The reproductive biology of two poorly known relatives of the fig (*Ficus*) and insights into the evolution of the fig syconium.

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Running title: Reproductive biology of fig relative *Dorstenia*

ABSTRACT

We conducted the first detailed investigation of the floral architecture and reproductive biology of two species from the genus *Dorstenia,* which are poorly known relatives of *Ficus* (Moraceae). Our aims were to extend and refine knowledge of the understudied genus *Dorstenia* and to explore possible insights into the evolution of the fig syconium. We characterised four key stages of floral development using light microscopy, scanning electron microscopy and histological staining. Reproductive biology was found to be complex and species-specific. Both study species are monoecious and produce an inflorescence of minute male and female flowers. Protogyny, associated with a spatial separation of male and female flowers and asynchronous stamen development, was species-specific, as was seed set. Our results reveal novel insights into the complex reproductive biology of an under-studied genus in the family Moraceae. We propose that exploring the reproductive biology of *Dorstenia* and other poorly known *Ficus* relatives will provide insights into the evolution of the fig syconium – the unique reproductive structure of this economically and ecologically important genus.

KEYWORDS: Developmental Biology; *Dorstenia;* Fig; Moraceae; Reproductive Biology; Syconium

INTRODUCTION

The Moraceae shows an extraordinary diversity in floral morphology, pollination modes and breeding systems (Datwyler and Weiblen, 2004). The family has fascinated botanists for generations, Corner (1962) describing ‘the monography of the Moraceae [as] one of the most exciting chapters in angiosperm taxonomy’ (Corner, 1962, Clement and Weiblen, 2009). The floral architecture of the Moraceae ranges from flattened disks (coenanthia) and globular or clavate inflorescences with flowers on the external surface, to completely enclosed inflorescences (syconia) of figs (*Ficus* spp.). The family contains several economically and ecologically important genera including the fig (*Ficus*), mulberry (*Morus*) and breadfruit (*Artocarpus*). Figs are noteworthy for their interesting modes of pollination which comprise obligate symbioses with specific species of wasp or thrip (Galil 1977, Janzen 1979, Zerega et al. 2004). Figs are also among the earliest domesticated crops and have been cultivated for over 11,000 years (Lansky, Paavilainen et al. 2008). There have been extensive studies into the reproductive biology of figs due to their importance as a model for coevolution with pollinating fig wasps (Cook and Rasplus, 2003, Cook & Segar 2010, Cook et al. 2017), their ecological importance as keystone plant resources (KPR) for frugivores in tropical forests (Janzen 1979, Mabberley, 1992, Diaz-Martin et al. 2014) and their economic importance.

Dorstenia is a diverse genus (Fig. 1) that is widely distributed across tropical regions of Central & South America and Africa (Berg and Hijman 1999, Datwyler and Weiblen, 2004) with an almost equal transatlantic distribution (Misiewicz and Zerega, 2012). Typical of the family Moraceae, *Dorstenia* possess a milky latex, minute, unisexual flowers, a fruit of aggregated drupes (Datwyler and Weiblen, 2004, Judd 2008), and is the only genus with woody, herbaceous and succulent species (Misiewicz and Zerega, 2012) (Fig. 1 F-I). The size and shape of the coenanthia are variable, particularly with respect to the prominent vegetative appendages (Fig. 1 A-F) (Misiewicz and Zerega, 2012) the function of which remains unclear. Interestingly, *Dorstenia* is predominantly monoecious with bisexual coenanthia (Berg 1977) however dioecy has been reported in the genus (McCoy and Massara, 2008). It is believed that Dioecy is the ancestral condition in the Moraceae and that monoecy has evolved up to four times independently in the family (Datwyler and Weiblen 2004).

The majority of studies on *Dorstenia* have focused on taxonomy, chemical properties and medicinal uses (Berg and Hijman 1999, Rojas-Lima et al. 1999, Abegaz et al. 2000, Bokesch, et al. 2004). Although no global monograph exists for the genus, new species have been described recently (Leal, 2014, Machado et al. 2014) and the first molecular phylogeny of the genus with substantial taxon sampling (42 of 105 taxa) constructed, which dates the radiation of the genus at 112.3 mya and suggests that a woody habit and large seeds are ancestral traits (Misiewicz and Zerega, 2012).

Few studies of *Dorstenia* have focused on reproductive biology, most being centred on classification. Early work explored mechanisms of fruit dispersal and development (Schleuss, 1958, De Granville, 1971). Hoen and Punt (1989) examined pollen morphology across the genus and described 13 different types, distinguished by aspis (outer pollen grain layer) characteristics and the number of pores. Berg (1999) described the floral morphology of different species for the purpose of classification. Most recently Araújo et al. (2017) characterised the reproductive biology of two species of *Dorstenia* in Brazil. Here we seek to revisit, extend and refine knowledge of the reproductive biology of the genus *Dorstenia* by characterising the floral architecture and reproductive biology of two poorly known species, and offer insights into the evolution of the fig syconium.

METHODS

Plant Material

Material was collected from two species of *Dorstenia*: *D. contrajerva* (native to Mexico, Central America, the Caribbean and South America) and *D. lujae* (native to tropical Africa), cultivated at the University of Bristol Botanic Garden. Identification was confirmed using the most comprehensive key to the genus available (Berg and Hijman 1999). Plants were cultivated under glass at 20oC, watered every other day with rainwater, under a day length regime of 12 hours light and 12 hours dark. Observations were made over the course of 14 weeks during which period both species completed their reproductive cycles. Specimens were observed weekly in an initial assessment using light microscopy which identified four stages of development. These were broadly consistent between species but were asynchronous (Table 1). Eight specimens were sampled at each of these stages for both species; four were then analysed using each of the treatments described below (half the samples were retained in case repeat analyses were required).

Sample Fixation for Light Microscopy

For each developmental stage (Table 1), samples were dissected from both the edge and the centre to span the entire radius of the receptacle. Samples were fixed according to Thorogood et al. (2009) in FAA (formaldehyde-acetic acid-alcohol) solution (50% ethanol + 5% formaldehyde + 10% glacial acetic acid + 35% ddH2O) overnight. Fixed samples were then dehydrated in an ethanol series over 7 hours and maintained in 100% ethanol overnight. Samples were taken through a HistoclearTM series over 5 hours and maintained in 100% Histoclear TM overnight. Samples then were taken through a paraffin series in a vacuum oven (Hybaid) at 60°C until saturated, and then set as blocks in moulds. 10µm sections were cut with a rotary microtome (Leica RM2245) and mounted in water on microscope slides on a hot plate (Raymond A Lamb Hotplate) at 50°C. Paraffin was removed in HistoclearTM by immersing for 10 minutes, then samples were rehydrated through a graded series of ethanol solutions (100%, 100%, 80%, 70%, 50%, 30%, ddH2O; 5 minutes in each) before staining (see below). Following staining, samples were dehydrated through the ethanol-dilution series and immersed in HistoclearTM before mounting. Slides were mounted with HistomountTM.

Light and UV Microscopy

Slides were observed using an optical microscope (Nikon SMZ800) and under UV at 450-490 nm (Leica DMLB; Leica I3 filter set) (Thorogood et al. 2009), and photographed with a digital camera (Nikon Coolpix E995).

Staining

Toluidine Blue (0.5% Toluidine Blue dissolved in 0.05 M potassium phosphate buffer, pH7.4, for 2 minutes) was used as a general stain of plant tissue cell walls. Samples were also stained for 1 hour with a filtered solution of 1% Alcian Green and 1% Safranin O in 60% ethanol (Tomlinson 2003) to observe lignin and cellulose. For dual-observation of lipids in pollen tubes and cuticle, samples were stained with Auramine O and Aniline Blue (3:1 in 0.05m phosphate buffer, pH7.2) (Hiscock et al. 2002). To measure stigma receptivity, samples were immersed in a Guiacol solution (0.1M Guiacol dissolved in 0.1M H2O2 and phosphate buffer (20mM, pH4.3) for 3 minutes to observe peroxidase activity, indicated by the presence of red staining (McInnis et al. 2006).

Scanning Electron Microscopy

Material for SEM was fixed with 2.7% glutaraldehyde buffer in 0.1M sodium cacodylate buffer (pH7.2) overnight and continuously agitated with a rotary mixer. Specimens were washed in 0.1M sodium cacodylate buffer (4 x 10 mins) then dehydrated in an ascending Ethanol series (10%, 30%, 50%, 70%, 100%; minimum 10 minutes each), finishing with 100% ethanol containing a cupric sulphate drying agent. Samples were critical-point dried (Samdri©-780) then mounted on aluminium stubs using carbon sticky pads. Samples were sputter-coated with gold palladium and observed using SEM (Phillips 501b).

RESULTS

Gross inflorescence *morphology*

Both species of *Dorstenia* are monoecious and produce coenanthia with unisexual flowers. Inflorescences of *D. contrajerva* are quadrangular and irregularly lobed with a narrow margin (Fig. 2A). *Dorstenia lujae* has a discoid coenanthium with a broad fringe and numerous vegetative appendages (Fig. 2B). There is a difference in the distribution of pistillate and staminate flowers on the coenanthium of the two species: flowers of *D. contrajerva* are intermixed and widely distributed across the coenanthium, whilst the pistillate flowers in *D. lujae* are central, forming a regular pattern, surrounded by the staminate flowers. There are 9- 17 pistillate flowers on the coenanthium of *D. lujae*, whilst the pistillate flowers of *D. contrajerva* are much more numerous (20-70). The cross-sectional morphology of coenanthia of *D. contrajerva* and *D. lujae* is similar. The staminate flowers are located on the surface, whilst pistillate flowers containing the ovaries are enclosed within the receptacle mantle (Fig. 2C). The surface of *D. lujae* coenanthia is covered in globular structures, visible under SEM, that appear to be cellular extensions. These were not observed in *D. contrajerva* and their function is unknown. *Dorstenia lujae* is protogynous (consistent with the spatial pattern of male and female flowers), however the pistillate and staminate flowers of *D. contrajerva* flower simultaneously. Red staining with Guiacol confirmed stigma receptivity due to peroxidase activity. Anther ripeness was confirmed by the presence of shed pollen observed using light microscopy. Stigmas were seen to be receptive before anther dehiscence in *D. lujae*, but in synchrony with pollen shed in *D. contrajerva*.

Androecium morphology and development

Stamen development is similar in *D. contrajerva* and *D. lujae* (Fig. 3). Initially (stage 1: bud) staminate flowers are enclosed by a tetrad collar, from which premature anthers then emerge (Fig. 3A, 3B). As the filaments uncurl the anthers are elevated (Fig. 3C; 3D). Unlike other genera in the family Moraceae stamens are not urticaeous (in which the pollen is released from the anthers via a springing mechanism). Rather, the pollen is released when the two theca of the anther dehisce longitudinally (Fig. 3E, 3F). After the pollen is released (stages 2-3), the filaments lose turgidity and the stamens collapse (Fig. 3G, 3H). In *D. contrajerva*, each staminate flower produces one to three stamens. However, in *D. lujae* the stamens are always produced in groups of three. Asynchronous stamen development was observed in *D. lujae*, in which one filament may be fully erect and releasing pollen whilst another in the group is emergent.

The developing anthers are enclosed by a thin layer of perianth tissue at the surface of the coenanthium. In the developing coenanthia (stage 2: early) the anthers were observed attached to the developing, curled filaments (Fig. 4A-C). The filaments have a discreet, central vascular bundle which stained red with Safranin O (Fig. 4D, 4E). Each anther consists of four pollen sacs, in which the pollen grains develop (Fig. 4F, 4G). Pollen morphology is species-specific. A previous study by Hoen and Punt (1989) classified *Dorstenia* pollen into 13 types. *Dorstenia contrajerva* has been assigned its own pollen type, which is distinguished by distinct, angular aspides around the pore (Fig. 4G). This was confirmed in our study. *Dorstenia lujae*, however, produces *D. zenkeri*-type pollen. This pollen type is characterised by circular, sunken pores which are much more numerous than those of *D. contrajerva* and are inaspidate (Fig. 4H). *Dorstenia zenkeri*-type pollen is characteristic of the subdivision Lomatophora (Hoen and Punt 1989), to which *D. zenkeri* and *D. lujae* are both ascribed.

Gynoecium morphology and development

Styles of both species are bipartite. At the bud stage (stage 1: bud), the apices of the developing styles are visible in SEM micrographs, emerging from a raised collar of tissue (Fig. 5A, 5B). The styles then extend until fully emerged (stage 2: early) (Fig. 5C, 5D). In *D. contrajerva* the styles later retract below the surface of the coenanthium as the ovules develop (stage 3: mature) (Fig. 5E), in contrast with *D. lujae* in which they remain fully extended (Fig. 5F), however in the former species they remain attached and visible on mature fruits (stage 4: late) (Fig. 5G). The coenanthium surface of pistillate flowers of both species is densely papillose and numerous trichomes were observed in *D. contrajerva* in particular.

No seed was produced in any specimens of *D. lujae*. Styles emerged early during development (stage 2: early) (Fig. 6A), and remained present on the surface during the course of observations (10 weeks). Clear differentiation within the ovaries was not apparent (Fig. 6B), and in post-anthetic specimens (stage 4: late) internal components disintegrate (Fig. 6C). In *D. contrajerva*, the styles also emerge early during development (stage 2: early) (Fig. 6D) however they then retracted below the receptacle surface following pollination, during seed ripening (Fig. 6E, 6F). At this point (stage 3: mature) there is clear differentiation of the mature ovule into identifiable components of a seed (Fig. 6G). The mature seed ruptures the surface of the coenanthium as it emerges and is dispersed by ballistic autochory.

DISCUSSION

Our results show that the reproductive biology *Dorstenia* is complex and species-specific for the two species studied. Both species are monoecious and produce a bisexual coenanthium of minute unisexual flowers; however distinct differences in floral architecture and seed set were observed. Our results complement recent findings in two poorly known species in Brazil which also showed distinct differences in floral architecture and breeding system (Araújo et al. 2017). Whilst a phylogenetic framework including half of all known *Dorstenia* species now exists, the reproductive biology and ecology of most of the ca. 105 taxa still remain virtually unexplored.

Pollination biology and compatibility

Studies on the pollination mechanisms in the genus *Dorstenia* are scarce. Our study demonstrates that pollen is released by a longitudinal dehiscence of the anther theca. This is not conducive to long distance pollen transfer, therefore it may be expected that a pollination agent could be involved in cross-pollination. Some species of *Dorstenia* are hypothesised to be insect-pollinated based on floral structure and scents (Datwyler and Weiblen, 2004); visual and olfactory cues were not obvious in either of our study species. Interestingly Araújo et al. (2017) recently observed small flies (family Lauxaniidae) visiting the coenanthia of *D. arifolia* which opens up a new line of investigation. Our study specimens were not enclosed from potential pollinators, however no flower visitors were observed during the course of 10 weeks, which is not surprising given that the plants were cultivated as part of ex situ collections under glass, and because pollination may be pollinator species-specific. Furthermore all pollen examined in this study was from the same species (identified by the distinct pollen types), even though the two species were grown in close proximity. This suggests that cross pollination may be rare or absent in cultivated plants. Our specimens were cultivated under controlled conditions in which potential pollinators were scarce; observations using natural populations *in situ* would establish pollination modes with more certainty. It is possible that some species of *Dorstenia* may be dependent on specific pollinator species, such as flies in association with *D. arifolia* (Araújo et al. 2017) and the symbiotic relationships studied in the closely related lineages, Ficeae and Castilleae. Interestingly beetles have been observed visiting some African species of

Dorstenia (Berg and Hijman 1999), however there is no evidence of their role in pollination. We saw no evidence of nectaries or nectar production in our specimens and there seem to be no records of nectar production in the two species in the literature. We repeatedly observed a lack of seed set across all specimens of *D. lujae*, however seed production was observed in *D. contrajerva*. Early work on *D. contrajerva* (De Granville, 1971) identified the absence of seed production following the excision of the styles. More extensive experimental work on the pollen-pistil interactions in these species is now required to build on these initial observations, and to objectively assess their breeding systems.

Fruit Development and Dispersal

Dorstenia fruits have been described as explosive drupes (Judd 2008). The mechanism of seed dispersal in the genus was examined by Schleuss (1958). Increased turgidity in tissue surrounding the developing fruit exerts pressure and ejects the endocarp body, tearing through the surface as it is released from the coenanthium. Our results confirm these observations of autochory (self-dispersal). Indeed numerous seedlings of *D. contrajerva* were observed in the immediate area surrounding our study specimens in the glasshouse. It has been suggested that the distal position of ovaries within the inflorescence may have a function in protecting the developing fruits from phytophagous insects (Berg and Hijman 1999). Our results also build on the recent findings of Araújo et al. (2017) who identified autochory in *Dorstenia*, as well as an unusual form of hydrochory, in which seeds are expelled when the long-stalked, funnel-shaped fruiting head fills with water and then tips to release the seeds.

Insights into fig syconium biology and evolution

The Dorstenieae lineage is sister to the Ficeae to which the fig genus *Ficus* belongs. Understanding the reproductive biology of this little-studied sister lineage may help to build incrementally our understanding of the evolution of the syconium, which is central to the evolution of the unique fig-fig wasp symbiosis (Galil, 1977). This obligate symbiosis is an extreme case of coordinated long-term co-diversification that arose at least 60 million years ago (Rønsted et al. 2005, Cruaud et al. 2012). The syconium comprises an infolded receptacle, apically closed off by numerous bracts which tightly seal an entrance hole - the ostiole (Verkerke, 1989). Fig wasps (Agaonidae: Hymenoptera) transport pollen through the ostiole and simultaneously fertilise some female flowers whilst ovipositing in others within the syconium. Flowers without ovules which receive an egg develop a gall on which the larva feeds; flowers with ovules, if pollinated, produce a seed (Harrison and Yamamura, 2003). The resulting aggregate of fruits composed of individual drupelets is accompanied by a rapid burst in syconium size (Freiman et al. 2014). Emergent wingless male wasps mate with gall-encased females which later emerge and collect pollen. Meanwhile male wasps cut a tunnel through the syconium wall and the females disperse (Harrison and Yamamura, 2003). Whilst numerous studies have explored this unique and fascinating symbiotic relationship between fig and fig wasp (Cook and Rasplus, 2003, Weiblen, 2002), the evolutionary origins of the syconium in fact remain enigmatic. This is in part because the morphology of the syconium is so distinct from other genera (Datwyler and Weiblen, 2004).

Analyses based on DNA sequence data coupled with information on life history and morphology have offered insights into the evolutionary relationships and adaptive significance of complex reproductive structures in the Moraceae (Clement and Weiblen, 2009). It is now hypothesized that whilst the inflorescence of ancestors to the Moraceae, Urticaceae, and Cannabaceae were bisexual cymes, the ancestor of Moraceae appears to have been dioecious, wind-pollinated, and lacked an involucre of bracts (Datwyler and Weiblen 2004, Clement and Weiblen, 2009). Clement and Weiblen (2009) suggest a major reorganization of floral morphology in the ancestral lineage to Dorstenieae, Ficeae, and Castilleae was associated with a shift from unisexual to bisexual inflorescences, from a dioecious to monoecious breeding system, and a reduction in base chromosome number from 14 to 13. Studying the reproductive biology of relatives of the fig could be a means of refining our rudimentary understanding of the evolution of the syconium. Corner (1978) hypothesized that a discoid head, with many external bracts which closed up to conceal the flowers in a primitive syconium, may have been a forerunner to the fig. The pathway from a dioecious, wind-pollinated receptacle to a complex syconium may have been accompanied by an involucre of bracts, a shift in pollination mode, and a structural transition to a fully enclosed structure. Living relatives with features reminiscent of these characteristics exist in the genus *Antiaropsis* (Corner, 1978). It has also been proposed by Misiewicz and Zerega, (2012) that *Dorstenia* may represent an intermediate form in the evolution of a putative open inflorescence and wind pollination in *Morus* (mulberries), to the specialised syconium and obligate pollination mutualism seen in *Ficus*. This hypothesis is corroborated by very recent observations of flies appear to oviposit in the flowers of *Dorstenia arifolia* (Araújo et al. 2017). The coenanthia appeared to be used as a source of food for the larvae and phytophagy of a species of Lauxaniidae. This novel and exciting observation adds weight to earlier hypotheses that oviposition is a shared trait among Moraceae tribes that predates the origin of the fig pollination mutualism (Zerega et al. 2004). Taken together, we propose that investigating the reproductive biology of extant lineages such as *Dorstenia* (Fig. 7A) and *Antiaropsis* (Fig. 7b) may offer valuable insights into the evolutionary pathway of the fig (Fig. 7C).

Investigating the developmental genetics of floral structure in these lineages is a logical next step in understanding the diversification of the Moraceae (Clement and Weiblen, 2009). In recent years, Next Generation Sequencing (NGS) technologies have enabled high throughput sequencing and broad gene expression studies in both model and non-model plants (Ikegami et al. 2013, Freiman et al. 2014). For example transcriptome sequencing has been used to examine four stages of syconium development in *Ficus carica* which provides a platform for further studies in the family Moraceae (Freiman et al. 2014). Recently Mori et al. (2017) used a whole genome sequencing approach to identify a sex determinant candidates in the fig genome. The authors used a restriction site-associated DNA sequencing (RAD-seq) analysis of an F1 population to construct a high-density linkage map based on genome-wide single nucleotide polymorphisms (SNPs). This led to the discovery of a prime candidate gene RESPONSIVE-TO-ANTAGONIST1 (RAN1), which the authors speculate may offer insights into sex determination systems in the genus *Ficus*. Extending the application of sequencing technologies to poorly known lineages will no doubt offer useful insights into the evolution of the fig.

CONCLUSIONS

Little is known about the evolution of the fig but characterising the reproductive biology of *Dorstenia,* and other poorly known fig relatives, will offer important insights into the evolution of the ecologically and economically important family Moraceae. Our results reveal novel insights into the reproductive biology of two such species. To further our understanding, a combined approach using phylogenetic data, NGS data, developmental genetics and characterisation of the reproductive biology across the family Moraceae should now be a priority.

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FIGURE LEGENDS

Table 1: Developmental stages identified in an initial weekly assessment over 14 weeks which were then more intensively sampled for each species for analysis by microscopy at the time points indicated. Specimens from each of these four developmental stages are shown in Fig. 3 and Fig. 5 for both species.

Figure 1 Inflorescence (coenanthium) (A-E) and vegetative (G-I) diversity in the genus *Dorstenia*: (A) *D. contrajerva*, native to Mexico, Central America, the Caribbean and South America; (B) *D. cilliata*, native to Cameroon; (C) *D. arifolia*, native to Brazil; (D) *D. lujae*, native to Africa; (E) *D. bahiensis*, native to Brazil; (F) *D. acangatara* native to Brazil; (G) *D. milaneziana* (syn. *D. gracilis*), a herbaceous perennial native to Brazil; (H) *D. hildebrandtii*, a herbaceous perennial native to tropical Africa; (I) *D. gigas*, a succulent tree native to Socotra Island in Yemen, in cultivation at UC Botanic Garden (Photographs C, E, F and G by Anderson F. P. Machado; photograph H by George Weiblen; photographs B & I by Tracy Misiewicz).

Figure 2 Inflorescence gross morphology. (A) late-phase *D. contrajerva* inflorescence at 10 weeks showing quadrangular receptacle and emergent seeds; (B) late-phase *D. lujae* inflorescence at 10 weeks showing surrounding fringe of vegetative appendages; (C) cross-section through a mature *D. lujae* inflorescence stained with Toluidine Blue at 6 weeks showing relative position of staminate and pistillate flowers at the receptacle surface and embedded within the receptacle respectively. A = Anther, O = Ovary.

Figure 3 Macro- and micro-morphology characterisation of androecium morphology and development in staminate flowers of *D. contrajerva* and *D. lujae* (light micrographs in left-hand panels show gross morphology at each developmental phase and SEMs in right-hand panels show micro-morphology respectively): (A) emergent immature anthers in a tetrad formation in *D. contrajerva* bud at 2 weeks; (B) immature anthers of *D. lujae* surrounded by papillate tissue at 2 weeks; (C) mature, dehiscent anthers in *D. contrajerva* at 6 weeks (D) emergent filaments of *D. lujae* which elevate anthers at 4 weeks; (E-F) fully dehiscent anthers with pollen grains visible in clefts in mature *D. contrajerva* and *D. lujae* specimens (8 and 6 weeks respectively); (G) collapsed stamens of late stage, post-anthetic inflorescence of *D. contrajerva* at 10 weeks; (H) collapsed stamens of late stage, post-anthetic *D. lujae* from which pollen already shed at 8 weeks.

Figure 4 Anther morphology and pollen micromorphology: (A) developing stamens of *D. contrajerva* inflorescence stained with Alcian Green & Safranin O (AGS) at 2 weeks showing curvature of filament; (B) developing stamens of *D. lujae* inflorescence stained with Toluidine Blue at 2 weeks showing curvature of filament; (C) transverse section through developing stamen of *D. lujae* inflorescence at 4 weeks stained with AGS; (D) transverse section through developing stamen of *D. contrajerva* inflorescence at 4 weeks stained with AGS showing intense signal at the vascular bundle; (E) transverse section through developing stamen of *D. lujae* inflorescence at 4 weeks stained with AGS showing intense signal of the vascular bundle; (F) *D. contrajerva* inflorescence at 4 weeks stained with Auramine O & Aniline Blue photographed under UV showing intense signal from developing pollen grains in anther tetrad; (G) SEM of *D. contrajerva* pollen showing sparse pores with prominent aspides; (H) SEM of *D. lujae* pollen showing numerous sunken pores without prominent aspides. A = Anther, P = Perianth, F = Filament, VB = Vascular Bundle, Po = Pollen.

Figure 5 Macro- and micro-morphology characterisation of gynoecium morphology and development in staminate flowers of *D. contrajerva* and *D. lujae* (light micrographs in left-hand panels show gross morphology at each developmental phase and SEMs in right-hand panels show micro-morphology respectively): (A) emergent immature styles surrounded by a papillate collar in *D. contrajerva* bud at 2 weeks; (B) immature styles of *D. lujae* completely enclosed by papillate tissue at 2 weeks; (C) mature, deflexed styles of *D. contrajerva* at 6 weeks (D) emergent (just receptive) styles of *D. lujae* at 4 weeks; (E) papillate collar into which styles are retracted in fertilised flowers of *D. contrajerva* at 8 weeks; (F) fully extended (post-receptive) bipartite styles of D. lujae at 6 weeks; (G) emergent seed with withered styles still attached in late stage, post-anthetic inflorescence of *D. contrajerva* at 10 weeks; (H) fully retracted (post-receptive) style of late stage, post-anthetic *D. lujae* at 8 weeks (note spatially segregated stamens on the left).

Figure 6 Ovary developmental morphology: (A) early *D. lujae* ovary at 4 weeks, with style emerging, stained with Toluidine Blue; (B) ovary from mature *D. lujae* inflorescence at 6 weeks, showing little differentiation, stained with Aniline Blue & Auramine O, viewed under UV; (C) ovary from late *D. lujae* inflorescence at 8 weeks, showing ovule deteriorating, stained with Aniline Blue & Auramine O, viewed under UV; (D) early *D. contrajerva* ovary at 4 weeks, with style emerging, stained with Toluidine Blue; (E) mature *D. contrajerva* ovary at 8 weeks with style retracted below receptacle surface, stained with Toluidine Blue; (F) late *D. contrajerva* ovary stained with AGS at 10 weeks with fully developed ovary; (G) mature *D. contrajerva* ovary at 8 weeks stained with Aniline Blue & Auramine O viewed under UV, showing developing embryo sac and surrounding structures. E = Endocarp; ES = Embryo Sac; N = Nucellus; M = Mesocarp; O = Ovary; P = Perianth; S = Style.

Figure 7 Reproductive structures (cross section above, gross structure below) of extant Moraceae lineages which may offer insights into the evolutionary pathway of the fig, from a putative flat, bractless receptacle, to a syconium fully enclosed by bracts: (A) flat discoid receptacle with flowers fully exposed on the upper surface (*Dorstenia*) (B) urn-shaped receptacle with flowers on the upper surface partially infolded by numerous bracts (*Antiaropsis*) (C) fully infolded receptacle with internal flowers tightly enclosed by a series of apical bracts (*ostiole*, black arrow), called a syconium (*Ficus*). Diagrams not to scale.