elytraria crenata	AGCTGCCGAATCTTCTACTGGTACATGGACAACCGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Thunbergia usambarica	AGCTGCCGAATCTTCTACTGGTACATGGACAACCGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Barleria prionitis	AGCTGCCGAATCTTCCACTGGTACATGGACAACCGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Jacaranda sparrei	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Kigelia africana	AGCTGCCGAATCTTCTACCGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Catalpa speciosa	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Schlegelia parviflora	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Synapsis ilicifolia	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Petrea racemosa	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Stachytarpheta dichotoma	AGCTGCCGAATCTTCTACTGGTACATGGACAACCGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Verbena bonariensis	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Proboscidea louisianica	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Hemimeris sabulosa	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTATAA
Scrophularia californica	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACGAGTCTTGATCGTTACAA
Retzia capensis	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGAT????TTACCAGCCTTGATCGTTACAA
Halleria lucida	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGCCTTGATCGTTACAA
Angelonia pubescens	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Veronica catenata	AGCAGCCGGATCTTCAACCGGTACATGGACAACTGTGTGGACCGATGG?CTTACCAGCCTTGATCGTTACAA
Streptocarpus holstii	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGAT????TTACCAGCCTTGATCGTTACAA
Nematanthus hirsutus	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Calceolaria mexicana	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGTCTTGATCGTTACAA
Jovellana sp.	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGGCTTACCAGCCTTGATCGTTACAA
Ligustrum vulgare	AGCTC??GAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Borago officinalis	AGCCGCTGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACCAGCCTTGATCGTTACAA
Gentiana procera	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTGTGGACCGATGGACTTACTAGCCTTGATCGTTACAA
Nicotiana tabacum	AGCTGCCGAATCTTCTACTGGTACATGGACAACTGTATGGACCGATGGACTTACCAGCCTTGATCGTTACAA

Thomandersia hensii	AGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Thomandersia laurifolia	AGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAGCAGATCAATATATCTGTTATGTAGCTTACCCTTT
Bartsia alpina	AGGACGATGCTACCACATCGAGCCTGTTCCTGGAGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Lindenbergia phillipensis	AGGACGATGCTACCACATCGAGCCCGTTCCTGGAGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Paulownia tomentosa	AGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Mimulus aurantiacus	AGGGCGATGCTACCACATCGAGCCCGTTCTTGGAGAAAAAGATCAATATATCTGTTATGTAGCTTACCCTTT
Callicarpa dichotoma	AGGGCGATGCTACCACATCGAGCCCGTTCTTGGAGAAAAGGATCAATATAT?????ATGTAGCTTACCCTTT
Lamium purpureum	AGGGCGATGCTACAACATCGAGCCGGTTCCTGGAGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Sesamum indicum	AGGGCGATGCTACCACATCGAGCCGGTTCCTGGAGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Ceratotheca triloba	AGGGCGATGCTACAACATCGAGCCCGTTCCTGGCGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Elytraria crenata	AGGGCGATGCTACAACATCGAGCCCGTTCCTGGCGAAACGGATCAATATATCTGTTATGTAGCTTACCCTTT
Thunbergia usambarica	AGGGCGATGCTACAACATCGAGCCCGTTGCTGGCGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Barleria prionitis	AGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAGCAGATCAATATATCTGTTATGTAGCTTATCCTTT
Jacaranda sparrei	AGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAGCAGATCAATATATCTGTTATGTAGCTTACCCTTT
Kigelia africana	AGGGCGATGCTACCACATCGAGCCCGTTGTTGGAGAAGCGGATCAATATATCTGTTATGTAGCTTACCCTTT
Catalpa speciosa	AGGGCGATGCTACCACATCGATCCCGTTCTTGGAGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Schlegelia parviflora	AGGGCGATGCTACCACATCGATCCCGTTCTTGGAGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Synapsis ilicifolia	AGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAGCAGATCAATATATCTGTTATGTAGCTTACCCTTT
Petrea racemosa	AGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAGCAGATCAATATATCTGTTATGTAGCTTACCCTTT
Stachytarpheta dichotoma	AGGGCGATGCTACCACATTGAGCCCGTTCCTGGAGACCCAGATCAATATATTTGTTATGTAGCTTACCCTTT
Verbena bonariensis	AGGGCGATGCTACCACATCGAGCCAGTTCCTGGAGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Proboscidea louisianica	AGGTCGATGCTACCACATCGAGCCCGTTCCTGGAGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Hemimeris sabulosa	AGGGCGATGCTACCACATCGAACCCGTTCCGGGAGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Scrophularia californica	AGGG??ATGCTACCACATCGAGCCCGTTCCTGGAGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Retzia capensis	AGGGCGATGCTACAACATCGAGCCCGTTCCTGGAGAACCAGATCAATATATCTGTTATGTAGCTTACCCTTT
Halleria lucida	AGGGCGATGCTACAACATCGAACCCGTTCCTGGAGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Angelonia pubescens	AGGGC??TGCTACCACATCGAGCCCGTTCCTGG???ACCAGATCAATATATCTGTTATGTAGCTTACCCTTT
Veronica catenata	AGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAACAGATCAATATATCTGTTATGTAGCTTACCCTTT
Streptocarpus holstii	AGGG??ATGCTACCACATCGAGCCCGTTCCTGGAGAAGCAGATCAATATATCTGTTATGTAGCTTACCCTTT
Nematanthus hirsutus	AGGGCGATGCTACCACATCGAGCCCGTTCTTGGAGAAGCAGATCAATATATCTGTTATGTAGCTTACCCTTT
Calceolaria mexicana	AGGGCGATGCTACCACATCGAGCCCGTTCTTGGAGAAGCAGATCAATATATCTGTTATGTAGCTTACCCTTT
Jovellana sp.	AGGGCGATGCTACCATATTGAGCCCGTTCCTGGAGAAGCAGATCAATATATCTGTTATGTAGCTTACCCTTT
Ligustrum vulgare	AGGGCGATGCTACCACATCGATGCCGTTCCGGGAGAAGAAAATCAATATATATGTTATGTAGCTTATCCTTT
Borago officinalis	AGGGCGATGCTACCACATTGAGCCCGTTCCTGGAGAAGAAGATCAATTTATTGCTTATGTAGCTTATCCCCT
Gentiana procera	AGGGCGATGCTACCGCATCGAGCGTGTTGTTGGAGAAAAAGATCAATATATTGCTTATGTAGCTTACCCTTT
Nicotiana tabacum	

Thomandersia hensii	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Thomandersia laurifolia	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Bartsia alpina	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAACGTATTTGGATTCAAAGC
Lindenbergia phillipensis	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Paulownia tomentosa	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Mimulus aurantiacus	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Callicarpa dichotoma	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Lamium purpureum	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Sesamum indicum	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Ceratotheca triloba	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Elytraria crenata	AGACCTTTTTGAAGAAGGTTCCGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Thunbergia usambarica	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Barleria prionitis	AGACCTTTTTGAAGAAGGTTCTGTTACCAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Jacaranda sparrei	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Kigelia africana	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Catalpa speciosa	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Schlegelia parviflora	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Synapsis ilicifolia	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Petrea racemosa	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Stachytarpheta dichotoma	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Verbena bonariensis	AGACCTTTTTGAAGAAGGTTCTG?TACGAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Proboscidea louisianica	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Hemimeris sabulosa	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAACGTATTTGGATTCAAAGC
Scrophularia californica	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTCACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Retzia capensis	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Halleria lucida	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Angelonia pubescens	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Veronica catenata	AGATCTTTTTGAAGAAGGGTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Streptocarpus holstii	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGATTCAAAGC
Nematanthus hirsutus	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGATTCAAAGC
Calceolaria mexicana	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGAAATGTATTTGGATTCAAAGC
Jovellana sp.	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGCAATGTATTTGGATTCAAAGC
Ligustrum vulgare	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGC
Borago officinalis	AGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCCATCGTAGGTAATGTATCTGGGTTCAAAGC
Gentiana procera	AGACCTTTTTGAAGAAGGTCCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGC
Nicotiana tabacum	AGACCTTTTTGAAGAAGGTTCTGTTACCAACATGTTTACTTCCATTGTAGGTAACGTATTTGGGTTCAAAGC

Thomandersia hensii	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCAACTATTAAAACTTTCCAACGCCCGCCTCA
Thomandersia laurifolia	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATATTAAAACTTTCCAAGGCCCGCCTCA
Bartsia alpina	CCTGCGTGCGCTACGTCTGGAAGATCTGCGAATCCCCCCTGCTTATATTAAAACTTTCCAAGGACCGCCTCA
Lindenbergia phillipensis	CCTGCGTGCTCTACGTCTGGAAGATTTGCGAATCCCTCCTGCTTATATTAAAACTTTCCAAGGCCCGCCTCA
Paulownia tomentosa	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATATTAAAACTTTCCAAGGCCCGCCTCA
Mimulus aurantiacus	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTACTGCTTATGTTAAAACTTTCCAAGGCCCGCCTCA
Callicarpa dichotoma	CCTACGTGCTCTACGTCTGGAAGATCTGCGAATCCCTACTGCTTATGTTAAAACTTTCCAAGGCCCGCCTCA
Lamium purpureum	CCTACGTGCTCTACGTCTGGAAGATCTGCGAAT?CCTCCTGCTTATGTTAAAACTTTCCAAGGCCCACCTCA
Sesamum indicum	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTACTGCTTATGTTAAAACTTTCCAAGGCCCGCCTCA
Ceratotheca triloba	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATATTAAAACTTTCCAAGGCCCGCCTCA
Elytraria crenata	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATGTTAAAACTTTCCAAGGTCCGCCTCA
Thunbergia usambarica	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATATTAAAACTTTCCAAGGTCCACCTCA
Barleria prionitis	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTACTGCTTATGTTAAAACTTTCCAAGGTCCGCCTCA
Jacaranda sparrei	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATATTAAAACTTTCCAAGGCCCGCCTCA
Kigelia africana	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAGGCCCGCCTCA
Catalpa speciosa	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATATTAAAACTTTCCAAGGCCCGCCTCA
Schlegelia parviflora	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATATTAAAACTTTCCAAGGTCCGCCTCA
Synapsis ilicifolia	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATATTAAAACTTTCCAAGGTCCGCCTCA
Petrea racemosa	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAGGCCCGCCTCA
Stachytarpheta dichotoma	CCTACGTGCTCTACGTCTGGAAGATCTGCGAATCCCTGTTGCTTATGTTAAAACTTTCCAAGGCCCGCCTCA
Verbena bonariensis	CCTACGCGTGCTACGTCTGGAAGATCTGCGAATCCCCGTTGCCTATACTAAAACTTTCCAAGGCCCGCCTCA
Proboscidea louisianica	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTACTGCTTATATTAAAACTTTCCAAGGCCCGCCTCA
Hemimeris sabulosa	CCTACGTGCTCTACGTCTGGAAGATHTGCGCATCCCTGTTGCTTATGTTAAAACTTTCCAAGGCCCGCCTCA
Scrophularia californica	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATACCTGTTGCTTATGTTAAAACTTTCCAAGGCCCTCCTCA
Retzia capensis	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATGTTAAAACTTTCCAAGGCCCGCCTCA
Halleria lucida	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTACCTCTTATGTTAAAACTTTCCAAGGCCCGCCTCA
Angelonia pubescens	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTGTTGCTTATGTTAAAACTTTCCAAGGCCCGCCTCA
Veronica catenata	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTGTTGCTTATGTTAAAACTTTCCAAGGCCCACCTCA
Streptocarpus holstii	CCTTCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTACTGCTTATGTTAAAACTTTCCAAGGCCCGCCTCA
Nematanthus hirsutus	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATGTTAAAACTTTCCAAGGCCCGCCTCA
Calceolaria mexicana	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATGTTAAAACTTTCCAAGGCCCCCCTCA
Jovellana sp.	CCTGCGTGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATGTTAAAACTTTCCAAGGCCCCCCTCA
Ligustrum vulgare	CCTGCGTGCTCTACGTCTGGAAGATCTGCGA?TCCCTACTGCTTATATTAAAACTTTCCAAGGCCCGCCTCA
Borago officinalis	CTTGCGCGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATGTTAAAACTTTCCAAGGCCCACCTCA
Gentiana procera	CCTACGTGCTCTACGCCTGGAAGATTTGCGAATCCCACCCGCGTATGTTAAAACTTTCCAAGGCCCTCCTCA
Nicotiana tabacum	CCTGCGCGCTCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATGTTAAAACTTTCCAAGGTCCGCCTCA

Thomandersia hensii	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATGAAACCTAA
Thomandersia laurifolia	TGGTATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGCCCCCTGTTGGGATGTACTATTAAACCTAA
Bartsia alpina	CGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCACTGTTGGGATGTACTATTAAACCTAA
Lindenbergia phillipensis	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTACGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAA
Paulownia tomentosa	TGGGGTCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAA
Mimulus aurantiacus	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAA
Callicarpa dichotoma	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCTCTGTTGGGATGTACTATTAAACCGAA
Lamium purpureum	TGGGATCCAAGTTGAGAGAGAT?AATTGA?CAAGTATGGTCGTCCTCTGTTGGGATGTACTATTAAACCTAA
Sesamum indicum	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAATATGGTCGTCCTCTGTTGGGATGTACTATTAAACCTAA
Ceratotheca triloba	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAATATGGTCGTCCTCTGTTGGGATGTACTATTAAACCTAA
Elytraria crenata	TGGGATCCAATCTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGCTGGGATGTACTATTAAACCTAA
Thunbergia usambarica	TGGGATCCAATCTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGCTGGGATGTACTATTAAACCTAA
Barleria prionitis	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAATATGGTCGTCCTCTGCTGGGATGTACTATTAAACCTAA
Jacaranda sparrei	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAA
Kigelia africana	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAA
Catalpa speciosa	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAA
Schlegelia parviflora	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAA
Synapsis ilicifolia	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAA
Petrea racemosa	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAA
Stachytarpheta dichotoma	TGGGATACAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCTCTGTTGGGATGTACTATTAAACCTAA
Verbena bonariensis	TGGGATCCAATCTGAGAGAGATAAATTAAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAA
Proboscidea louisianica	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCTCTGATGGGATGTACTATTAAACCTAA
Hemimeris sabulosa	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAA
Scrophularia californica	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACTATTAAACCTAA
Retzia capensis	CGGGATCCAAGTTGAGAGAGATAAATTGAATAAGTATGGGCGTCCCCTGTTGGGATGTACTATTAAACCTAA
Halleria lucida	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACAATTAAACCTAA
Angelonia pubescens	CGGGATCCAAAGTGAGAGAGATAAGTTGAACAAGTACGGTCGTCCTCTGTTGGGATGTACTATTAAACCTAA
Veronica catenata	CGGGATTCAGGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCTCTGTTGGGATGTACTATTAAACCTAA
Streptocarpus holstii	TGGGATCCAAGTTGAAAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCAAA
Nematanthus hirsutus	TGGGATCCAAGTTGAAAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCAAA
Calceolaria mexicana	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACTATTAAACCGAA
Jovellana sp.	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTATTGGGATGTACTATTAAACCTAA
Ligustrum vulgare	TGGGATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCAAA
Borago officinalis	TGGGATCCAAGTTGAAAGAGATAAATTGAACAAGTACGGCCGTCCTTTGTTGGGATGTACTATTAAACCTAA
Gentiana procera	TGGCATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGCCCCCTGTTGGGATGTACTATTAAACCGAA
Nicotiana tabacum	TGGGATCCAAGTTGAAAGAGATAAATTGAACAAGTATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAA

Thomandersia hensii	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Thomandersia laurifolia	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Bartsia alpina	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Lindenbergia phillipensis	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Paulownia tomentosa	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Mimulus aurantiacus	ACTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Callicarpa dichotoma	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCCGTTTATGAATGTCTTCGCGGTGGGCTTGATTTTACCAA
Lamium purpureum	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Sesamum indicum	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCATGTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Ceratotheca triloba	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Elytraria crenata	ATTGGGGTTATCCGCTAAAAACTATGGTAGGGCATGTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Thunbergia usambarica	GTTGGGGTTATCCGCTAAAAACTATGGTAGAGCGTGCTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Barleria prionitis	ATTGGGGTTATCCGCTAAAAACTATGGTAGAGCATGTTATGAATGTCTTCGTGGTGGACTTGATTTTACGAA
Jacaranda sparrei	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Kigelia africana	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Catalpa speciosa	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Schlegelia parviflora	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Synapsis ilicifolia	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Petrea racemosa	ATTGGGGTTATCTGCTAAAAACTACGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Stachytarpheta dichotoma	ATTGGGGTTATCTGCTAAAAACTACGGTAGAGCATGTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Verbena bonariensis	ATTGGGTTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Proboscidea louisianica	ATTGGGGCTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Hemimeris sabulosa	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Scrophularia californica	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGTGGTGGACTTGATTTTACCAA
Retzia capensis	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Halleria lucida	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Angelonia pubescens	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Veronica catenata	ATTGGGTTTATCTGCTAAAAACTACGGTAGAGCATGTTATGAATGTCTTCGTGGTGGACTTGATTTTACCAA
Streptocarpus holstii	ACTGGGGTTATCCGCTAAAAACTACGGTAGAGCGGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Nematanthus hirsutus	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCGGTTTATGAATGTCTTCGCGGCGGACTTGATTTTACCAA
Calceolaria mexicana	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGCGGACTTGATTTTACCAA
Jovellana sp.	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTATGAATGTCTTCGCGGCGGACTTGATTTTACCAA
Ligustrum vulgare	ATTGGGGTTATCTGCTAAAAACTATGGTAGAGCAGTTTTTGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Borago officinalis	ATTGGGCTTATCCGCTAAAAACTACGGTAGAGCCGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACCAA
Gentiana procera	ATTGGGGTTATCTGCTAAAAACTACGGTAGAGCAGTTTATGAATGTCTTCGTGGTGGACTTGATTTTACTAA
Nicotiana tabacum	ATTGGGGTTATCTGCTAAAAACTACGGTAGAGCCGTTTATGAATGTCTTCGCGGTGGACTTGATTTTACTAA

Thomandersia hensii	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAAT
Thomandersia laurifolia	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACT
Bartsia alpina	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAAT
Lindenbergia phillipensis	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACT
Paulownia tomentosa	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACT
Mimulus aurantiacus	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAAT
Callicarpa dichotoma	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGAC?GCTTCTTATTTTGTGCCGAAGCACT
Lamium purpureum	AGATGATGAAAACGTGAACTCACAGCCATTTATGCGTTGGAGAGATCGCTTCTTGTTTTGTGCCGAAGCAAT
Sesamum indicum	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAAT
Ceratotheca triloba	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAAT
Elytraria crenata	AGATGATGAGAACGTGAACTCTCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAAT
Thunbergia usambarica	AGATGATGAGAATGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAAT
Barleria prionitis	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAAT
Jacaranda sparrei	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCCAT
Kigelia africana	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCCCT
Catalpa speciosa	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCCCT
Schlegelia parviflora	AGATGATGAGAACGTGAACTCCCAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACT
Synapsis ilicifolia	AGATGATGAGAACGTGAACTCCCAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACT
Petrea racemosa	AGATGATGAGAACGTAAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACT
Stachytarpheta dichotoma	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACT
Verbena bonariensis	AGATGATGAGAACGTGAACTCTCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAAT
Proboscidea louisianica	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACT
Hemimeris sabulosa	AGATGATGAAAACGTGAACTCCCAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAAT
Scrophularia californica	AGATGATGAGAATGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAAT
Retzia capensis	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAAT
Halleria lucida	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCAAT
Angelonia pubescens	AGATGATGAGAACGTGAACTCCCAGCCGTTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGGAAT
Veronica catenata	AGATGATGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCTGAAGCCAT
Streptocarpus holstii	AGATGACGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCTCT
Nematanthus hirsutus	AGATGACGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACT
Calceolaria mexicana	AGATGACGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCTGAAGCAAT
Jovellana sp.	AGATGACGAGAACGTGAACTCCCAGCCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGCGCCGAAGCACT
Ligustrum vulgare	AGATGATGAGAACGTGAACTCCCAACCGTTTATGCGTTGGAGAGACCGTTTCTTATTTTGTGCCGAAGCTCT
Borago officinalis	AGATGATGAAAACGTGAACTCCCAACCATTTATGCGTTGGAGAGCTGGTTTCCTATTTTGTGCCGAAGCAGT
Gentiana procera	AGATGATGAGAACGTGAACTCACAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACT
Nicotiana tabacum	

Thomandersia hensii	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Thomandersia laurifolia	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Bartsia alpina	TTATAAAGCACAGGCTGAAACAGGCGAAATCAAAGGTCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Lindenbergia phillipensis	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Paulownia tomentosa	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACGGCTGGTACATGCGAAGA
Mimulus aurantiacus	TTATAAATCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Callicarpa dichotoma	TTATAAAGCACAGTGTGAAACAGGCGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Lamium purpureum	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTATTTGAATGCTATTGCGGGTACATGCGAAGA
Sesamum indicum	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Ceratotheca triloba	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Elytraria crenata	TTATAAATCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Thunbergia usambarica	TTATAAATCACAGGCTGAAACAGGCGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Barleria prionitis	TTATAAATCACAGGCCGAAACAGGCGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Jacaranda sparrei	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Kigelia africana	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Catalpa speciosa	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Schlegelia parviflora	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Synapsis ilicifolia	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Petrea racemosa	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCAGGTACATGCGAAGA
Stachytarpheta dichotoma	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAAGA
Verbena bonariensis	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCAGGTACATGCGAAGA
Proboscidea louisianica	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Hemimeris sabulosa	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAGGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Scrophularia californica	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCAGGTACATGCGAAGA
Retzia capensis	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Halleria lucida	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCAGGTACATGCGAAGA
Angelonia pubescens	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Veronica catenata	TTATAAATCACAGGCGGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Streptocarpus holstii	TTATAAATCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAATA
Nematanthus hirsutus	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Calceolaria mexicana	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Jovellana sp.	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Ligustrum vulgare	TTATAAATCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCGGGTACATGCGAAGA
Borago officinalis	TTATAAAGCACAAGCTGAAACAGGTGAAATCAAAGGACATTACTTAAATGCTACTGCAGGTAATTGCGATGA
Gentiana procera	TTATAAAGCACAGGCTGAAACAGGTGAAATAAAGGGGCATTACTTGAATGCTACTGCAGGTACATGTGAAGA
Nicotiana tabacum	TTATAAAGCACAGGCTGAAACAGGTGAAATCAAAGGGCATTACTTGAATGCTACTGCAGGTACATGCGAAGA

Thomandersia hensii	AATGATGAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCATAATGCATGACTACTTAACAGGAGG
Thomandersia laurifolia	AATGATAAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Bartsia alpina	AATGATGAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Lindenbergia phillipensis	AATGATGAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Paulownia tomentosa	AATGATCAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Mimulus aurantiacus	AATGATGAAAAGAGCTATATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACGGGAGG
Callicarpa dichotoma	AATGATGAAAAGAGCTATATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Lamium purpureum	AATGATCAAAAGGGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Sesamum indicum	AATGATGAAAAGAGCTATATTTGCTAGAGAATTGGGAGTTCCTATCATAATGCATGACTACTTAACAGGAGG
Ceratotheca triloba	AATGATCAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Elytraria crenata	AATGATCAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Thunbergia usambarica	AATGATCAAAAGAGCTGTATTTGCTAGAGAATTGGCAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Barleria prionitis	AATGATGAAAAGAGCTGTATTTGCTAGGGAATTGGGAGTTCCTATCATAATGTTTGACTACTTAACAGGAGG
Jacaranda sparrei	AATGATCAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Kigelia africana	AATGATGAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Catalpa speciosa	AATGATCAAAAGAGCTGTATTTGCTAGAGAATTAGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Schlegelia parviflora	CATGATGAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Synapsis ilicifolia	AATGATGAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Petrea racemosa	AATGATCAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Stachytarpheta dichotoma	AATGATGAAAAGAGCTATATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Verbena bonariensis	AATGATGAAAAGAGCTATATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGTATGACTACTTAACAGGAGG
Proboscidea louisianica	AATGATGAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTGCCTATCATAATGCATGACTACTTAACAGGAGG
Hemimeris sabulosa	AATGATGAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCACGATTACTTAACAGGAGG
Scrophularia californica	AATGATGAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Retzia capensis	AATGATGAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCGTTTGTAATGCATGACTACTTAACAGGAGG
Halleria lucida	AATGATGAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Angelonia pubescens	AATGATAAAAAGAGCTGTATTTGCTAGAGAATTGGGGGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Veronica catenata	AATGATGAAAAGAGCGATATTTGCTAGGGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Streptocarpus holstii	AATGATGAAAAGGGCTATATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Nematanthus hirsutus	AATGATGAAAAGGGCGGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTACTTAACAGGAGG
Calceolaria mexicana	AATGATCAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTATATAACAGGAGG
Jovellana sp.	AATGATCAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGACTATATAACAGGAGG
Ligustrum vulgare	AATGATCAAAAGAGCTGTATTTGCTAGAGAATTGGGAGCTCCTATCGTAATGCATGACTACTTAACAGGAGG
Borago officinalis	AATGATAAAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTTATGCACGACTACCTAACAGGGGG
Gentiana procera	AATGATCAAAAGAGCCGTATTTGCTAGAGAATTGGCAGTTCCTATTGTAATGCACGATTACTTAACAGGGGG
Nicotiana tabacum	AATGATCAAAAGAGCTGTATTTGCTAGAGAATTGGGCGTTCCGATCGTAATGCATGACTACTTAACGGGGGG

Thomandersia hensii	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCACCGTGCAAT
Thomandersia laurifolia	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCACCGTGCAAT
Bartsia alpina	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Lindenbergia phillipensis	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAACGGCCTACTTCTTCACATTCACCGTGCAAT
Paulownia tomentosa	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Mimulus aurantiacus	ATTCACTGCAAATACTACCTTGGCTCATTATTGCCGAGATAATGGCCTGCTTCTTCACATTCACCGTGCAAT
Callicarpa dichotoma	ATTCACTGCAAATACTAGCTTGTCTCATTATTGCCGAGATAATGGCTTACTTCTTCACATTCACCGTGCAAT
Lamium purpureum	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCTTACTTCTTCACATTCACCGTGCAAT
Sesamum indicum	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Ceratotheca triloba	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Elytraria crenata	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAACGGCCTACTTCTTCACATTCACCGTGCAAT
Thunbergia usambarica	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Barleria prionitis	ATTCACTGCAAATACGACTTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCACCGTGCAAT
Jacaranda sparrei	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Kigelia africana	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Catalpa speciosa	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Schlegelia parviflora	ATTCACTGCAAATACTACCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Synapsis ilicifolia	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Petrea racemosa	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Stachytarpheta dichotoma	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Verbena bonariensis	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCACCGTGCAAT
Proboscidea louisianica	ATTCACTGCAAATACTAGCTTGTCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Hemimeris sabulosa	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Scrophularia californica	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Retzia capensis	ATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Halleria lucida	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATTCACCGTGCAAT
Angelonia pubescens	ATTCACTGCAAATACTAGTTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Veronica catenata	ATTCACTGCAAATACTACCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATCCACCGTGCAAT
Streptocarpus holstii	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Nematanthus hirsutus	ATTCACTGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATTCACCGTGCAAT
Calceolaria mexicana	ATTCACTGCAAATACTACCTTGTCTAATTATTGCCGGGATAATGGTCTACTTCTTCACATTCACCGTGCAAT
Jovellana sp.	ATTCACCGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGCCTACTTCTTCACATCCACCGTGCAAT
Ligustrum vulgare	ATTCACCGCAAATACTAGCTTGGCTCATTATTGCCGAGATAATGGTCTACTTCTTCACATCCACCGTGCAAT

Thomandersia hensii	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGGGTTACGTATGTC
Thomandersia laurifolia	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Bartsia alpina	GCACGCCGTTATTGATAGACAGAAGAATCACGGTATACACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Lindenbergia phillipensis	GCATGCAGTTATTGATAGACAGAAGAACCATGGGATACACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Paulownia tomentosa	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATACACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Mimulus aurantiacus	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCGTTACGTCTGTC
Callicarpa dichotoma	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGACCTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Lamium purpureum	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCGCTACGTCTGTC
Sesamum indicum	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGMMCTTCCGTGTACTASSTAAAGCGTTACGTATGTC
Ceratotheca triloba	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCGTTACGTCTGTC
Elytraria crenata	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Thunbergia usambarica	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCCTTACGTCTGTC
Barleria prionitis	GCATGCAGTTATTGATAGACAGAAGAATCACGGTATGCACTTCCGTGTACTAGCTAAAGCGTTACGGATGTC
Jacaranda sparrei	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Kigelia africana	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Catalpa speciosa	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATC??CTTCCGTGTACTA??TAAAGCGTTACGTATGTC
Schlegelia parviflora	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Synapsis ilicifolia	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Petrea racemosa	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Stachytarpheta dichotoma	GCACGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTC
Verbena bonariensis	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCCTTACGTATGTC
Proboscidea louisianica	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATACACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Hemimeris sabulosa	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATACACTTCCGTGTACTAGCTAAAGCCTTACGTATGTC
Scrophularia californica	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATACACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Retzia capensis	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATACACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Halleria lucida	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATACACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Angelonia pubescens	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATACACTTCCGCGTATTGGCTAAAGCGTTACGTATGTC
Veronica catenata	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATCCACTTCCGTGTATTAGCTAAAGCGTTACGTATGTC
Streptocarpus holstii	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATACATTTCCGTGTATTA??TAAAGCGCTACGTATGTC
Nematanthus hirsutus	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATACACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Calceolaria mexicana	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTACTAGCTAAAGCGTTACGGATGTC
Jovellana sp.	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATACACTTCCGTGTACTAGCTAAAGCGTTACGGATGTC
Ligustrum vulgare	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATACACTTCCGTGTACTAGCTAAAGCGTTACGTATGTC
Borago officinalis	GCATGCGGTTATTGATAGACAGAAGAATCATGGTATGCACTTCCGTGTATTAGCTAAAGCGTTACGTATGTC
Gentiana procera	GCATGCAGTTATTGATAGACAGAAGAATCATGGTATCCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTC
Nicotiana tabacum	GCATGCGGTTATTGATAGACAGAAGAATCATGGTATCCACTTCCGGGTATTAGCAAAAGCGTTACGTATGTC

Thomandersia hensii	TGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Thomandersia laurifolia	TGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Bartsia alpina	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGATATCACTTTGGGCTT
Lindenbergia phillipensis	TGGTGGGGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Paulownia tomentosa	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Mimulus aurantiacus	TGGTGGAGATCATATTCATGCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGATATCACTTTGGGGTT
Callicarpa dichotoma	TGGTGGAGATCATATTCACGCTGGTACTGTAGTAGGTAAACTTGAAGGAGAAAGAGATATTACTTTAGGCTT
Lamium purpureum	CGGTGGAGATCATATTCACTCCGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGAAATTACTTTA?????
Sesamum indicum	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Ceratotheca triloba	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Elytraria crenata	TGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGATT
Thunbergia usambarica	TGGTGGAGATCATATTCACTCTGGTACCGTTGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Barleria prionitis	TGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATTACTTTGGGCTT
Jacaranda sparrei	TGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Kigelia africana	TGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Catalpa speciosa	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Schlegelia parviflora	TGGTGGAGATCATATTCACGGTGGTACCGTAGTCGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Synapsis ilicifolia	TGGTGGAGATCATATTCACGGTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Petrea racemosa	TGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Stachytarpheta dichotoma	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Verbena bonariensis	TGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Proboscidea louisianica	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Hemimeris sabulosa	TGGGGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Scrophularia californica	TGGTGGAGATCATATTCACTCCGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Retzia capensis	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Halleria lucida	TGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Angelonia pubescens	TGGCGGAGACCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAGGGAGAAAGAGACATCACTTTGGGATT
Veronica catenata	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAGGGAGAAAGAGACATCACTTTGGGCTT
Streptocarpus holstii	TGGTGGAGATCACATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACGTTGGGCTT
Nematanthus hirsutus	TGGTGGAGATCATATTCACGCTGGTACCGTAGTAGGTAAGCTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Calceolaria mexicana	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Jovellana sp.	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACTTTGGGCTT
Ligustrum vulgare	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGGAAACTTGAAGGGGAAAGAGACATCACTTTGGGCTT
Borago officinalis	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGTGGAAGAAATATCACTTTGGGTTT
Gentiana procera	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGAGAAAGAGACATCACCTTAGGCTT
Nicotiana tabacum	TGGTGGAGATCATATTCACTCTGGTACCGTAGTAGGTAAACTTGAAGGTGAAAGAGACATAACTTTGGGCTT

Thomandersia hensii	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGTGGTATTTATTTCACTCAAGATTGGGT
Thomandersia laurifolia	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGTGGTATTTATTTCACTCAAGATTGGGT
Bartsia alpina	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Lindenbergia phillipensis	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTTACTCAAGATTGGGT
Paulownia tomentosa	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Mimulus aurantiacus	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Callicarpa dichotoma	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Lamium purpureum	????????????????GTGATGATTTTATTGAAAAAGATCGAAGCCGCGGTATTTATTTCACTCAAGATTGGGT
Sesamum indicum	TGTTGATTTACTGCGTGATGATTTTGTTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Ceratotheca triloba	TGTTGATTTACTGCGTGATGATTTTGTTGAAAAAGATCTAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Elytraria crenata	TGTTGATTTACTGCGCGATGATTTTGTTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Thunbergia usambarica	TGTTGATTTACTGCGTGACGATTTTGTTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Barleria prionitis	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Jacaranda sparrei	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Kigelia africana	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Catalpa speciosa	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGCCGCGGTATTTATTTCACTCAAGATTGGGT
Schlegelia parviflora	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATGAAGATCGCGGTATTTATTTCACTCAAGATTGGGT
Synapsis ilicifolia	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATGCAGATCGCGGTATTTATTTCACTCAAGATTGGGT
Petrea racemosa	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Stachytarpheta dichotoma	TGTTGATTTACTGCGCGATGACTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Verbena bonariensis	TGTTGATTTACTGCGTGATGATTATATTGAAAAAGATCGAAGCCGCGGTATCTATTTCACTCAAGATTGGGT
Proboscidea louisianica	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Hemimeris sabulosa	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATAGAAGTCGCGGTATTTATTTCACCCAAGATTGGGT
Scrophularia californica	TGTTGATTTATTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGACTGGGT
Retzia capensis	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Halleria lucida	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Angelonia pubescens	TGTTGATTTACTGCGCGATGATTTTATTGAAAAAGATCGAAGCCGCGGTATTTATTTCACTCAAGATTGGGT
Veronica catenata	TGTTGATTTATTGCGTGATGATTTTATTGAGAAAGATCGTAGTCGCGGGATTTATTTCACTCAAGATTGGGT
Streptocarpus holstii	TGTTGATTTACTGCGTGATGATTTTATTGCAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Nematanthus hirsutus	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Calceolaria mexicana	TGTTGATTTACTTCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Jovellana sp.	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Ligustrum vulgare	TGTTGATTTACTGCGCGATGATTTTATTGAAAAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Borago officinalis	TGTTGATTTATTGCGTGATGATTTTATTGAAAAAGATAGAACTCGCGGTATCTATTTCACTCAAGATTGGGT
Gentiana procera	TGTTGATTTACTGCGTGATGATTTTATTGAAAAAGACCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT
Nicotiana tabacum	TGTTGATTTACTGCGTGATGATTTTGTTGAACAAGATCGAAGTCGCGGTATTTATTTCACTCAAGATTGGGT

Thomandersia hensii	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Thomandersia laurifolia	CTCTTTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Bartsia alpina	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Lindenbergia phillipensis	CTCTCTACCAGGTGTTATTCCCGTGGCTTCGGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACTGAGAT
Paulownia tomentosa	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Mimulus aurantiacus	CTCTCTACCAGGTGTTATTCCCGGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Callicarpa dichotoma	CTCTCTACCGGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTT?GCATATGCCTGCTCTGACCGAGAT
Lamium purpureum	CTCTCTACCGGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Sesamum indicum	CTCTCTACCAGGTGTTATTCCTGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTAACCGAGAT
Ceratotheca triloba	CTCTCTACCAGGTGTTATTCCTGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTAACCGAGAT
Elytraria crenata	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Thunbergia usambarica	CTCTCTACCGGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACTGAGAT
Barleria prionitis	CTCTTTACCTGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Jacaranda sparrei	CTCTCTACCAGGTGTTATTCCTGTGGCTTCAGGGGGTATTCACGTATGGCATATGCCTGCTCTGACCGAGAT
Kigelia africana	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Catalpa speciosa	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTT?GCATATGCCTGCTCTGACCGAGAT
Schlegelia parviflora	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Synapsis ilicifolia	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Petrea racemosa	CTCTCTACCGGGTGTTATCCCTGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTTTGACCGAGAT
Stachytarpheta dichotoma	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Verbena bonariensis	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACTGAGAT
Proboscidea louisianica	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Hemimeris sabulosa	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGGATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Scrophularia californica	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Retzia capensis	CTCTCTACCAGGTGTTATTCCTGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Halleria lucida	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Angelonia pubescens	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGC?TGCTCTGACCGAGAT
Veronica catenata	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTT?GCATATGCCTGCTCTGACCGAGAT
Streptocarpus holstii	CTCTCTACCGGGTGTTATTCCGGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Nematanthus hirsutus	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Calceolaria mexicana	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Jovellana sp.	CTCTCTACCAGGTGTTATTCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Ligustrum vulgare	CTCTCTACCAGGTGTTCTGCCCGTGGCTTCAGGGGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT
Borago officinalis	CTCTCTACCAGGTGTGCTGCCTGTAGCTTCAGGAGGTATTCACGTTTGGCATATGCCGGCTTTGGTTGAAAT
Gentiana procera	CTCTCTACCGGGTGTTATACCCGTTGCTTCAGGGGGTATTCACGTTTGGCATATGCCCGCTCTGACCGAGAT
Nicotiana tabacum	CTCTTTACCAGGTGTTCTACCCGTGGCTTCAGGAGGTATTCACGTTTGGCATATGCCTGCTCTGACCGAGAT

Thomandersia hensii	CTTTGGAGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Thomandersia laurifolia	CTTTGGAGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Bartsia alpina	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCACCGGGTGC
Lindenbergia phillipensis	CTTTGGGGACGATTCCGTACTACAGTTTGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Paulownia tomentosa	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Mimulus aurantiacus	CTTTGGGGATGATTCCGTACTACAGTTTGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Callicarpa dichotoma	CTTTGGGGATGATGCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Lamium purpureum	CTTTGGGGATGATTCCGTACTACAGTTCGGCGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Sesamum indicum	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Ceratotheca triloba	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Elytraria crenata	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Thunbergia usambarica	CTTCGGGGATGATTCCGTTCTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCCGGTGC
Barleria prionitis	CTTTGGGGATGATTCCGTACTACAGTTTGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Jacaranda sparrei	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Kigelia africana	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCACCAGGTGC
Catalpa speciosa	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Schlegelia parviflora	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Synapsis ilicifolia	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Petrea racemosa	CTTTGGGGATGATGCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Stachytarpheta dichotoma	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Verbena bonariensis	CTTTGGAGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Proboscidea louisianica	CTTTGGGGATGATTCCGTACTACAGTTCGGCGGCGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Hemimeris sabulosa	CTTTGGGGATGATTCATGTTTGCAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Scrophularia californica	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Retzia capensis	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Halleria lucida	CTTTGGGGATGATTCCGTACTACAGTTTGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCACCAGGTGC
Angelonia pubescens	CTTTGGGGACGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCACCAGGTGC
Veronica catenata	CTTTGGGGATGATTCCGTATTACAGTTTGGTGGCGGAAC???AGGACACCCTTGGGGTAATGCGCCGGGTGC
Streptocarpus holstii	CTTTGGGGATGATTCTGTACTACAGTTCGGTGGAGG?ACTTTAGGACACCCTTGGGGTAATGCACCAGG???
Nematanthus hirsutus	CTTTGGGGATGATTCCGTACTTCAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Calceolaria mexicana	CTTTGGTGATGATTCCGTCCTACAGTTTGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Jovellana sp.	CTTTGGTGATGATTCCGTCCTACAGTTTGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Ligustrum vulgare	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGGCACCCTTGGGGTAATGCGCCAGGTGC
Borago officinalis	CTTTGGAGACGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACACCCTTGGGGTAATGCGCCAGGTGC
Gentiana procera	CTTTGGGGATGATTCTGTACTACAGTTCGGTGGAGGAACTTTAGGACATCCTTGGGGTAATGCCCCAGGTGC
Nicotiana tabacum	CTTTGGGGATGATTCCGTACTACAGTTCGGTGGAGGAACTTTAGGACATCCTTGGGGTAATGCGCCAGGTGC

Thomandersia hensii	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Thomandersia laurifolia	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Bartsia alpina	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Lindenbergia phillipensis	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Paulownia tomentosa	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Mimulus aurantiacus	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Callicarpa dichotoma	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Lamium purpureum	TGTAGCTAACCGAGTCGCTCTAGAAGCATGTGTCCAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Sesamum indicum	CGTAGCTAACCGAGTAGCTCTAGA??????????????????AATG?AGGA?GTGATCTTGCTGCTGAGGG
Ceratotheca triloba	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Elytraria crenata	CGCGGCTAACCGAGTAGCTCTAGAAGCTTGTGTACAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Thunbergia usambarica	CGCGGCTAACCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGGCGTGATCTTGCTGCTGAGGG
Barleria prionitis	CGTAGCTAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Jacaranda sparrei	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Kigelia africana	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Catalpa speciosa	TGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Schlegelia parviflora	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Synapsis ilicifolia	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGAAATGAAGGACGTGATCTTGCTGCTGAGGG
Petrea racemosa	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Stachytarpheta dichotoma	TGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGTAATGAAGGACGTGATCTTGCTAGTGAGGG
Verbena bonariensis	TGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Proboscidea louisianica	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Hemimeris sabulosa	CGTAGCTAACCGAGTAGCTCTAGAGGCATGTGTACAAGCTCGTAACGAAGGACGTGATCTTGCTGCTGAGGG
Scrophularia californica	CGTAGCTAACCGAGTCGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Retzia capensis	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Halleria lucida	CGTAGCTAACCGAGTAGCTCTAGAAGCGTGTGTAAAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAAGG
Angelonia pubescens	?GTAGCTAACCGAGTAGCTTTAGAAGCATGTGTACAAGCTCGTAATGAAGGACGTGATCTTGCTACTGAGGG
Veronica catenata	CGTAGCTAACCGAGTAGCTCTAGA?G??TGTGTACAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Streptocarpus holstii	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Nematanthus hirsutus	TGTAGCTAACCGCGTAGCTCTAGAAGCATGTGTGCAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Calceolaria mexicana	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTGCAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Jovellana sp.	CGTAGCTAACCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGACGTGATCTTGCTGCTGAGGG
Ligustrum vulgare	CGTAGCTAATCGAGTAGTTCTAGAAGCATGTGTACAAGCTCGTAATGAGGGACGCGATCTTGCTAGTGAGGG
Borago officinalis	CGTAGCGAATCGAGTAGCTCTAGAAGCATGTGTACAAGCTCGTAATGAAGGGCGTGATCTTGCTCGTGAAGG
Gentiana procera	CGTAGCTAATCGAGTAGCTCTAGAAGCATGTGTAAAAGCTCGTAATGAAGGACGTGATCTTGCTCAGGAAGG
Nicotiana tabacum	

Thomandersia hensii	TAATGCAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Thomandersia laurifolia	TAATGCAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Bartsia alpina	TAATGCAATTATCCGCGAGGCTAGCAAAT??	
Lindenbergia phillipensis	TAATGCAATTATCCGTGAGGCTAGCAAAT??	
Paulownia tomentosa	TAATGCAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Mimulus aurantiacus	TAATGCAATTATCCGTGAGGCTTGCAAAT??	
Callicarpa dichotoma	TAATGTAATTATCCGTGAG?CTAGCAAATGGAGTCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Lamium purpureum	TAATGCAATTATCCGCGAGGCTTGCAAGTGGAGTCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Sesamum indicum	TAATGCAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Ceratotheca triloba	TAATGCAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Elytraria crenata	TAATGCAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCTGCCGCTTGTGAGATATGGAAAGAGAT	
Thunbergia usambarica	CAATGCAATTATTCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Barleria prionitis	TAATGCAATTATCCGTGAAGCTAGCAAATGGAGCCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Jacaranda sparrei	TAATGTAATTATCCGTGAGGCTAGCAAATGG??????????????????????????????????????	
Kigelia africana	TAATGCAATTATCCGTGAGGCTAGCAAATGGAGTCCGCAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Catalpa speciosa	TAATGCAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACT???GGCCGCTTGTGAGGTATGGAAAGAGAT	
Schlegelia parviflora	TAATACAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Synapsis ilicifolia	TAATACAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGA???	
Petrea racemosa	TAATACAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Stachytarpheta dichotoma	TAATGTAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Verbena bonariensis	TAATGTAATTATCCGTGAGGCTTGCAAATGGAGTCCTGAACTATCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Proboscidea louisianica	TAATGCAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCTGCTGCTTGTGAGGTATGGAAAGAGAT	
Hemimeris sabulosa	TAATGCAATTATCCGTGAGGCTTGCAAAT??	
Scrophularia californica	TAATACAATTATCCGTGA?GCTAGCAAAT??	
Retzia capensis	TAATGCAATTATCCGTGAGGCTTGCAAATGGAGTCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Halleria lucida	TAATGCAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCTGCTGCTTGTGAGATATGGAAAGAGAT	
Angelonia pubescens	TAATGCAATTATCCGCGAGGCTAGCAAAT??	
Veronica catenata	TAATACAATTATCCGTGAGGCTTGCAAGT??	
Streptocarpus holstii	TAACGCAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCTGCCGCTTGTGAGGTATGGAAAGAGAT	
Nematanthus hirsutus	TAATGTGATTATCCGTGAG?CTAGCAAATGGAGTCCCGAACTAGCTGCTGCTTGTGAGATATGGAAAGAGAT	
Calceolaria mexicana	TAATGCAATTATCCGTGAGGCTTGCAAAT??	
Jovellana sp.	TAATGCAATTATCCGTGAAGCTAGCAAAT??	
Ligustrum vulgare	TAATGAAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAGCTAGCTGCTGCTTGTGAGGTATGGAAGGAGAT	
Borago officinalis	TRRTGATATTRTTCGTGAGGCTACCAAATGGAGTCCTGAACTAGCTGCTGCTTGTGAAGTATGGAAGGAGAT	
Gentiana procera	TAATGAAATTATCCGTGAGGCTAGCAAATGGAGTCCTGAACTAGCCGCTGCTTGTGAGGTATGGAAGGAGAT	
Nicotiana tabacum	TAATGAAATTATTCGCGAGGCTTGCAAATGGAGCCCGGAACTAGCTGCTGCTTGTGAAGTATGGAAAGAGAT	

Thomandersia hensii	CAGATTTGAGTTTCAAGCAATGGATACTTTGGAT	[1,402]
Thomandersia laurifolia	CAGATTTGAGTTTCAAGCAATGGATACTTTGGAT	[1,402]
Bartsia alpina	??????????????????????????????????	[1,402]
Lindenbergia phillipensis	??????????????????????????????????	[1,402]
Paulownia tomentosa	CAAATTTGAGTTTAAAGCGGTGGATACTTTGGAT	[1,402]
Mimulus aurantiacus	??????????????????????????????????	[1,402]
Callicarpa dichotoma	CAAATTTGAGTTTAAAGCAGTGGATACTTTGGAT	[1,402]
Lamium purpureum	CAAATTTGAATTTAAAGCAATGGATACTTTGGAT	[1,402]
Sesamum indicum	CAAATTTAAGTTTAAAGCAGTGGATACTTTGGAT	[1,402]
Ceratotheca triloba	CAAATTTGAGTTTCAAGCAGTGGATACTTTGGAT	[1,402]
Elytraria crenata	CCGATTTGAGTTTAAAGCAGTGGATACTTTAGAT	[1,402]
Thunbergia usambarica	CAGATTTGAGTATGCAGCAGTGGATACTTTGGAT	[1,402]
Barleria prionitis	CAAATTTGAATTTAAAGCAGTGGATACTTTGGAT	[1,402]
Jacaranda sparrei	??????????????????????????????????	[1,402]
Kigelia africana	CAAATTTGAGTTTAAGGCAGTGGATACTTTGGAT	[1,402]
Catalpa speciosa	CAAATTTGAGTTTAAGGCAGTGGATACTTTGGAT	[1,402]
Schlegelia parviflora	CAAATTTGAGTTTGAGGCAGTGGATAAATTGGAT	[1,402]
Synapsis ilicifolia	??????????????????????????????????	[1,402]
Petrea racemosa	CAAATTCGAGTTCAAAGCAGTGGATACTTTGGAT	[1,402]
Stachytarpheta dichotoma	CAAATTTGAGTTCGCAGCAGTGGAT??TTTGGAC	[1,402]
Verbena bonariensis	CAAATTTGAGTTCGAAGCAATGGATACTTTGGAT	[1,402]
Proboscidea louisianica	CAAATTTGAGTTTAAAGCAGTGGATACTTTGGAT	[1,402]
Hemimeris sabulosa	??????????????????????????????????	[1,402]
Scrophularia californica	??????????????????????????????????	[1,402]
Retzia capensis	CAAATTTGATGTTAAAGCAGTGGATACTTTGGAT	[1,402]
Halleria lucida	CAAATTTGAATTTAAAGCAGTGGATACTTTGGAT	[1,402]
Angelonia pubescens	??????????????????????????????????	[1,402]
Veronica catenata	??????????????????????????????????	[1,402]
Streptocarpus holstii	CAAATTTGAATTTCAAGCGGTGGATACTTTGGAT	[1,402]
Nematanthus hirsutus	CAAATTTGAGTTTGCAGC?GTGGATACTTTGGAT	[1,402]
Calceolaria mexicana	??????????????????????????????????	[1,402]
Jovellana sp.	??????????????????????????????????	[1,402]
Ligustrum vulgare	CAAATTTGAGTTTCAGGCAATGGATACTTTGGAT	[1,402]
Borago officinalis	TACATTTACTTTCGAGGAAGTGGATAAATTGGAT	[1,402]
Gentiana procera	CAAATTTGAGTATGAAGCAGTGGATAAGTTGGAT	[1,402]
Nicotiana tabacum	CGTATTTAATTTTGCAGCAGTGGACGTTTTGGAT	[1,402]

Appendix 11 Specimens examined in anatomical and morphological studies.

Species and authority	Voucher/collection	Source	Collected	Analyses
Aphelandra 'Eppes Findling'	AW5	FHO	HK	Sectioning, SEM
Asystasia calycina Benth.	AW2	FHO	HK	Sectioning, SEM
Ballochia rotundifolia Balf.f.	AW3	FHO	HK	Sectioning
Barleria senensis Klotzsch	*Greenway* 7962	K	Zambia	Sectioning, SEM
Callichlamys riparia Miq.	*Broadway* s.n. (1927)	FHO	Trinidad	Observations
Eccremocarpus scaber Ruiz & Pav.	AW8	FHO	U.K.	Sectioning
Elytraria acaulis (Linn.f.) Lindau	*Brenan* s.n.	K	Nigeria	SEM
Petrea volubilis L.	K spirit collection #16936	K	-	Sectioning
Pseuderanthemum tunicatum (Afzel.) Milne-Redh.	AW1	FHO	HK	Sectioning, SEM
Ruellia tuberosa L.	AW4	FHO	HK	Sectioning, SEM
Schlegelia fuscata A.H.Gentry	*Bernal & Sugden* 93	FHO	Colombia	Pollen acetolysis
Schlegelia violacea Griseb.	AW7	FHO	HK	Sectioning, SEM
Sesamothamnus gurichii (Engl.) E.A.Bruce	*Sydon & Kings* 3	K	Namibia	Observations
Thomandersia anachoreta Heine	*Aké Assi* s.n. (1964)	MO	Côte d'Ivoire	Observations
	Baldwin 11264	K	Liberia	Pollen acetolysis
	Geerling & Bokdam 2436	K	Côte d'Ivoire	Sectioning
Thomandersia butayei De Wild.	*Gillardin* 235	BR	Democratic Republic of Congo	Pollen acetolysis
	McPherson 16790	MO	Gabon	Observations
Thomandersia congolana De Wild. & T.Durand	*Breteler* 6942	BR	Gabon	Pollen acetolysis
	Claessens s.n. (1921)	BR	Democratic Republic of Congo	Observations
Thomandersia hensii De Wild. & T.Durand	*Harris* s.n. (2000)	-	Central African Republic	Sectioning, SEM
	Onochie 9314	BM	Cameroon	Pollen acetolysis
	Robyns 972	BR	Democratic Republic of Congo	Wood anatomy
	Sapin s.n. (1907)	BR	Democratic Republic of Congo	Wood anatomy
	Sapin s.n. (1912)	BR	Democratic Republic of Congo	Observations
	Vanderyst 2964	BR	Democratic Republic of Congo	Observations
Thomandersia laurentii De Wild.	*Robyns* 4279	BR	Democratic Republic of Congo	Observations
Thomandersia laurifolia (T. Anderson ex Benth.) Baill.	*Bos* 6080	K	Cameroon	Observations
	De Wilde 9400	WAG	Gabon	Observations
	Talbot 381	BM	Nigeria	Pollen acetolysis
	Wieringa 3883	WAG	Gabon	Observations
Thunbergia grandiflora Roxb.	AW6	FHO	HK	sectioning

Appendix 12 Herbarium collections studied (all at FHO).

Species and authority	Voucher/collection	Collected
Angelonia pubescens Benth.	s.n.	n/a
Barleria prionitis L.	*Simpson* 9392	Sri Lanka
Bartsia alpina L.	*Halliday* 369/69	France
	Kesby 148 & 149.	Iceland
Borago officinalis L.	*Saunders* s.n.	n/a
Calceolaria mexicana Benth.	*Hartweg* 356	Mexico
Callicarpa caudata Maxim.	*Mabberley* 2402	Papua New Guinea
Catalpa speciosa Warder	*Coll. Syst.* 23	USA
	Gates 16281	USA
Ceratotheca sesamoides Endl.	*Angus* 2885	Zimbabwe
Elytraria marginata Vahl.	*Onochie & Okafor* 36057	Nigeria
Gentiana lutea L.	*Huguenin* 154	France
	Stratton s.n.	Switzerland
Halleria lucida L.	*Lawton* 1296	Malawi
	White 3226	Zambia
Hemimeris montana L.	*Drege* s.n.	South Africa
Jacaranda mimosifolia D.Don.	*Greenway* 965	Kenya
	White 6096	Zimbabwe
Jovellana punctata Ruiz & Pav.	*Bridges* 657 & 796	Chile
Kigelia africana (Lam.) Benth.	*White* 11043	Kenya
Lamium purpureum L.	*Schmidt* s.n.	n/a
Ligustrum vulgare L.	*Savory* 28	UK
Lindenbergia philippensis Benth.	*Cuming* 708 & 909	n/a
Martynia annua L.	*Sofoluwe* 38169	Nigeria
Mimulus aurantiacus Curtis	*Thompson* 1116 & 1993	USA
Nicotiana tabacum L.	*Brown* 2164	Ghana
Paulownia tomentosa (Thunb.) Steud.	*Chiao* 3163	China
	White 9121	UK
Petrea volubilis L.	*Emwiogbon* 57964	Nigeria
	White 9016	Mexico
Proboscidea parviflora (Woot.) Woot. & Standl.	*Damrel* 2525	USA
Schlegelia parviflora (Oerst.) Monach.	*Smith* 145	Costa Rica
Scrophularia nodosa L.	*Munroe* s.n.	n/a
	s.c. s.n.	n/a
Sesamum indicum L.	*Angus* 2615	Zimbabwe
	Kennedy 151	Nigeria
Stachytarpheta dichotoma Ruiz & Pav.	*s.c.* s.n.	n/a
Streptocarpus dolicanthus Hilliard & Burtt.	*Chapman, J.D. & E.G.* 8496	Malawi
Thunbergia alata Boj.	*Greenway* 8603	Tanzania
	S.L.F.D. 153	Sierra Leone
Verbena bonariensis L.	*Bourgeau* 288	Canary Islands
Veronica traversii Hook.f.	*Macklean* s.n.	UK

Appendix 13 Additional literature and websites studied for morphological characters.

Abrams (1951)
Aiken (1999)
Aluri (1990)
Armstrong (1985)
Baas et al. (1998)
Barringer & Burger (2000)
Bhandari & Natesh (1985)
Bigazzi & Selvi (2000)
Bocher et al. (1968)
Bremekamp (1942)
Bremer et al. (1994)
Burger (2000)
Cantino (1990)
Carlquist (1986)
Carlquist (1987)
Carlquist & Zona (1988)
Cheung et al. (2000)
Dahlgren et al.(1979)
Den Outer & Van Veenendaal (1983)
Dos Santos & Miller (1997)
Durkee (1986)
Ehrhart (1997)
Flora of China Editorial Committee (1994)
Galati & Strittmatter (1999)
Galetto (1995)
Gasson & Dobbins (1991)
Gatesy et al. (2003)
Gentry (1977)
Gentry (1992a)
Gentry & Tomb (1979)
Ghorbel & Nabli (1998)
Gibson (1970)
Gibson (1974a)
Gibson (1974b)
Gleason (1952)
Gleason & Cronquist (1991)
Goldblatt & Keating (1976)
Grayer-Barkneijer (1978)
Grisebach (1866)
Guedes (1974)
Harrison et al. (1999)
Hickman (1993)
Hjertson (1995)
Holmgren & Molau (1984)
Jensen et al. (1998)
Jensen et al. (1975)
Johri & Singh (1959)
Juan et al. (1997)
Kampny & Dengler (1997)
Kooiman (1975)
Land Protection (2001)
Lindsey (1940)
Long & Lakela (1971)
MacBride (1961)
Mathew & Shah (1988)
Meszaros et al. (1996)
Minkin & Eshbaugh (1989)
Molau (1988)
Möller & Cronk (2001)
Moro et al. (2001)
Moss (1959)
Munir (1992)
Munz (1959)
Olmstead et al. (1993)
Parvati & Narayana (1978)
Raj (1961)
Raj (1983a)
Rueda (1994)
Saitoh et al. (1985)
Schonenberger (1999)
Schonenberger & Endress (1998)
Scoggan (1979)
Scogin (1980)
Seibert (1948)
Singh (1960)
Smith (1996)
Smith (2000a)
Srinivasan (1940)
Standley & Williams (1969)
Standley & Williams (1973)
Steiner (1996)
Straka & Ihlenfeldt (1965)
Turgeon et al. (1993)
Verdcourt (1992)
Vogel & Machado (1991)
Von Poser et al. (1997)
Wallender & Albert (2000)
Wilson (1974a)
Wilson (1974b)
Yeo (1989)
Yuan (1993)
Yuen & Dehgan (1982)

http://www.botanical.com/botanical/mgmh/b/borage66.html#des
http://hua.huh.harvard.edu/china/mss/volume18/SCROPHULARIACEAE.published.pdf
http://polyland.lib.calpoly.edu/overview/Archives/derome/scrub.html#monkeyflower
http://www.ppws.vt.edu/scott/weed_id/lampu.htm
http://www.fs.fed.us/global/iitf/Barleria%20prionitis.pdf
http://www.cnr.vt.edu/dendro/dendrology/syllabus/cspeciosa2.htm
http://www.wa.gov/agr/weedboard/weed_info/unicornplant.html
http://waynesword.palomar.edu/ww0801.htm#Americas
http://www.hort.uconn.edu/plants/l/ligvul/ligvul3.html
http://pharm1.pharmazie.uni-greifswald.de/systematik/7_bilder/bar_al_1.jpg
http://www.forestryimages.org/browse/subthumb.cfm?sub=2426&start=1

http://www.mindbird.com/mimulus_aurantiacus.htm
http://www.mpiz-koeln.mpg.de/~stueber/thome/band4/tafel_062_small.jpg
http://www.mobot.org/MOBOT/research/library/kohler/1758_054.jpg
http://plantsdatabase.com/showpicture/5732.html
http://image.nybg.org/herbim/2970/v-297-00114978big.jpg
http://florawww.eeb.uconn.edu/images/byspecies/THUNBERGIA_GRANDIFLORA01.JPG
http://image.nybg.org/herbim/2970/v-297-00278958big.jpg
http://mobot.mobot.org/cgi-bin/search_vast
http://www.botany.hawaii.edu/faculty/carr/images/kig_pin.jpg
http://www.forestryimages.org/browse/detail.cfm?imgnum=0008308
http://caliban.mpiz-koeln.mpg.de/~stueber/thome/band4/tafel_062_small.jpg
http://www.pharmakobotanik.de/systematik/7_bilder/yamasaki/yamas382.jpg
http://digitalis.mobot.org/mrsid/bin/mosid/mosid.pl
http://www.csdl.tamu.edu/FLORA/dcs420/c/
http://botany.cs.tamu.edu/FLORA/hdwimages1/
http://elib.cs.berkeley.edu/imgs/512x768/6249_3022/3534/0087.jpeg
http://waynesword.palomar.edu/plmay99.htm
http://www.dipbot.unict.it/orto/0585-1.html
http://image.nybg.org/herbim/2880/v-288-00067833big.jpg
http://www.gesneriads.ca/txtrbge.htm
http://image.nybg.org/herbim/2880/v-288-00067937big.jpg
http://www.calhortsociety.org/Plant_Forums/apr2001/source/dscn5197.htm
http://www.mpiz-koeln.mpg.de/~stueber/thome/band4/tafel_075_small.jpg
http://www.mpiz-koeln.mpg.de/~stueber/thome/band4/tafel_020_small.jpg
http://www.sciencebuff.org/BOTANYCATALOGUE/gentproc.jpg
http://www.mpiz-koeln.mpg.de/~stueber/thome/band4/tafel_033_small.jpg
http://mobot.mobot.org/cgi-bin/search_vast?w3till=MOA-05081_001.jpg
http://mobot.mobot.org/cgi-bin/search_vast?w3till=MOA-05421_001.jpg
http://www.botgard.ucla.edu/html/MEMBGNewsletter/Volume1number4/Gorgeousbignons.html

Appendix 14 Characters excluded from large dataset for logical dependency.

Character removed	Characters (not already removed) with shared dependency
Habit	Support, life form, life cycle
Life form	Support, life cycle
Life style	Succulence, leaf epidermis, stomata, stomata position, leaf hairs, leaf margin, corolla fleshiness
Leaf width	Leaf length
Flower arrangement	Bracteoles, superposed buds
# perianth parts	# calyx lobes, # corolla lobes
Flower symmetry	Calyx shape, calyx symmetry, corolla symmetry, corolla shape
Gamosepaly degree	Calyx lobes
Calyx symmetry	Calyx shape
Gamopetaly degree	Corolla lobes
Corolla shape	Corolla symmetry
# androecial members	# fertile stamens, # staminodes
Androecial members (reduction)	# corolla lobes
Androecium	# fertile stamens, # staminodes
Androecial members (equality)	Androecium, # fertile stamens, # staminodes, stamen didynamy
# stigma lobes	# pistil cells
Placentation	# locules
Pollination	Numerous floral characters
Fruit (type)	Fruit (fleshiness), loculicidal dehiscence, septicidal dehiscence, dispersal unit
Dehiscence	Retinaculum
Dispersal unit	Loculicidal dehiscence, septicidal dehiscence
Seed diameter/width	Seed length

Appendix 15 Morphological characters included in large matrix.

	Character	States
1	Support	0, self; 1, twiner; 2, vine; 3, climber; 4, creeping; 5, epiphyte
2	Life cycle	0, annual; 1, biennial; 2, perennial
3	Succulence	0, not succulent; 1, stem succulent
4	Stem internode	0, terete; 1, square or tetragonal
5	Nodes	0, not articulated; 1, articulated
6	Included/interxylary phloem	0, absence; 1, presence
7	Internal/intraxylary phloem	0, absence; 1, presence
8	Medullary bundles	0, absence; 1, presence
9	Ray width	0, uniseriate; 1, biseriate; 2, multiseriate; 3, mixed
10	Xylem fibres	0, septate libriform; 1, tracheids or fibre-tracheids; 2, mixed
11	Wood	0, ring-porous; 1, diffuse porous; 2, semi-ring-porous
12	Wood elements	0, not storied; 1, storied
13	Axial parenchyma	0, paratracheal/scanty paratracheal; 1, banded; 2, aliform; 3, confluent; 4, vasicentric/scanty vasicentric; 5, apotracheal; 6, absent
14	Leaf length	(quantitative)
15	Leaf arrangement	0, opposite (including opposite-decussate); 1, alternate; 2, whorled/verticillate/rosette
16	Leaves of a pair	0, equal; 1, anisophyllous
17	Petioles	0, leaves sessile; 1, petiolate
18	Petiole base	0, not articulated; 1, articulated/invaginated
19	Leaf structure	0, simple; 1, compound
20	Leaf venation	0, pinnate; 1, palmate or at least trinerved; 2, linear; 3, obscure or no secondary venation
21	Midrib	0, embedded/immersed/impressed above; 1, prominent above
22	Leaf epidermis	0, not mucilaginous; 1, mucilaginous
23	Stomata	0, anomocytic; 1, anisocytic; 2, paracytic; 3, diacytic; 4, actinocytic; 5, diallelocytic
24	Stomata position	0, amphistomatic; 1, hypostomatous
25	Calcium oxalate crystals	0, absence; 1, presence
26	Domatia	0, absence; 1, presence
27	Domatia type	0, pits; 1, tufts; 2, pockets; 3, sacs
28	Leaf hairs	0, absence; 1, presence
29	Glandular hairs	0, absence; 1, presence
30	Multicellular hairs	0, absence; 1, presence
31	Branched multicellular hairs	0, absence; 1, presence
32	Branched unicellular hairs	0, absence; 1, presence
33	Non-hair leaf glands	0, absence; 1, presence
34	Leaf margin	0, flat; 1, involute; 2, revolute
35	Leaf margin	0, toothed; 1, entire (including undulate)
36	Terminal inflorescence unit	0, indeterminate; 1, determinate; 2, cauliflorus groups
37	Bracteoles	0, absence; 1, presence
38	Superposed buds	0, absence; 1, presence
39	Pedicels	0, flowers pedicellate; 1, flowers sessile
40	# calyx lobes	0, 4; 1, 5; 2, >5; 3, <4
41	Calyx lobes	0, longer than tube; 1, shorter than tube; 2, about same length as tube
42	Calyx ridged	0, no; 1, yes
43	Abaxial calycine swelling	0, absence; 1, presence
44	Calyx gland	0, absence; 1, presence
45	Gland opening	0, shallow; 1, channelled
46	Calyx shape	0, tubular; 1, campanulate; 2, spathulate; 3, cupular; 4, rotate

47	Calyx persistence	0, not persistent; 1, persistent/accrescent
48	# corolla lobes	0, 4; 1, 5; 2, 2
49	Corolla lobes	0, shorter than tube; 1, longer than tube; 2, same length as tube
50	Corolla symmetry	0, actinomorphic; 1, zygomorphic
51	Corolla tube	0, straight (tubular); 1, gibbous
52	Corolla	0, not spurred; 1, spurred
53	Corolla fleshiness	0, not fleshy; 1, fleshy
54	Corolla aestivation	0, imbricate; 1, contort; 2, induplicate-valvate; 3, plicate; 4, valvate/reduplicate-valvate; 5, quincuncial
55	Filament hairs/ trichomes	0, absence; 1, presence
56	Stamens	0, included; 1, exserted; 2, subexserted
57	Androecial members	0, free; 1, coherent
58	# fertile stamens	0, 5; 1, 4; 2, 2
59	Fertile stamens	0, as all except posterior (adaxial) member; 1, as adaxial pair; 2, as lateral pair; 3, as anterior (abaxial) pair; 4, all fertile
60	# staminodes	0, 0; 1, 1; 2, 2; 3, 3
61	Staminode vascular bundle	0, absence; 1, presence
62	Stamen didynamy	0, all same length; 1, didynamous
63	Degree of stamen fusion	(quantitative)
64	Anthers	0, separate; 1, cohering; 2, connivent
65	Anthers	0, dorsifixed; 1, basifixed; 2, apicifixed
66	# thecae	0, 1; 1, 2
67	Anther appendages	0, absence; 1, presence
68	Anther endothecium	0, without fibrous thickenings; 1, with fibrous thickenings
69	Tapetum type	0, amoeboid; 1, glandular
70	Pollen grains when shed	0, 2-celled; 1, 3-celled
71	Pollen shape	0, triangular; 1, globose, spheroidal or nearly so; 2, ellipsoid/elliptic
72	# pollen apertures	0, 3; 1, 5-6; 2, >6; 3, possibly inaperturate
73	Aperture type	0, colporate; 1, colpate; 2, spiroaperturate; 3, porate; 4, colporoidate
74	Hypogynous nectary	0, absence; 1, presence
75	Annular disk nectary	0, absence; 1, presence
76	# pistil cells	0, 1; 1, 2; 2, 4
77	# locules	0, 1; 1, 2; 2, 4
78	Style derivation	0, terminal; 1, attenuate; 2, gynobasic
79	Style length	(quantitative)
80	Style	0, solid; 1, hollow
81	Stigma surface	0, dry; 1, wet
82	# ovules per carpel	0, >2; 1, 2; 2, <2
83	Ovule orientation	0, anatropous; 1, hemianatropous; 2, amphitropous; 3, pendulous; 4, orthotropous; 5, ascending; 6, pendulous and ascending in same locule
84	Endothelium	0, not differentiated; 1, differentiated
85	Embryogeny	0, onagrad; 1, chenopodiad; 2, solanad
86	Polar nuclei fusion	0, before fertilisation; 1, after only one fertilised; 2, after both fertilised
87	Antipodal cells	0, persistent; 1, ephemeral/degenerating
88	Synergids	0, pyriform; 1, hooked; 2, slender
89	Seed wing	0, not winged; 1, winged
90	Endosperm at seed	0, absence; 1, presence
91	Endosperm amount	0, copious; 1, scanty
92	Chalazal endosperm haustoria	0, absence; 1, presence
93	Micropylar endosperm haustoria	0, absence; 1, presence
94	Funicular obturator	0, absence; 1, presence
95	Fruit	0, fleshy; 1, dry
96	Loculicidal dehiscence	0, not dehiscent; 1, dehiscent
97	Septicidal dehiscence	0, not dehiscent; 1, dehiscent

98	Retinaculum	0, absence; 1, presence
99	Embryo	0, straight; 1, curved
100	Seed length	(quantitative)
101	Seed testa	0, ridged/furrowed; 1, hairy; 2, reticulate; 3, scaly; 4, smooth
102	Iridoids	0, absence; 1, presence
103	Iridoid type	0, aucubin derivatives; 1, seco-iridoids
104	Iridoid alkaloids (all types)	0, absence; 1, presence
105	Chromosome number	0, 2n=24; 1, 2n=20; 2, 2n=36; 3, 2n=32; 4, 2n=18; 5, 2n=28; 6, 2n=14; 7, 2n=40; 8, 2n=34; 9, 2n=48; 10, 2n=16; 11, 2n=30; 12, 2n=26

Appendix 16 Large matrix of morphological character states.

T. hensii	020000??301??J01110000?1?1110100101011{01}120111111211015110101010150010???111101107O?11?????0????01101?A3????
T. laurifolia	02000????????I01110000???1111100101011011111111101101511010 1?160010???111101105??01?????0?????1101?92????
Bartsia	02010?????--?1000-011????0-11111100000000110-11101000?110100-150011???10100110A??0???????1????1??0?5010?0
Lindenbergia	02000?????--?80010000????1111111100000011100-11101000?000100-130210???100001106??0???????1?????100?B210?3
Paulownia	02000??11?0?{234}K0010010????101111??0100001000??11101000?0001?0-1?0110???10???110???0???????1111?1100???10?7
Mimulus	02001????????6000-0001???0-1111??21000011100-01101000?000100-150110???00010000E??04??????0-???1100?4????1
Callicarpa	020000???0???B000-000?{034}1?10111101001000011100-31000001?010140-010010???201??21???0151??????????0--0???{01}??4
Lamium	000110???0-04000010010?{0345}0?0-101001001??012100-11101000?100100-15011011{01}?2?01212??0?510?????111?000???1??4
Sesamum	000100??30-04F00110010??0-11100100-01010100-11101100000010111300111111211121 8?100?001001111?1100?801?0?
Ceratotheca	020100??30?0440111001?0??0-11110100-?1010000-411011000000101?120010111?21012219?100?00100??11?1110?701???
Elytraria	0?00-1???0-04E2-10001?3?0131110010001?11??????01?1?000??02231-???1?11010{01}0010????02101100101111101112????
Thunbergia	1200111?3010570010010?3?10-10110101-10020100-2?1010001100101?1?00110101?20111??1?100?110?0-01??1001?210?4
Barleria	0200111?30?04C001100{01}?3?00-10000001011111100-31101000?110100-131010010103101 10B??000?10000-11?11011C11???
Jacaranda	020100??0010{01}P00111000??10-1111?001001011100-10101100?100101?131100?00?0110111C??001000110-1010100?D010?4
Kigelia	020000??0011{23}N00101010???0-10100101000011001030101100?1201?1?1?0210?00?0111111G??0??001100-10?0000???10??
Catalpa	020000??10010M00110000???10101001010??01???????101?00???0??????????00????1??1???0??001110-10?1010?B110??
Schlegelia	32000?0???10{04}D011100{01}0???0-1011012101?011?010{13}11?1001?1{01}01?11{01}??01????10410110???00??????11???0000082???7
Synapsis	0200?????????0?1100???????0----?200??011????1?1?????0?001?1?1???1????????11????0??????????????????????
Petrea	320100??20???H0011001?{0234}1?0-11100001000010100-41120000000010111?0010110{02}0111110?112000??1?0-11??000???10?8
Stachytarpheta	020010???0???B001000{01}?{034}0?0-110001000001{01}1100-011010000100232?-?00101112{01}100110??1{01}000??1?0-11?1??0???1{01}?9
Verbena	000110???0-1?C000-0001{04}1?0-1111012011?110100-01101000010010??1????0110200??22???11000??1?0-11?1000???11?5
Proboscidea	001000?0?0-04F001001010??0-11110101 0??012100-20101?00??001???1?1110??113-??00?B???01?000?11???0??0???10??
Martynia	020000?030?04C001001110??0-1111010000?010000-20101000??002?2?-?1?10??013-01000D??0010000?111??0000???100?
Hemimeris	40011?0???--?20010001????0-110-0120?00010000-41010000?101220--21000??????10??01??????????1???????0???1??6
Scrophularia	02010?0???--?H0010000????12111001200110110 00-11101001?100101013010 0??????01??03??0???????1?11??010??010??
Retzia	0??0000?32?0452-0-02??0????1110??2101????????0010000?4??0040-0??01????1000111????0{36}??????10????1100?210?{05}
Halleria	0200{01}0????2?04900000000???0-10110121210012000-11101101?110100-150210??????00??0B??????????1????0??0???????
Angelonia	00000?????--?60010001????1211100121-00011100-41111110??001???1????????1000?110???00???1?00-00?1100062???1
Veronica	0{12}001?????--?A000-030????0?10000000100?000100-11010000?010230--40210?????1011104??0???????????1100?341{01}?2
Streptocarpus	0?00-0?0-1-14O001000001??0-10111021010010000-411010000?002???-?1???1101??01???H??00?0?12?0-11?1100??????B
Nematanthus	5?0??0?1?1?14?0?1?0?1?11???111??0??????1?????????????0?????????????110????1??????00?0?12???11?0??0??????A
Calceolaria	40101??0-?-1-C0010000????0-110001001{01}0001000-412110000?002?0--???1????100??11????0???????????1110?22???3
Jovellana	0?001????????A0010000????1111100100?00000000-41201001?100230--30010??????101102??0???????????1??0??????2
Ligustrum	0200000?3220030010000 0???0-000000010?0001000-310200002?{01}02???-?0010110???00110??0101?????????00000??1112
Borago	000100???1-1?G1-100011???0-10111101100010000-41110010?111040-0?1010111?2?00222710 1??1?1???????1--0???0-??
Gentiana	0?10011?-1-1{15}L000-0210??10-00000101000001000-210000003010040-02011011{01}???011002??0012010110??01010??2111C
Nicotiana	0000001??1-0?Q1-0-00?1???0-11100001000011000-11100000?110040-0700101?{01}???01110F0?0?12010??????1?10???0-1?

Appendix 17 Characters excluded from small matrix for failing similarity criteria.

Character omitted	Reason
Habit	Effectively presence/absence of lignin: absence can arise through different transformations
Succulence	Effectively presence/absence of storage tissues: absence can arise through different transformations
Nodes (articulation)	Effectively presence/absence of lines or hairs: absence can arise through different transformations
Ray width	Effectively presence/absence of additional rows of cells: absence can arise through different transformations
Xylem fibres	States fail conjunction test
Wood (porosity)	States fail conjunction test
Wood elements	Effectively presence/absence of additional layers of ray cells: absence can arise through different transformations
Axial parenchyma	States fail conjunction test
Leaf length	Continuous
Leaf width	Continuous
Leaves of a pair	Effectively continuous
Petioles	Absence can arise through different transformations
Petiole base	Absence can arise through different transformations
Midrib	Effectively continuous
Leaf epidermis	Effectively presence/absence of mucilage: absence can arise through different transformations
Stomata	States fail conjunction test
Calcium oxalate crystals	Absence can arise through different transformations
Domatia	States are not similar in composition
Domatia type	States fail conjunction test
Leaf hairs	Absence can arise through different transformations
Glandular hairs	Absence can arise through different transformations
Multicellular hairs	Absence can arise through different transformations
Branched multicellular hairs	Absence can arise through different transformations
Branched unicellular hairs	Absence can arise through different transformations
Non-hair leaf glands	Absence can arise through different transformations
Leaf margin (folding)	States are not similar in composition/ontogeny
Leaf margin (teeth)	States are not similar in composition/ontogeny
Flower arrangement	States are not similar in composition
Terminal inflorescence unit	States are not similar in composition
Bracteoles	Absence can arise through different transformations
Superposed buds	Absence can arise through different transformations
Pedicels	Absence can arise through different transformations
# perianth parts	Effectively presence/absence of additional tepals: absence can arise through different transformations
# calyx lobes	Effectively presence/absence of additional sepals: absence can arise through different transformations
Gamosepaly degree	Continuous
Calyx lobes (length)	Continuous
Calyx ridges	Absence can arise through different transformations
Abaxial calycine swelling	Absence can arise through different transformations
Calyx gland	Absence can arise through different transformations
Gland opening	Effectively continuous (curvature of gland)
Calyx persistence	States are not similar in ontogeny
# corolla lobes	Effectively presence/absence of additional petals: absence can arise through different transformations
Corolla lobes (length)	Continuous
Gamopetaly degree	Continuous

Corolla shape	States are not similar in composition
Corolla tube	Effectively presence/absence of swelling: absence can arise through different transformations
Corolla spurs	Absence can arise through different transformations
Corolla fleshiness	Effectively presence/absence of storage tissues: absence can arise through different transformations
Corolla aestivation	Effectively presence/absence of overlaps: absence can arise through different transformations
Filament hairs	Absence can arise through different transformations
Stamens (exsertion)	Continuous
# androecial members	Effectively presence/absence of additional stamens: absence can arise through different transformations
Androecial members (reduction)	Effectively presence/absence of additional stamens: absence can arise through different transformations
Androecium	Effectively presence/absence of staminode: absence can arise through different transformations
Androecial members (equality)	Continuous
# fertile stamens	Effectively presence/absence of additional stamens: absence can arise through different transformations
# staminodes	Effectively presence/absence of staminodes: absence can arise through different transformations
Stamen didynamy	Continuous
Degree of stamen fusion	Continuous
Anthers (coherence)	Effectively presence/absence of gap: absence can arise through different transformations
Anthers (fixation)	Could fail conjunction test
# thecae	Effectively presence/absence of additional thecae: absence can arise through different transformations
Anther appendages	Absence can arise through different transformations
Tapetum type	Effectively presence/absence of cell walls: absence can arise through different transformations
Pollen grains when shed	Effectively presence/absence of additional cells: absence can arise through different transformations
# pollen apertures	Effectively presence/absence of additional apertures: absence can arise through different transformations
Aperture type	States are not similar in composition/ontogeny
Hypogynous nectary	Absence can arise through different transformations
Annular nectary	Absence can arise through different transformations
# pistil cells	Effectively presence/absence of additional cells: absence can arise through different transformations
# locules	Effectively presence/absence of additional locules: absence can arise through different transformations
Style length	Continuous
Style	Effectively presence/absence of cavity: absence can arise through different transformations
Stigma surface (hydration)	Effectively presence/absence of hydrating tissues: absence can arise through different transformations
# stigma lobes	Effectively presence/absence of additional lobes: absence can arise through different transformations
# ovules per carpel	Effectively presence/absence of additional ovules: absence can arise through different transformations
Endothelium	Effectively presence/absence of extra cells: absence can arise through different transformations
Polar nuclei fusion	Continuous
Antipodal cells	States are not similar in ontogeny
Synergids	States fail conjunction test
Seed wing	States are not topographically correspondent
Endosperm at seed	Absence can arise through different transformations
Endosperm amount	Continuous

Funicular obturator	States are not topographically correspondent
Pollination	States may fail conjunction test
Fruit (fleshiness)	Effectively presence/absence of storage tissues: absence can arise through different transformations
Fruit (type)	States are not topographically correspondent
Dehiscence	Absence can arise through different transformations
Dispersal unit	States are not topographically correspondent
Seed length	Continuous
Seed width/diameter	Continuous
Seed testa	States fail conjunction test
Iridoids	Absence can arise through different transformations
Iridoid type	States fail conjunction test
Iridoid alkaloids	Absence can arise through different transformations
Chromosome number	Effectively presence/absence of certain chromosomes: absence can arise through different transformations

Appendix 18 Characters excluded from small matrix for logical dependency.

Character removed	Characters (not already removed) with shared dependency
Life form	Support, life cycle
Stomata position	Life style
Flower symmetry	Calyx shape, calyx symmetry, corolla symmetry
Calyx symmetry	Calyx shape

Appendix 19 Morphological characters included in small matrix.

	Character	States
1	Support	0, self; 1, twiner; 2, vine; 3, climber; 4, creeping; 5, epiphyte
2	Life cycle	0, annual; 1, biennial; 2, perennial
3	Life style	0, mesophyte; 1, helophyte; 2, hydrophyte; 3, hygrophyte; 4, xerophyte
4	Stem internode	0, terete; 1, square or tetragonal
5	Included/interxylary phloem	0, absence; 1, presence
6	Internal/intraxylary phloem	0, absence; 1, presence
7	Medullary bundles	0, absence; 1, presence
8	Leaf arrangement	0, opposite (including opposite-decussate); 1, alternate; 2, whorled/verticillate/rosette
9	Leaf structure	0, simple; 1, compound
10	Leaf venation	0, pinnate; 1, palmate or at least trinerved; 2, linear; 3, obscure or no secondary venation
11	Calyx shape	0, tubular; 1, campanulate; 2, spathulate; 3, cupular; 4, rotate
12	Corolla symmetry	0, actinomorphic; 1, zygomorphic
13	Androecial members	0, free; 1, coherent
14	Fertile stamens	0, as all except posterior (adaxial) member; 1, as adaxial pair; 2, as lateral pair; 3, as anterior (abaxial) pair; 4, all fertile
15	Staminode vascular bundle	0, absence; 1, presence
16	Anther endothecium	0, without fibrous thickening; 1, with fibrous thickenings
17	Pollen shape	0, triangular; 1, globose, spheroidal or nearly so; 2, ellipsoid/elliptic
18	Style derivation	0, terminal; 1, attenuate; 2, gynobasic
19	Placentation	0, axile; 1, parietal; 2, superficial; 3, basal; free-central
20	Ovule orientation	0, anatropous; 1, hemianatropous; 2, amphitropous; 3, pendulous; 4, orthotropous; 5, ascending; 6, pendulous and ascending in same locule
21	Embryogeny	0, onagrad; 1, chenopodiad; 2, solanad
22	Chalazal endosperm haustoria	0, absence; 1, presence
23	Micropylar endosperm haustoria	0, absence; 1, presence
24	Loculicidal dehiscence	0, not dehiscent; 1, dehiscent
25	Septicidal dehiscence	0, not dehiscent; 1, dehiscent
26	Retinaculum	0, absence; 1, presence
27	Embryo	0, straight; 1, curved

Appendix 20 Small matrix of morphological character states.

T. hensii	0200???0001100?1001???101?088
T. laurifolia	0200???0001100??1001???101?099
Bartsia	0221???0011100-?100??????0?11213
Lindenbergia	02{01}0???0001100-?100????100?11011
Paulownia	02?0??1001110?-?10???11100?11011
Mimulus	02?0???0000100-?0014???100?11011
Callicarpa	02000??0003004-?2?35???--0?31114
Lamium	00?10??0011100-1?2350110000?167
Sesamum	00?10??000110011110001110 0?044
Ceratotheca	02010??0004100?1?100011110?055
Elytraria	0?001??200?102111?120111011055
Thunbergia	120011?0012100?01?00?011001044
Barleria	02?011?0003100-01000?111011145
Jacaranda	02?10??0101100???1?0010100?088
Kigelia	02?00??010310????10?010000?099
Catalpa	02?00??000?10????1??010010?01111
Schlegelia	32?0?0?000{13}10?1?1000???0000088
Synapsis	02?0???0001?0????????????????01919
Petrea	32?10??000400011{02}000011000?044
Stachytarpheta	02?00??0000103?12010011??0?077
Verbena	00?10??0000100?12??0011000?077
Proboscidea	00?00?0001210???1?10?????0?01212
Martynia	02?00?0001210???101001?000?077
Hemimeris	40?1?0?0004012-??00??????0?11314
Scrophularia	02?1?0?00011000??00??11010?088
Retzia	0??000?2020004-?1??{36}???110011011
Halleria	02?00??0001100-??0???????0?11415
Angelonia	00?0???000410???1000?001000088
Veronica	0{12}20???0031003-??0?????100?11213
Streptocarpus	0?300?0000410??11??0011100?077
Nematanthus	5???0?100??????1???0011??0?01616
Calceolaria	4030??0000410?-?1??????110?11213
Jovellana	0??0???0004103-??00??????0?11516
Ligustrum	020000?0003003?1?000???0000066
Borago	00410??1004014-1?2??1??--0?31114
Gentiana	0??011?0022004-1?0202??010?189
Nicotiana	000001?1001004-1?00?2???10?189