Host-plants of leaf-miners in Australian subtropical rainforest

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Abstract

Leaf-miners are endophytic insect herbivores that are considered to be relatively host-specific compared with other types of insect herbivores, often depending on one or a few congeneric hosts. Due to their degree of host-specificity, they may be particularly vulnerable to environmental change. Despite this, little is known about the host-plants and life histories of the Australian leaf-mining fauna. Here, we present new information on the host-plant use of leaf-miners occurring in Australian subtropical rainforest. We repeatedly hand-collected leaf-miners at 14 sampling sites within the 'Tweed Caldera' subtropical rainforest region of south-eastern Queensland and north-eastern New South Wales, Australia. Leaf-miners and their host-plants were identified to species (or morphospecies in the case of some leaf-miners). Within the region, a total of 106 plant species was recorded as leaf-miner hosts, on which a total of 12679 individual leaf-miners was counted, belonging to 50 different species. We measured the local host-plant range of each leaf-miner species for which we had reliable incidence records across sampling sites (24 species). Local host-specificity was relatively high with 66.7 % of species recorded from a single or two congeneric host-plants. 16.7 % of

species were restricted to a single plant family and 16.7 % were recorded on a few to several plants within the same plant order or across a range of unrelated host-plants.

Key words: herbivory, host specificity, insect-plant interactions

Introduction

Leaf-miners are herbivorous insects that pass their larval stages inside the leaf of their host-plant, using it as a permanent 'captured' food source, a strategy that occurs among Lepidoptera, Diptera, Coleoptera and Hymenoptera (Hespenheide 1975). Little is known about the ecology of leaf-miners occurring in natural ecosystems in Australia (Sinclair & Hughes 2010), and even less is known about species inhabiting rainforest. Basset (1991) recorded leaf-miner damage on the rainforest tree *Argyrodendron actinophyllum* (Malvaceae) in subtropical rainforest at Lamington National Park in south-eastern Queensland. Four species of *Roscidotoga* Hoare (Lepidoptera: Nepticulidae), which mine plants of the order Oxalidales, have been collected and described from eastern Australian rainforest, including from Lamington and Border Ranges National Parks (Hoare 2000, van Nieukerken 2011, Hoare & van Nieukerken 2013).

As part of a larger study including other forest types, Sinclair & Hughes (2008) recorded the incidence of leaf-mining on trees at six rainforest sites along the east Australian coast, between Sydney and Broken Head (28°–33°S, New South Wales). In their study, for 89 % of thirty-six mined plant species no previous records of leaf-mining existed, indicating how little is known about the range of plants mined by insects in Australia (Sinclair & Lesley 2008). Beyond rainforest communities, Bairstow *et al.* (2010) recorded ninety-seven leaf-miner morphospecies (using mine shape as an indication of identity) on forty-six different species of *Acacia* along a rainfall gradient from eastern to western New South Wales. Lambkin *et al.* (2008) reared three different agromyzid flies and their parasitoids from three plant species (two Asteraceae and one Plantaginaceae) around the Canberra region.

Some host-plants of leaf-miners are listed in '*Moths of Australia'* (Common 1990), '*Insects of Australia'* (Naumann 1991) and scattered through the taxonomic literature (see for example Spencer 1977; Kaila 2011; Pullen *et al.* 2014; van Nieukerken & Geertsema 2015). Kaila

(2011), for example, described many leaf-mining associations in elachistine moths, which mine monocotyledonous plants. Some host-plant information is also attached to specimens of leaf-miners deposited in collections such as the Australian National Insect Collection (ANIC). Here we present new information on the host-plant use of leaf-miners occupying subtropical rainforest in Australia. These data were obtained during a wider study that investigated the effects of elevation on the diversity and composition of leaf-miners and their interactions with host-plants (Maunsell *et al.* 2016) as well as the food web structure of leaf-miners and their parasitoids (Maunsell *et al.* 2015).

Materials and Methods

During 2011 and 2012, leaf-miners and their host-plants were sampled in Australian subtropical rainforest at fourteen sites, situated at elevations between 260 and 1160 m above sea level (see Supplementary Material Table S1). Four sites were situated in Lamington National Park in south-eastern Queensland (Qld), nine in Border Ranges National Park and one in Mebbin National Park in north-eastern New South Wales (NSW) (28°S, 153°E). We sampled all sites for leaf-miners in August and October 2011 and February and May 2012, apart from two low-elevation sites (approximately 300 m), which were not sampled in August. Five sites within the Border Ranges National Park were also additionally sampled in December 2011 and April 2012 (see Supplementary Material, Table S1).

On each sampling occasion, leaf-miners were sampled by examining all plants along eight 50-by-3 m transects (a total of 1200 m^2) at each site. Leaves with active mines were removed from the plant, and the host-plant species was identified in the field or later from a pressed specimen. Leaf-miners (larvae and/or pupae) were reared to adults, mounted or stored in ethanol, sorted into morphospecies and then identified to species where possible. Leaf-miners that were not reared were assigned to species or morphospecies based on a combination of host-plant and mine structure. Some of the lepidopteran leaf-miners were DNA barcoded in order to confirm identifications. Two legs were removed from pinned specimens and crushed. DNA extractions were performed using a modified spin column extraction protocol (Epoch Life Sciences). The mtDNA cytochrome C oxidase 1 (CO1) barcode region was sequenced using LepF1 and LepR1 primers (Hebert 2004). The PCR cycle (40 cycles) consisted of 3 minutes initial denaturation at 94°C, 15 seconds denaturation at 94°C, 30 seconds annealing at 50°C, 40 seconds extension at 72°C, 5 minutes final extension at 72°C. PCR products were purified using FastAp and Exonuclease (Thermofisher Scientific). Sequencing (forward only)

was outsourced to Macrogen, Korea. Geneious R8 software was used to manually trim sequences and high quality sequences compared to all barcode records available through the Barcode of Life (BOLD) platform (http://www.boldsystems.org).

Host-plant categories were designated for leaf-miner species for which we had reliable incidence records, that is for those that occurred in more than 10 transects (out of a total of 114) and in at least three sites (out of a total of fourteen) (see Supplementary Material, Figure S1 and S2). Rarely sampled species were not categorised because measures of host range are highly dependent on sampling effort (Novotny & Basset 2005).

Results

Leaf-miner and host-plant records

Throughout this study, we recorded 12679 active leaf-miner individuals. All but 162 (1.3 %) of these individual miners were assigned to a morphospecies. The 162 unassigned miners were associated with twenty-nine different plant species, most of which yielded only one to two individual leaf-miners over the course of the study. In total 12517 leaf-miners were assigned to morphospecies or species (where possible). Fifty-five morphospecies of leaf-miner were encountered in this study (Table 1). Most were Lepidoptera (41 species), the others being Coleoptera (5 species), Diptera (4 species) and a single species of sawfly (Hymenoptera: 'Symphyta'). Four species could not be reared to adult stage and therefore could not be identified to order, but were considered to be unique based on their characteristic mine shapes and host-plant associations. Lepidoptera and Coleoptera voucher specimens have been deposited in the Australian National Insect Collection (ANIC) and Diptera and Hymenoptera voucher specimens in the Queensland Museum.

Twenty of the 41 lepidopteran leaf-miner morphospecies were identified to family, comprising 17 Gracillariidae, two Heliozelidae and one Lyonetiidae. All five species of leaf-mining beetles collected in this study belong to the family Curculionidae (weevils). Three of the fly species encountered were agromyzids, a family primarily composed of leaf-miners. We also reared a leaf-mining drosophilid, *Scaptodrosophila* sp., from the fern *Blechnum wattsii* (Blechnaceae). We recorded a single species of leaf-mining sawfly, *Leptoperga brunnea* Riek (Pergidae: Phylacteophaginae), mining the leaves of *Ripogonum* sp. (Ripogonaceae) (Table 1).

We obtained high-quality CO1 barcodes for 23 individuals belonging to 11 different species (indicated with [B] in Table 1). Similarities between our sequences and those available through the BOLD database ranged between 88.82 % and 99.23 %, with an average of 91.4 % (Supplementary Material, Table S2). Sequences allowed us to confirm identification at the generic or family level only. Even when sequence matches were high (> 98 %), only families or genera were assigned to sequences in the BOLD database.

Leaf-mining was recorded for 106 plant species belonging to 44 different families (Table 2). Of these 106 species, four (3.8 %) could not be identified; three of these were represented by a single record and one had a generalist leaf-miner associated with it (*Tropicomyza polyphyta* (Kleinschmidt)). Three plant species were identified only to genus, but all were unique in the study and therefore treated as distinct species. Two genera were grouped (*Carex* and *Gahnia* (Cyperaceae)) as they were not reliably distinguishable in the field. In four cases, two similar plant species in the same genus (of *Beilschmiedia*, *Clematis*, *Neolitsea* and *Quintinia*) were also grouped because they could not always be distinguished in the field. In most of these cases we were unsure whether the recorded leaf-miner fed on only one or both of the pair of species, except for a miner that was definitely reared from both *Quintinia verdonii* and *Q. sieberi*. Most plant species that were mined had only one or two leaf-miner species associated with them, apart from *Croton verreauxii* (Euphorbiaceae), for which we recorded four associated leaf-miner species (Table 2).

Host-plant range

Host-plant ranges were designated for 24 out of a total of 56 recorded leaf-miner species (43 %). Of these 24 species, 16 used one or two host-plants and the remainder used between three and 17 hosts (Figure 1). Ten species were recorded from a single host-plant, six from two congeneric hosts, four from several confamilial hosts, one from hosts in the same order, and three species used a range of hosts from different orders and were therefore classified as generalists (Table 1).

Discussion

This study adds substantially to our knowledge of the biology and ecology of leaf-miners in Australia. We discuss host-plant records from this study for each of the four orders of leaf-mining insects (Lepidoptera, Coleoptera, Diptera and Hymenoptera) below and compare our findings to previous records.

Lepidoptera

Leaf-mining occurs in ten lepidopteran families in Australia (Common 1990; Sinclair & Hughes 2010), three of which were recorded here. For the five lepidopteran leaf-miners identified to species, at least one or a few host-plants were already known, including *Caloptilia bryonoma* (Turner) (Gracillariidae) on *Nothofagus moorei* (Nothofagaceae) and *Lyonetia lechrioscia* Turner (Lyonetiidae) on *Quintinia* spp. (Quintiniaceae) (based on specimen labels at ANIC). However, we also recorded some new host-plants. *Acrocercops chionosema* (Turner) (Gracillariidae), for example, has been recorded from *Macadamia integrifolia*, *M. tetraphylla* and *Stenocarpus salignus* (Common 1990), all of which belong to the family Proteaceae. We recorded *A. chionosema* feeding on three additional species of Proteaceae: *Orites excelsus*, *Helicia glabriflora* and *Stenocarpus sinuatus*. Another gracillariid, *Macarostola formosa* (Stainton), which has previously been recorded from *Acmena smithii* (Common 1990), was also recorded in our study on *Lophostemon confertus* (both Myrtaceae) (Table 2). Heliozelidae sp. A, which was recorded on *Psychotria simmondsiana* and *P. loniceroides*, may belong to the genus *Holocacista*, as a similar or perhaps the same species of moth has previously been collected from *P. simmondsiana* (van Nieukerken & Geertsema 2015).

Coleoptera

We only observed leaf-mining curculionids (weevils) and did not encounter any species of Chrysomelidae or Buprestidae, families known to also contain leaf-mining species (Hespenheide 1991). Leaf-mining is quite widespread in the Curculionidae globally but has not been comprehensively assessed. The lifestyle occurs in at least eight tribes of the subfamily Curculioninae (Caldara *et al*. 2014). It has also been recorded in the supertribes Bariditae and Ceutorhynchitae of the subfamily Conoderinae (Prena *et al*. 2014) and for the tribes Gonipterini and Hyperini, currently unassigned to subfamily (Oberprieler *et al*. 2014). The larvae of three species of *Cydmaea* (Curculioninae: Storeini) have been reported to mine in leaves of *Hakea* (Proteaceae) (Moore 1966), but the larvae also develop in buds and flowers of these plants and do not specifically appear to be leaf-miners. However, larvae of the genera *Syarbis* (Gonipterini) and *Gerynassa* (Hyperini), evidently are true leaf-miners (Oberprieler *et al*. 2014), as are those of *Austrocis* and *Platynotocis* (Viticiini) (Caldara *et al*. 2014).

We made 1156 observations of live mines of *Platynotocis angulipennis* Zimmerman & Oberprieler feeding on a single host-plant, *Polyosma cunninghamii* (Escallioniaceae) (Table 1). There are two other described species of *Platynotocis* in Australia, *P. albomaculatus* Zimmerman & Oberprieler and *P. pyriformis* Lea, for which there are no host records (Pullen *et al*. 2014), but a related species, *Austrocis bicaudatus* Zimmerman & Oberprieler, has been reared from leaf-mines on *Elaeocarpus reticulatus* (Elaeocarpaceae) (Pullen *et al*. 2014). There are additional species belonging to the same tribe (Viticiini) in Australia (Pullen *et al*. 2014), and it is likely that most members of this tribe are leaf-miners (Pullen *et al*. 2014).

We recorded one species of *Orchestes* mining in leaves of two species of *Argyrodendron* (Malvaceae). Although the lifestyle of this genus was previously largely unknown in Australia, *Orchestes* belongs to the tribe Rhamphini of Curculioninae, which is well known globally for its leaf-mining habits (Caldara *et al*. 2014). Three further species of weevils collected in this study belong to undescribed genera, and nothing was previously known about their biology. Two belong to the tribe Storeini *sensu lato* (Storeini sp. A and sp. B), and the third (Genus nr. *Thaumastophasis* sp.) (Table 1) belongs to the *Eristinus* group recently classified in the subfamily Molytinae (Pullen *et al*. 2014).

Diptera

We recorded three species of Agromyzidae. *Tropicomyia polyphyta*, which was collected from 17 host-plants in this study, has been previously recorded feeding on many hosts in Australia, including several vegetable crops (Spencer 1977). We provide additional host records for this species, which utilised a range of unrelated plant taxa in our study (Table 1). We recorded *Phytoliriomyza queenslandica* Spencer mining in leaves of two species of *Pittosporum* (Pittosporaceae) (Table 2). Another species of *Phytoliriomyza* has previously been recorded from an unknown species of the same plant family (Spencer 1977), but, as far as we are aware, ours are the first host records for *Pittosporum multiflorum* and *P. oreillyanum*. *Phytomyza vitalbae* Kaltenbach, which we recorded from *Clematis glycinoides/pickeringii*, has previously been recorded from *Clematis aristata* (Spencer 1963), a species that does occur in the study region (Queensland Herbarium 2013) but was not included in our dataset. *Phytomyza vitalbae* is a naturalised species introduced from Europe (Spencer 1977).

Leaf-mining is common in the family Agromyzidae, however, we also recorded a leaf-mining species of Drosophilidae from the fern *Blechnum wattsii* (Blechnaceae). The leaf-mining habit is rare in Drosophilidae and has been reported for only three genera, *Gitona*, *Drosophila* and *Scaptomyza*, and it is prevalent only in species of *Scaptomyza (Scaptomyza)* (Ferrar 1987). As far as we are aware, ours is the first record of leaf-mining in the genus *Scaptodrosophila*. Interestingly, two Hawaiian species of *Drosophila*, *D. apicipuncta* Hardy and *D. sadleria* Bryan, have been bred from the rachises of ferns of the genus *Sadleria* (also in the family Blechnaceae), with larvae of the former species living in mines (Magnacca & O'Grady 2009). Larvae of three other Australian species of *Scaptodrosophila* have been found to feed on living ferns. Two species feed on young fronds of bracken ferns (*Pteridium* species, Dennstaedtiaceae), groups of larvae of *Scaptodrosophila notha* (Bock) destroying the central tissues of the rachis and single larvae of *Scaptodrosophila megagenys* (Bock) feeding externally on the circinate tips (Thomson *et al.* 1982). *Scaptodrosophila inornata* (Malloch) larvae recovered from eucalypt litter in the crowns of tree ferns have been shown to feed on the ferns in the laboratory (Bock & Parsons 1978).

Hymenoptera

We recorded a single species of leaf-mining sawfly, *Leptoperga brunnea*, belonging to the subfamily Phylacteophaginae of the family Pergidae. This subfamily is restricted to Australia (Riek 1970; Naumann 1983) and contains only three genera, one of which (*Phylacteophaga*) includes the eucalypt leaf-blister sawflies, the larvae of which mine the leaves of some myrtaceous species (Mayo *et al.* 1997).

Conclusions

Our findings indicate that leaf-miners in Australian subtropical rainforest are, at least locally, relatively host-specific. Insect herbivores involved in such specific interactions may be particularly vulnerable to environmental change (Moir *et al.* 2015). In Australia, however, we have a limited understanding of host-plant use of insects in natural systems, and a better knowledge of such relationships will enable us to predict how insect-host interactions may be affected by environmental change. Our dataset contained many instances in which only one or two mines were encountered on particular plant species, which may have been because these plant species are rare in our study sites or because they are rarely mined. Given the relative host-specificity of leaf miners, diversity is likely to increase from local to regional scales tracking turnover in plant composition (Novotny & Miler 2014). Numerous singleton

observations in our study imply that further field sampling of leaf-miners in Australian rainforest is likely to generate many more host-plant records.

Supporting Information

Table S1. Data collection sites and dates.

Table S2. Blast results (top 10 similarity hits) of top genetic barcodes.

Figure S1. Occurrence of leaf miner morpho/species at sampling sites.

Figure S2. Occurrence of leaf miner morpho/species at sampling sites

Acknowledgments

This study was funded by the Griffith School of Environment, as part of SM's PhD program. Additional funding was provided by the Environmental Futures Research Institute, The Ecological Society of Australia and the NCCARF Terrestrial Biodiversity Network. RJM was funded by a Royal Society University Research Fellowship. Sincere thanks go to all the volunteers who helped with this project, particularly Elliot Leach, Mariana Pinto, Carlos Torrente, Jonathan McCallum, Casey Hall, Sandra Preston-Hatcher, Patrick O'Connor, Francesca Dem and Heath Cambie. We also thank Louise Ashton, Stephanie Horton and John Hunter for establishing some of the plots that were used in this study. We thank Erik van Nieukerken and Camiel Doorenweerd for identification and DNA barcoding advice. We thank the Queensland Government for providing a permit to work in Lamington National Park (WITK07650710) and the New South Wales Government and the Githabul National Parks Management Committee for providing a license to work in Border Ranges National Park (SL100007).

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Tables

Table 1. Leaf-miner species and host-plant records from Australian subtropical rainforest (Lamington, Border Ranges and Mebbin National Parks (Queensland and New South Wales)). Species or morphospecies in bold are those that were recorded at > 10 transects in ≥ 3 sampling sites (see Supplementary Material, Figure S1) and therefore assigned to host-specificity categories. Higher taxa of plants (families and orders) are shown for confamilial, within-order and generalist feeding categories. [B] indicates where the identification (at the level preceding [B]) was confirmed with DNA barcoding (see Supplementary Material Table S2).

Order Family	Morpho/species	Host category	Host-plant/s
Coleoptera			
Curculionidae	**Storeini sp. A.**	confamilial	**Sapindaceae:** *Arytera distylis, A. divaricata, Sarcopteryx stipata, Cupaniopsis flagelliformis* var. *australis*
	***Orchestes* sp.**	congeneric	*Argyrodendron actinophyllum* subsp. *actinophyllum, A. trifoliolatum*
	***Platynotocis angulipennis* Zimmerman & Oberprieler**	host-specific	*Polyosma cunninghamii*
	Storeini sp. B.	-	*Tasmannia insipida*
	Genus nr. *Thaumastophasis* sp.	-	*Triunia youngiana*
Diptera			
Drosophilidae	*Scaptodrosophila* sp.	-	*Blechnum wattsii*
Agromyzidae	***Tropicomyia polyphyta* (Kleinschmidt)**	generalist	**Apiales:** *Cephalaria cephalobotrys, Pennantia cunninghammii;* **Laurales:** *Wikliea huegeliana, W. austroqueenslandica;* **Malpighiales:** *Streptothamnus moorei, Croton verreauxii;* **Liliales:** *Ripogonum* sp.; **Gentianales**: *Atractocarpus benthamianus;* **Proteales:** *Helicia glabriflora;* **Cornales:** *Alangium villosum* subsp. *polysmoides;* **Celastrales:** *Denhamia celastroides,Celastrus subspicata;* **Sapindales:** *Anthocarapa nitidula, Synoum glandulosum* subsp. *glandulosum;* **Ericales:** *Planchonella australis;* **Berberidopsidales:** *Berberidopsis beckleri,* and Vine 1 (unknown taxon)
	***Phytoliriomyza queenslandica* Spencer**	congeneric	*Pittosporum multiflorum, P. oreillyanum*
	Phytomyza vitalbae Kaltenbach	-	*Clematis glycinoides/pickeringii*
Hymenoptera			

Pergidae	*Leptoperga brunnea* Riek	-	*Ripogonum* sp.
Lepidoptera			**Sapindaceae:** *Arytera distylis, A. divaricata, Mischocarpus anodontus*
Gracillariidae	**Gracillariidae sp. F.**	confamilial	
	***Acrocercops*[B] *chionosema* (Turner)**	confamilial	**Proteaceae:** *Orites excelsus, Helicia glabriflora, Stenocarpus salignus , S. sinuatus*
	Gracillariidae[B] sp. A.	generalist	**Laurales:** *Wilkiea huegeliana, W. austroqueenslandica, Cryptocarya erythroxylon, Beilschmiedia obtusifolia/elliptica*
	***Phyllocnistis*[B] sp. A.**	generalist	**Vitales:** *Cissus antarctica, C. hypoglauca, C. sterculiifolia*; **Canellales:** *Tasmannia insipda*; **Malpighiales:** *Breynia oblongifolia*
	Gracillariidae[B] sp. B.	congeneric	*Atractocarpus benthmianus, A. chartaceous*
	Gracillariidae sp. C.	congeneric	*Planchonella australis, P. myrsinfolia*
	Gracillariidae sp. D.	host-specific	*Melodinus australis*
	***Phyllocnistis*[B] sp. B.**	host-specific	*Eupomatia laurina*
	***Caloptilia*[B] *bryonoma* (Turner)**	host-specific	*Nothofagus moorei*
	Gracillariidae sp. H.	-	*Acradenia eudiiformis*
	Phyllocnistis[B] sp. D.	-	*Stenocarpus salignus, S. sinatus*
	Acrocercops[B] *trapeziodes* (Turner 1894)	-	*Embelia australiana*
	Phyllocnistinae sp. C.	-	*Dysoxylum fraserianum*
	Gracillariidae[B] sp. E.	-	*Myrsine subsessilis* subsp. *subsessilis*
	Macarostola formosa (Stainton)	-	**Myrtaceae:** *Acmenia smithii, Lophostemon confertus*
	Gracillariidae sp. G.	-	*Achronychia bauerlenii, Achronychia pubescens*
	Phyllocnistinae sp. E.	-	Vine 1 (unknown taxon)
Heliozelidae	**Heliozelidae[B] sp. A.**	congeneric	*Psychotria simmondsiana, P. loniceroides*
	Heliozelidae sp. B.	-	*Cissus antarctica*
Lyonetiidae	***Lyonetia lechrioscia* Turner**	congeneric	*Quintinia verdonii, Q. sieberi*
Lepidoptera (unknown family)	**Lep48**	confamilial	**Cyperaceae:** *Cyperus tetraphyllus*, *Carex* sp., *Gahnia* sp.
	Lep49	within order	**Poales:** *Oplismenus imbecillus*, *Cyperus tetraphyllus*
	Lep27	host-specific	*Croton verreauxii*
	Lep31	host-specific	*Gynochthodes jasminoides*
	Lep35	host-specific	*Cleistanthus cunninghamii*
	Lep46	host-specific	*Elaeodendron australe*

	Lep5	host-specific	*Psychotria simmondsiana*
	Lep51	-	**Sapindaceae:** *Mischocarpus australis, M. anodontus, Rhysotoechia* sp.
	Lep32	-	*Polyosma cunninghamii*
	Lep34	-	*Neolitsea australiensis/dealbabta*
	Lep37	-	*Argyrodendron trifoliolatum*
	Lep44	-	*Sloanea woollsii*
	Lep54	-	*Rhysotoechia bifoliolata* subsp. *bifoliolata*
	Lep50	-	*Syzigium francisii*
	Lep55	-	*Hibbertia scandens*
	Lep39	-	*Pollia crispate*
	Lep29	-	*Croton verreauxii*
	Lep43	-	*Anthocarpa nitidula*
	Lep53	-	*Polyscias murrayi*
	Lep56	-	*Sarcopteryx stipata*
	Lep28	-	*Croton verreauxii*
Unknown taxa	**M59** (only parasitoids reared)	host-specific	*Doryphora sassafrass*
	M64	-	*Elattostachys nervosa*
	M63	-	*Baloghia inophylla*
	M62	-	*Diospyros pentamera*

Table 2. List of host-plants recorded with leaf-miners from Australian subtropical rainforest (Lamington, Border Ranges and Mebbin National Parks (Queensland and New South Wales)). M? indicates a leaf-miner recorded on a host-plant but not identified to morphospecies because no adults reared and not distinguishable on mine structure

Plant Family	**Plant species**	**Leaf-miner morpho/species**
Anacardiaceae	*Euroschinus falcatus*	M?
Annonaceae	*Melodorum leichhardtii*	M?
Apocynaceae	*Melodinus australis*	Gracillariidae sp. D.
Araliaceae	*Cephalaralia cephalobotrys*	*Tropicomyza polyphyta*
	Polyscias murrayi	Lep53
Arecaceae	*Calamus muelleri*	M?
Berberidopsidaceae	*Berberidopsis beckleri*	*Tropicomyza polyphyta*
Blechnaceae	*Blechnum cartilagineum*	M?
	Blechnum wattsii	*Scaptodrosophila* sp.
	Doodia aspera	M?
Capparaceae	*Capparis arborea*	M?
Celastraceae	*Celastrus subspicata*	*Tropicomyza polyphyta*
	Denhamia celastroides	*Tropicomyza polyphyta*
	Elaeodendron australe var. *australe*	Lep46
Commelinaceae	*Pollia crispata*	Lep39
	Aneilema acuminatum	M?
Cornaceae	*Alangium villosum* subsp. *polyosmoides*	*Tropicomyza polyphyta*
Cucurbitaceae	*Zehneria cunninghamii*	M?
Cyperaceae	Cyperaceae sp. (*Carex* or *Gahnia*)	Lep48; Lep42
	Cyperus tetraphyllus	Lep49
Dilleniaceae	*Hibbertia scandens*	Lep55
Ebenaceae	*Diospyros pentamera*	Lep62
Elaeocarpaceae	*Sloanea woollsii*	Lep44
Escallioniaceae	*Polyosma cunninghamii*	*Platynotocis angulipennis*; Lep32
Euphorbiaceae	*Baloghia inophylla*	M63
	Croton verreauxii	Lep27; Lep28; Lep29; *Tropicomyza polyphyta*
	Mallotus philippensis	M?
	Alchornea ilicifolia	M?
Eupomatiaceae	*Eupomatia laurina*	*Phyllocnistis* sp. B
Fabaceae	*Austrosteenisia blackii*	M?
Flacourtiaceae	*Scolopia braunii*	M?
	Streptothamnus moorei	*Tropicomyza polyphyta*
Lauraceae	*Beilschmiedia obtusifolia/elliptica*	M1?
	Cinnamomum oliveri	M?
	Cryptocarya erythroxylon	Gracillariidae sp. A.; Lep7
	Endiandra crassiflora	M?
	Endiandra pubens	Gracillariidae sp. A.
	Neolitsea australiensis/dealbata	Lep34
Malvaceae	*Argyrodendron actinophyllum* subsp. *actinophyllum*	*Orchestes* sp.
	Argyrodendron trifoliolatum	*Orchestes* sp.; Lep37
Meliaceae	*Anthocarapa nitudula*	*Tropicomyza polyphyta*
	Dysoxylum fraserianum	Phyllocnistinae sp. C.
	Synoum glandulosum subsp. *glandulosum*	*Tropicomyza polyphyta*

Monimiacae	*Palmeria foremanii*	Gracillariidae sp. A.
	Wilkiea austroqueenslandica	Gracillariidae sp. A.
	Wilkiea huegeliana	Gracillariidae sp. A.
	Doryphora sassafras	M59
Myrsinaceae	*Embelia australiana*	*Acrocercops trapeziodes*
	Myrsine subsessilis subsp. *subsessilis*	Lep23
Myrtaceae	*Acmena ingens*	M?
	Acmena smithii	*Macarostola formosa*
	Gossia acmenoides	M?
	Gossia bidwillii	M?
	Lophostemon confertus	*Macarostola formosa*
	Syzygium francisii	Lep50
Nothofagaceae	*Nothofagus moorei*	*Caloptilia bryonoma*
Oleaceae	*Jasminum simplicifolium* subsp. *australiense*	M?
Pennantiaceae	*Pennantia cunninghamii*	*Tropicomyza polyphyta*
Phyllanthaceae	*Breynia oblongifolia*	*Phyllocnistis* sp. A.
	Cleistanthus cunninghamii	Lep35
	Glochidion ferdinandi	M?
Pittosporaceae	*Pittosporum lancifolium*	M?
	Pittosporum multiflorum	*Phytoliriomyza queenslandica*
	Pittosporum oreillyanum	*Phytoliriomyza queenslandica*
	Pittosporum revolutum	M?
Poaceae	*Oplismenus imbecillis*	Lep49
Proteaceae	*Helicia glabriflora*	*Acrocercops chionosema*; *Tropicomyza polyphyta*
	Orites excelsus	*Acrocercops chionosema*
	Stenocarpus salignus	*Acrocercops chionosema*; *Phyllocnistis* sp. D.
	Stenocarpus sinuatus	*Acrocercops chionosema*; *Phyllocnistis* sp. D.
	Triunia youngiana	Genus nr. *Thaumastophasis* sp.
Quintiniaceae	*Quintinia verdonii/sieberi*	*Lyonetia lechrioscia*
Ripogonaceae	*Ripogonum* sp.	*Leptoperga brunne*a; *Tropicomyza polyphyta*
Rubiaceae	*Atractocarpus benthamianus*	Gracillariidae sp. B.
	Atractocarpus chartaceous	Gracillariidae sp. B.
	Gynochthodes jasminoides	Lep31
	Psychotria loniceroides	Heliozelidae sp. A.
	Psychotria simmondsiana	Heliozelidae sp. A.; Lep5
Rununculaceae	*Clematis glycinoides/pickeringii*	*Phytomyza vitalbae*
Rutaceae	*Acradenia euodiiformis*	Gracillariidae sp. D.
	Acronychia baeuerlenii	Lep47
	Acronychia pubescens	Lep47
Sapindaceae	*Arytera distylis*	Storeini sp. A..; Gracillariidae sp. F.
	Arytera divaricata	Storeini sp. A.; Gracillariidae sp. F.
	Atalaya multiflora	M?
	Cupaniopsis flagelliformis var. *australis*	Storeini sp. A.
	Diploglottis australis	M?
	Elattostachys nervosa	M64
	Guioa semiglauca	M?

	Mischocarpus anodontus	Gracillariidae sp. F.; Lep51
	Mischocarpus australis	Lep51
	Rhysotoechia bifoliolata subsp. *bifoliolata*	Lep54; Lep51
	Sarcopteryx stipata	Storeini sp. A.
Sapotaceae	*Planchonella australis*	Gracillariidae sp. C.; *Tropicomyza polyphyta*
	Planchonella myrsinifolia	Gracillariidae sp. C.
Solanaceae	*Solanum aviculare*	M?
	Solanum inaequilaterum	M?
Vitaceae	*Cissus antarctica*	*Phyllocnistis* sp. A..; Heliozelidae sp. B.
	Cissus hypoglauca	*Phyllocnistis* sp. A.
	Cissus sterculiifolia	*Phyllocnistis* sp. A.; Heliozelidae sp. B.
Winteraceae	*Tasmannia insipida*	Storeini sp. B.; *Phyllocnistis* sp. A.
Unknown family	Climbing fern	M?
	Vine 1	*Tropicomyza polyphyta*
	Vine 2	M?
	Vine 3	Phyllocnistinae sp. E.

Figures

Figure 1. Number of host-plant species used by leaf-miner morpho/species, recorded at >10 transects (50*3m) and at ≥3 (out of 14) sampling sites situated in Australian subtropical rainforest (within Lamington, Border Ranges and Mebbin National Parks).