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Systematics and Floral Evolution in the Plant Genus Garcinia (Clusiaceae)

Patrick Wayne Sweeney
University of Missouri-St. Louis

SYSTEMATICS AND FLORAL EVOLUTION IN THE PLANT GENUS GARCINIA (CLUSIACEAE)

by

PATRICK WAYNE SWEENEY
M.S. Botany, University of Georgia, 1999
B.S. Biology, Georgia Southern University, 1994

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Advisory Committee

Elizabeth A. Kellogg, Ph.D.

Peter F. Stevens, Ph.D.

P. Mick Richardson, Ph.D.

Barbara A. Schaal, Ph.D.

Dissertation Abstract

The pantropical genus *Garcinia* (Clusiaceae), a group comprised of more than 250 species of dioecious trees and shrubs, is a common component of lowland tropical forests and is best known by the highly prized fruit of mangosteen (*G. mangostana* L.). The genus exhibits as extreme a diversity of floral form as is found anywhere in angiosperms and there are many unresolved taxonomic issues surrounding the genus.

To understand patterns of floral evolution within the group and to evaluate morphology-based classification schemes involving *Garcinia* and its relatives, relationships among a broad sample of *Garcinia* and close relatives were inferred by conducting Bayesian, parsimony, and likelihood analyses of 70 species using sequence data from two nuclear genes, granule-bound starch synthase I (GBSSI) and the nuclear ribosomal internal transcribed spacers (ITS). The phylogenies suggest that all species of *Garcinia* fall into two major lineages, one characterized by the occurrence of nectariferous floral structures of uncertain derivation such as antesepalous appendages and intrastaminal disks and rings, and the other by their absence. Several additional clades are supported each sharing particular combinations of floral characters (some being synapomorphies), and which generally correspond to sections recognized in the most recent taxonomic treatment of the genus. Additionally these results support a broad circumscription of *Garcinia* to include the segregate genera *Ochrocarpos*, *Pentaphalangium*, *Rheedia*, and *Tripetalum*. The monophyly of tribe Garcinieae is also supported.

The nectariferous floral structures that characterize one of the major lineages identified in the molecular phylogenetic analyses have been hypothesized to represent an outer whorl

of stamens. Similar structures are also found among other Clusiaceae and closely related families, and evidence from some species representing these groups supports that these structures represent an outer staminal whorl. However, the position of these structures in mature *Garcinia* flowers does not support the current hypothesis that they represent an outer whorl of stamens. To better understand the nature of the appendages, disks, and rings in *Garcinia*, floral development and anatomy were studied in a sample of six *Garcinia* species. An outer whorl, staminodal origin for the disks and appendages is not supported by timing of development or position. Disks and appendages are not apparent until late in development and the disks arise in the center of flower. Anatomical data is equivocal, disks are supplied by traces that arise from the vascular stele and appendages receive traces from the floral stele and from stamen trunk bundles. These data also reject a gynoecial origin for these structures, and suggest that they are intrastaminal receptacular nectaries. Other notable features of floral development include open carpel development and interspecific differences in floral developmental morphology being evident ab initio.

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Chapter 1

Phylogenetic relationships of *Garcinia* (Clusiaceae) and relatives with an emphasis on understanding patterns of floral evolution.

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Molecular Phylogenetics and Evolution

Patrick W. Sweeney

Abstract

Despite renewed interest in understanding the evolution of floral diversity, many groups with particularly diverse floral morphology have not yet been examined within a comparative phylogenetic framework. One such group is the pantropical genus *Garcinia*, a group comprised of more than 250 species of dioecious trees and shrubs that are a common component of lowland tropical forests. To understand patterns of floral evolution within the group and to evaluate morphology-based classification schemes involving *Garcinia* and its relatives, relationships among a broad sample of *Garcinia* and close relatives were inferred by conducting Bayesian, parsimony, and likelihood analyses of 70 species using sequence data from two nuclear genes, granule-bound starch synthase and the internal transcribed spacer. The phylogenies suggest that all species of *Garcinia* fall into two major lineages, one of which is characterized by the occurrence of floral organs of uncertain derivation such as central disks, antesepalous lobes, and intrastaminal ring-shaped disks, and the other by their absence. Several clades are supported each

sharing particular combinations of floral characters (some being synapomorphies), and which generally correspond to sections recognized in the most recent treatment of the genus. These results support the monophyly of tribe Garcinieae and a broad circumscription of *Garcinia*.

Key words: Clusiaceae; floral evolution; *Garcinia*; GBSSI; ITS; phylogeny

1. Introduction

The evolution of floral form has long interested biologists and recently great progress has been made towards our understanding of the evolution of floral diversity that has been spurred in part by the development of the fields of molecular phylogenetic systematics and of evolutionary developmental biology (Endress, 1994, 2006; Smyth, 2005). While comparative phylogenetic studies of floral evolution are accumulating for many clades (e.g., Jaramillo et al., 2004; McMahon and Hufford, 2005; Ronse De Craene et al., 2003; Endress and Matthews, 2006), they are lacking for many other groups across the angiosperm phylogeny, some of which exhibit striking floral variation. In this study I begin to unravel the evolution of floral form in an understudied, species rich, tropical tree genus, *Garcinia* L. (Clusiaceae, Malpighiales), by providing the first comprehensive higher-level phylogeny for the group and by examining patterns of floral variation within the resulting phylogenetic framework.

Garcinia comprises more than 250 species of dioecious, small shrubs to medium-sized trees that are a common component of lowland tropical forests. The genus exhibits several features that are of general interest to biologists. In many areas, particularly in

Madagascar and South East Asia where it has centers of diversity, the genus is notable for its high levels of sympatric species diversity (Ashton, 1988; Lee et al., 2002; Thomas et al., 2003; Whitmore, 1998), and this diversity is especially notable considering that species that are both dioecious and agamospermous may be widespread in the genus (Allem, 2004; Ashton, 1988; Richards, 1990a; Thomas, 1997). From an economic standpoint, *Garcinia* is probably best known as the highly prized fruit of mangosteen (*G. mangostana* L.), a tree native to southeast Asia; moreover, mangosteen and other species (e.g., *G. gummi-gutta* (L.) N. Robson or "*G. cambogia*") have become the focus of pharmacological studies (Heymsfield et al., 1998; Ho et al., 2002; Mackeen et al., 2000) and a large natural supplement industry has formed around these species. Finally, *Garcinia* and its close relatives exhibit some of the most extreme diversity of floral form, particularly in the androecium, as is found anywhere in angiosperms (Leins and Erbar, 1991).

While many species of *Garcinia* have four free sepals and four free petals (e.g., Fig. 1, A, B, C, D, E, J, L, N, O, P), others have two, three, or five or more (Fig. 1, F, G, I, K) perianth parts per whorl, and in some the sepals can be completely fused to each other in bud (Fig. 1, H). The stamens (or staminodes in pistillate flowers) vary in number (e.g., Fig. 1, L, M), in whether these organs are clustered into groups (i.e., fasciculate) and fused together (i.e., phalangiate - Fig. 1, E, F, G, H, K, L, M, O or as a ring - Fig. 1, C, D) or distributed evenly (i.e., non-fasciculate) and free (i.e. non-phalangiate - Fig. 1, A, B, P, Q), in degree of fusion to each other when clustered (e.g., Fig. 1, L, M, O), and in degree of fusion to the petals. The anthers vary in the shape of the thecae, number of loculi (thecae) per anther, and whether locelli are present or absent. Pistillodes may be

present (Fig. 1, C, D, E, F, H) or absent (Fig. 1, A, B, K, L, O, P, Q). In pistillate flowers, style branches can be present (Fig. 1, J) or absent (Fig. 1, I, N) and the surface ornamentation of the stigma is very diverse (Fig. 1, I, J, N). Some groups have additional disk-, lobe-, or ring-like structures in the flowers (Fig. 1, K, L, N, O, P, Q), called fasciclodes (Robson, 1972), that have been described as nectaries (Leins and Erbar, 1991) and that have been variously interpreted as sterile reproductive organs (Robson, 1972; Jones, 1980) or as of receptacular origin (Leins and Erbar, 1991; Pierre, 1883).

Despite haveing this remarkable floral diversity and many other attributes of evolutionary, ecological, and economic significance, important basic information about the genus is lacking, especially in regards to its phylogeny. While recent phylogenetic studies are beginning to provide insights into the pattern of morphological evolution within Clusiaceae, as well as allowing for a re-evaluation of previous classifications in the family, relationships among *Garcinia* and its close relatives, which represent up to a quarter of the species in the family, are largely unknown.

The diversity of floral form in *Garcinia* led to earlier workers relying on floral characters when delimiting the genus and in constructing infrageneric classifications. Based largely on floral morphology, several genera have been segregated from *Garcinia* (e.g., Planchon and Triana, 1860; Bentham, 1862; Engler, 1893, 1925; Vesque, 1893; Perrier de la Bâthie, 1948, 1951). These include *Ochrocarpos* Thours (two sepals fused in bud vs. four or more free sepals in *Garcinia* — Fig. 1, H), *Pentaphalangium* Warb. (five-merous, staminal phalanges adnate to petals vs. four-merous, phalanges free — Fig. 1, F), *Rheedia* L. (two sepals vs. four — Fig. 1, N, P), and *Tripetalum* Schumann (three-merous and staminal phalanges adnate to petals vs. four-merous and phalanges free). Despite

these morphological differences, many have argued that these genera should be united with *Garcinia* (*Rheedia* – Robson, 1958; Adams, 1970; Jones, 1980; *Tripetalum* – Jones, 1980; Turner and Stevens, 1999; *Pentaphalangium* – Kostermans, 1976; Jones, 1980; *Ochrocarpos* pro parte – Jones, 1980; Stevens, 2005, 2006). All these genera share with *Garcinia* baccate fruits with a single ovule per locule (Stevens, 2006).

The first major treatment of *Garcinia* itself (but excluding *Rheedia* and *Ochrocarpos*) is that of Planchon and Triana (1860) who used mostly characters of the androecium and pistillode in staminate flowers and of the stigma and style in pistillate flowers to recognize six sections. Pierre (1883) first monographed *Garcinia* (again, excluding *Rheedia* and *Ochrocarpos*), splitting the genus into 37 sections that were placed into six groups. Pierre's (1883) sections were circumscribed using largely flower and inflorescence characters and his groups were circumscribed using anther characters in particular. Engler (1893; 1925) based his treatment of the genus on the work of Pierre (1883), recognizing basically the same sections but grouping them differently in his key to sections. The latest monograph of *Garcinia* was provided by Vesque (1893) who treated 180 species (excluding *Rheedia*) and recognized three subgenera (based on characters of floral morphology and leaf stomata) and nine sections (based on floral morphology). Much of the data used by Vesque (1893) was drawn from his ambitious *Epharmosis* (Vesque, 1889), which presented the results of morphological and anatomical investigations of 118 species of *Garcinia* (including *Ochrocarpos* pro parte) and *Rheedia*. The most recent world-wide sectional treatment of *Garcinia* was provided by Jones (1980) in an unpublished Ph.D. thesis. Jones's (1980) treatment is influenced heavily by that of Pierre (1883) and Engler (1925) and differs most by her uniting many

of their sections to end up with only 14 sections; she relied heavily on the morphology of staminate flowers and of pollen. Two of Jones's (1980) sections correspond to the genera *Rheedia* and *Tripetalum*; she was the first to treat these as sections of *Garcinia*. She made the appropriate combinations, but they only appear in her Ph.D. thesis and are not validly published. Jones (1980) proposed a hypothetical scheme of relationships for the entire genus that was based in part on assumptions about morphological trends observed in the flowers and pollen and did not incorporate a formal cladistic analysis.

The placement of *Garcinia* into subfamily Clusioideae (Stevens, 2006) along with *Symphonia* and its relatives (the tribe Symphonieae) and *Clusia* and its relatives (the tribe Clusieae) is well supported in molecular phylogenetic studies (Bittrich et al., 2005; Gustafsson et al., 2002). Within Clusioideae, molecular phylogenetic studies (Bittrich et al., 2005; Gustafsson et al., 2002) find support for a monophyletic Clusieae sister to a strongly supported clade containing the tribes Symphonieae and Garcinieae, the latter containing *Garcinia*, its segregate genera, and the African endemic *Allanblackia*. While the above studies find strong support for a Symphonieae/Garcinieae clade, relationships within this clade are generally unresolved and the monophyly of Garcinieae and *Garcinia*, whether broadly or narrowly circumscribed, has not been demonstrated (Bittrich et al., 2005; Gustafsson et al., 2002).

Within *Garcinia*, two unpublished (Nazre, 1999; Sari, 2000) and one published (Yapwattanaphun et al., 2004) study, all with a largely southeast Asian focus, have examined evolutionary relationships among species of *Garcinia* and relatives. Nazre (1999) utilizing ITS and *trnL-F*, and Yapwattanaphun et al. (2004), utilizing ITS, both focused on the relationship between mangosteen and its putative close relatives and thus

had limited sampling. The study of Sari (2000), also utilizing ITS, included several species of *Garcinia* sensu stricto and representatives of *Pentaphalangium*, *Rheedia*, and *Tripetalum*, but overall the sampled species still represented only a portion of the floral diversity within the genus, only parts of its biogeographic range, and did not sample species from four of Jones's (1980) sections.

To establish a comparative phylogenetic framework within which interesting evolutionary and ecological attributes of *Garcinia* can be further explored, I conduct a comprehensive phylogenetic analysis to examine higher-level relationships within *Garcinia* and between this genus and its close relatives. Analyses use two nuclear genes, the internal transcribed spacer (ITS) of nuclear ribosomal DNA and granule-bound starch synthase (GBSSI or *waxy*), and include a morphologically, taxonomically, and biogeographically representative sample of *Garcinia*. Patterns of floral evolution are explored and unsettled taxonomic issues that surround the group are examined, with the specific objectives of this study being 1) to evaluate the monophyly of Garcinieae (sensu Stevens, 2006), 2) to determine if there is support for an expanded concept of *Garcinia* (i.e., *Garcinia* sensu lato), including *Ochrocarpos*, *Pentaphalangium*, *Rheedia*, and *Tripetalum*, 3) to evaluate previous infrageneric classifications of *Garcinia*, focusing in particular on that of Jones (1980) and on the phylogenetic utility of characters previously used to circumscribe major groups within the genus, and 4) to examine the floral diversity of *Garcinia* and its close relatives within a phylogenetic framework.

2. Materials and methods

2.1 Taxon sampling

Fifty-nine species were sampled that encompassed the morphological variation within *Garcinia* s.l., all major biogeographic areas in which *Garcinia* occurs, and that included representatives of the segregate genera *Ochrocarpos*, *Pentaphalangium*, *Rheedia*, and *Tripetalum*. All sections recognized by Jones (1980) were sampled and an attempt was made to sample the range of floral morphological variation within each section. To evaluate the monophyly of Garcinieae and *Garcinia*, representatives of *Allanblackia* and of five of the seven genera of Symphonieae were included. Representatives of *Clusia* and *Tovomita* were used as outgroups. Voucher specimen data, GenBank accession numbers, taxonomic authorities, and sectional placement (sensu Jones, 1980) for all sampled taxa are provided in the Appendix.

2.2 DNA sequencing

After preliminary studies evaluating the phylogenetic utility of various chloroplast and nuclear markers, sequencing efforts focused on two nuclear genes, ITS and GBSSI. Preliminary examination of several chloroplast markers commonly used in phylogenetic studies (*trnL-F*, *ndhF*, *psbA-trnH*) revealed that they provided little resolution, due to low variation or high homoplasy, among species within the Garcinieae/Symphonieae clade and thus would not be useful for elucidating relationships within this clade.

Genomic DNA was extracted from silica-gel dried material or from material preserved in a salt-saturated CTAB solution following the protocols of Doyle and Doyle

(1987), Lodhi et al. (1994), and Murray and Thompson (1980). The entire ITS region (i.e., ITS1, 5.8S, ITS2) was amplified using primers ITSLEU1 (Malcomber, 2002) and ITS4 (White, 1990). PCR products were sequenced directly or were cloned as described in Malcomber (2002). Sequencing reactions used primers ITSLEU1 and ITS4 (for direct products), ITS2 (White, 1990) and ITS3B (Baum et al., 1994), and SP6 and T7 (for plasmids). Between one and six inserts were sequenced per each cloned accession.

The amplified region of GBSSI spanned from the 3' end of exon three to the 5' end of exon six. This region was amplified using two novel primers, EXON3-F (5'-TAYAA AMGWG GRGTT GATCG-3') and EXON6-R (5'-GCCAR TCRTT GGCAA YGAAG-3') that were designed from the consensus of GBSSI sequences (downloaded from GenBank) of *Manihot esculenta* (accession number X74160), *Arabidopis thaliana* (AY123983), and *Solanum tuberosum* (X83220). Amplifications were conducted using a modified "Stepdown" procedure (Hecker and Roux, 1996). All GBSSI products were cloned following the procedures outlined above. GBSSI sequencing reactions used the amplification primers (EXON3-F & EXON6-R), the plasmid primers SP6 and T7, and the novel primers EXON4-F (5'-TSCGA TTYAG YTTGY TBTGC-3'), EXON5-R (5'-CCAMA CCATA TGGRC CASAG-3').

Initial exploratory GBSSI PCR reactions of a broad sample of ingroup taxa consistently produced two distinct bands per plant accession with sizes differing approximately 150 to 400 bp within an accession. Among Symphonieae and outgroup taxa, only one band was apparent. For each accession, the resulting bands (or band in the case of Symphonieae) were excised, cloned, and sequenced. The exon sequences of all bands were used as a query in a BLAST (Altschul et al., 1990) search against GenBank

and were verified as GBSSI with Expect values (E) of less than 9e-25, suggesting that two distinct copies of GBSSI were present. To test orthology/paralogy relationships, all of the GBSSI sequences were aligned as a single dataset and analyzed via maximum parsimony, using a sequence of *Manihot esculenta* (Euphorbiaceae, Malphigiales) downloaded from GenBank (X74160) as an outgroup. A MP bootstrap analysis produced two strongly supported (PB ≥ 80%) monophyletic groups one of which contained all of the larger copies ("copy A") from Garcinieae taxa and the other all of the smaller copies ("copy B"). Sequences of clones coming from a single band of PCR products from *Montrouziera sphaeroidea* and *Pentadesma butyracea* had two distinct GBSSI sequences, with one sequence grouping with Copy A and the other with Copy B. Topologies produced by each copy were congruent with each other. Each copy thus represents a set of orthologous sequences. Only one distinct GBSSI sequence was obtained from PCR clones of *Clusia flava*, *Moronobea coccinea*, and *Symphonia globulifera* and these fell within the Copy B clade. I focus on copy B alone for the remainder of the study.

For ITS and GBSSI direct PCR and cloned products were fluorescence-labeled using the Big Dye terminator cycle sequencing protocol (Applied Biosystems, Foster City, CA) and both strands were sequenced (>70% overlap) at the University of Missouri-St. Louis on either an ABI 377 or an ABI 3100 automated sequencer (Applied Biosystems) or at Macrogen Inc. (Seoul, South Korea) on either an ABI 3700 or an ABI 3730XL.

ITS sequences of *Symphonia globulifera* (AF479787), *S. urophylla* (Decne. ex Planch. and Triana) Vesque (AF479788), and the outgroups, *Clusia rosea* Jacq.

(AJ509203), *C. uvitana* Pittier (AJ509226), and *Tovomita weddelliana* Planch. & Triana (AJ509218), were downloaded from GenBank.

2.3 Phylogenetic analyses

Trace editing and contig assembly were conducted following methods described in Sweeney et al. (2004). Phylogenetic analyses employed maximum parsimony (MP), maximum likelihood (ML), and Bayesian (BI) methods. Models of evolution for the ML and Bayesian analyses were selected using MrModeltest version 2.2 (Nylander, 2004) and were those chosen by the Akaike information criterion (AIC). For all analyses characters were weighted equally and indels were coded as missing data. All analyses were conducted on the University of Missouri-Saint Louis Beowulf Cluster (http://www.umsl.edu/technology/hpcc/). To find the shortest parsimony tree, the Parsimony Ratchet (Nixon, 1999) was implemented in PAUP* version 4.0b10 for Unix (Swofford, 2001) using the setup and batch files generated in PAUPRat (Sikes and Lewis, 2001) and following the methodology outlined in Sikes and Lewis (2001). Likelihood analyses were conducted in PAUP* [random sequence addition (nreps=10), TBR swapping, multrees on]. Bayesian analyses [two independent runs of four chains, 10 000 000 generations, tree sampling every 1000 generations] were implemented with MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) using a parallel algorithm for Metropolis-coupled Markov Chain Monte Carlo (Altekar, 2004). Burn-in for each run was determined by plotting the log-likelihood against the number of generations. Trees whose parameters had not reached stationarity were discarded. To evaluate if runs had

proceeded an adequate number of generations, the average standard deviations of split frequencies were examined.

To obtain measures of statistical support for individual branches, 1000 MP [random sequence addition (nreps=10), TBR swapping, multrees off] and 100 ML [random sequence addition (nreps=10), TBR swapping, multrees off] bootstrap replicates were run using PAUP*. Bayesian posterior probabilities were obtained for each data set by generating the majority rule consensus tree of the sampled trees (burn-in trees excluded).

To create an ITS dataset that was comparable to GBSSI (and vice versa) for combined ITS/GBSSI analyses, the full ITS and GBSSI data sets were pruned, keeping the least derived (in the ML tree) clone from the same accession and keeping only those accessions common to both datasets. The pruned datasets had 50 terminals.

It should be noted that MP, ML, and BI have different theoretical underpinnings and assumptions, and each has its own particular strengths and weaknesses (Holder and Lewis, 2003; Felsenstein, 2004). Instead of relying on one method, results from all three are presented herein, as they all have the potential to provide information about the underlying organismal phylogeny. Additionally there are issues surrounding the use of bootstrap percentages and Bayesian posterior probabilities to evaluate clade support and the relationship between values that are obtained by these different methods is not well understood (Suzuki et al., 2002; Kolaczkowski and Thornton, 2004; Simmons et al., 2004, 2006; Lewis et al., 2005). Both types of support values are reported in this paper; however, they should not be interpreted as equivalent.

2.4 Tests of topological congruence and alternative hypotheses

For the individual ITS and GBSSI data sets**,** a Shimodaria-Hasegawa (S-H) test (Shimodaira and Hasegawa, 1999; Shimodaira, 2002) was employed to determine if the topologies produced by one data set were significantly different from phylogenetic hypothesis suggested by the other data set (cf., Doust and Drinnan, 2004) and to test whether certain taxonomic groupings (tribes, segregate genera, and sections) recognized by morphology are significantly different from the topologies suggested by the molecular data sets (Goldman et al., 2000). Additionally, the combined data set was used in an S-H test to evaluate the validity of the taxonomic groupings and to evaluate Jones's (1980) scheme of relationships. The S-H tests were performed with PAUP* using log-likelihood (RELL) optimizations and 1000 bootstrap replicates.

2.5 Morphological character reconstructions

Ancestral character states were reconstructed on ML phylogenies for selected morphological characters that appeared to be possible synapomorphies for major clades recognized by the molecular phylogenies. Most character states were determined by direct observation of specimens; however, some were taken from the literature (Pierre, 1883; Vesque, 1889; Jones, 1980). Characters were mapped onto ML phylogenies resulting from unconstrained and constrained analyses of the combined data set. Characters were treated as multistate and unordered and were mapped on the phylogenies

using Mesquite version 1.12 (Maddison and Maddison, 2006), under parsimony and maximum likelihood (i.e., Markov k-state one parameter) models.

3. Results

3.1 ITS

The full ITS dataset had 122 terminals representing 73 accessions and had an aligned length of 775 base pairs (bp). The parsimony ratchet analysis yielded 3718 trees of 1607 steps (consistency index (CI) excluding uninformative characters of 0.424 and a retention index (RI) of 0.846). The model selected by MrModeltest (by AIC) and used in the likelihood and Bayesian analyses was SYM+I+G. The 50% majority rule consensus tree of the trees sampled during the Bayesian analysis is shown in Figure 2 with Bayesian posterior probabilities and maximum parsimony and likelihood bootstrap replicate percentages presented. Across the phylogeny, an S-H test could not reject that multiple clones from single accessions were monophyletic (P = 0.19), and in most cases clones coalesced within individuals or species. However at a shallow level among some apparently closely related species, clones from different accessions were interspersed with each other (Fig. 2, Clades 2 and 8). These clones always coalesced at a shallow level in the phylogeny (i.e., within clades of closely related species), suggesting ITS is useful for examining relationships at deeper levels of the *Garcinia* phylogeny — the level at which this study focuses.

All methods of analysis gave trees with congruent results. The Bayesian (BI) and maximum likelihood (ML) analyses provided statistical support for the greatest number of clades, whereas maximum parsimony (MP) trees were less resolved by having only a subset of the clades present in the ML and BI analyses. In all analyses, species representing the tribes Symphonieae and Garcinieae together form a monophyletic group, with Bayesian posterior probability (PP) equal 1.0, and likelihood bootstrap support (LB) and parsimony bootstrap support (PB) equal to 100%. BI and ML analyses support (PP = 1.0 & LB = 74%) a monophyletic Garcinieae, but monophyly of Symphonieae is neither supported nor rejected (Fig. 2).

The BI and ML analyses group Garcinieae into two major clades. One clade (PP = 0.99; LB = 81%; also supported by MP, PB = 85%), designated Lineage A, contains mostly African and South American species of *Garcinia*, all sampled *Rheedia*, and most sampled species of sections (sensu Jones, 1980) *Rheedia*, *Rheediopsis* Pierre, *Teracentrum* Pierre, *Tetraphalangium* Engl., and *Xanthochymus* (Roxb.) Pierre (Fig. 2). In the Bayesian analysis, *Allanblackia floribunda* is supported as sister to Lineage A (PP = 0.99) but its position is unresolved (in the base of the Garcinieae clade) in the ML and MP analyses. Two major clades within Lineage A are supported. The BI analysis places (PP = 0.98) most of the sampled representatives of section *Xanthochymus* into a monophyletic group, designated Clade 1 (Fig. 2). In the ML and MP analyses, two African representatives of section *Xanthochymus*, *G. kola* and *G. lucida*, are unresolved at the base of Lineage A. One species of section *Xanthochymus*, *G. prainiana*, was apparently misclassified by Jones (1980); it falls within Lineage B (see below). All analyses (1.0/100/100, PP/PB/LB) support a group containing species representing

sections *Rheedia*, *Rheediopsis*, and *Teracentrum*, designated Clade 2 (Fig. 2). *G. ovalifolia* and *G. staudtii* of section *Rheediopsis* are supported as monophyletic (1.0/85/96), but the position of a third species from this section, *G. smeathmannii*, is unresolved at the base of the Clade 2. All analyses show that *Rheedia* is paraphyletic with the South American species forming a clade (1.0/100/100) that is sister (1.0/100/100) to a clade (1.0/99/98) containing the sampled Malagasy *Rheedia* and *G. livingstonei* (section *Teracentrum*).

The second major clade within Garcinieae (PP = 1.0 & LB = 82%), designated Lineage B (Fig. 2), contains most of the sampled Asian species and some African and Malagasy taxa. This clade contains species from the segregate genera *Ochrocarpos*, *Pentaphalangium*, and *Tripetalum*, and all sampled representatives of sections *Brindonia* (Thouars) Choisy, *Discostigma* (Hassk.) Hook. f., *Garcinia*, *Hebradendron* (Graham) Planch. & Triana, *Mungotia* Pierre, *Macrostigma* Pierre, *Paragarcinia* (Baillon) Vesque, *Tagmanthera* Pierre, and *Tripetalum*. Within Lineage B, all analyses supported the same basic relationship of seven strongly supported monophyletic groups. Six of these groups largely correspond to sections *Paragarcinia* (Clade 3; 1.0/100/100), *Discostigma* (Clade 4; 1.0/100/100), *Brindonia* (Clade 5; 1.0/88/99), *Garcinia* (Clade 6; 1.0/100/100), *Hebradendron* (Clade 7; 1.0/100/99), and *Tagmanthera* (Clade 8; 1.0/100/100). Clade 3 also contains all of the sampled species of the segregate genus *Ochrocarpos*. In the BI and ML analyses support (PP = 1.0 & LB = 78%) was obtained for the grouping of Clades 5, 6, and 7. The relationships among these three clades are unresolved. Clade 5 contains a strongly supported clade (1.0/94/97) containing the two Malagasy species, *G. asterandra* and *G. chapelieri*, the latter placed in section *Garcinia* and the former not

treated in Jones (1980). Within Clade 6, *G. mangostana* groups (1.0/99/93) with *G. malaccensis*, and this clade is sister to a clade (PP = 1.0 & LB = 79%) containing the remaining representatives of section *Garcinia* (excepting *G. chapelieri*). The final major strongly supported (1.0/87/86) monophyletic group (Clade 9) of Lineage B contains an assemblage of species representing sections *Macrostigma* (*Pentaphalagium latissimum*), *Tripetalum* (*Tripetalum cymosum*), *Mungotia* (*G. amplexicaulis*), *Xanothchymus* (*G. prainiana*), and *Discostigma* (*G. warrenii*). Within this group, *G. prainiana* is the first branching lineage (0.99/87/86); *G. warrenii* and *G. amplexicaulis* are strongly supported (1.0/98/96) as sisters as are *Pentaphalangium latissimum* and *Tripetalum cymosum* (0.99/85/83).

The topologies supported by the MP, ML, and BI analyses of the pruned ITS dataset were congruent with those produced by the full ITS dataset and all clades present in these trees were also present in trees resulting from analyses of the full dataset. As above for the full dataset, MrModeltest chose the SYM+I+G model for use in the Bayesian and ML analyses

3.2 GBSSI

Initial analyses of the GBSSI data set included sequences of all clones and included 92 sequences. An S-H test could not reject clones from the same accession as being monophyletic and the data set was pruned to create a data set comparable to ITS (see Materials and Methods). The pruned data set contained 50 terminals and had an aligned length of 1142 bp. Sequences of *Garcinia* and its close relatives (members of

Ochrocarpos, *Pentaphalangium*, *Rheedia*, and *Tripetalum*) were from 591 to 648 bp in length while the Symphonieae sequences ranged from 490 to 979 bp in length. The outgroup sequence, *Clusia flava*, was 1070 bp in length. The size discrepancy between Garcinieae, Symphonieae, and *Clusia* sequences was due largely to the presence of two large indels in intron 4. One was a 214 bp (in final alignment) indel in *Pentadesma butyracea* and the other was a variable length indel of up to 481 bp (in final alignment) that was represented as a gap in all taxa except *Clusia flava*. The parsimony ratchet analyses yielded 4019 trees of 803 steps in length (CI excluding uninformative characters of 0.622 and RI of 0.868). The model selected by MrModeltest and used in the likelihood and Bayesian analyses was HKY+G. The MP, ML, and BI analyses of the GBSSI dataset yield topologies that were congruent with each other (trees not shown).

Analyses of the GBSSI dataset yielded topologies that were congruent with the ITS dataset (see below); however, they were less resolved (trees not shown). Like the ITS analyses, the GBSSI results show that all sampled representatives of Garcinieae form a monophyletic group (1.0/100/94). Within Garcinieae, 9 major clades were supported as monophyletic that were also present in the ITS analyses. These clades were Clade 1 (0.99/71/70), Clade 2 (1.0/99/100), Clade 3 (1.0/100/100), Clade 4 (1.0/100/99), Clade 5 (0.99/84/84), Clade 6 (1.0/97/98), Clade 7 (1.0/99/99), Clade 8 (1.0/100/99), and Clade 9 (1.0/95/98). Similar to the ITS results, Clades 5 and 6 were grouped together but with stronger support (1.0/94/95).

The two major *Garcinia* lineages found in the ITS analyses (Lineages A & B) were not resolved in the GBSSI analyses. In two areas, the GBSSI topologies resolved clades that were unresolved in the ITS topologies. The GBSSI analyses provided more

resolution among the representatives of Symphonieae, which is shown to be paraphyletic with Garcinieae embedded within it. Unlike the ITS trees which did not resolve the position of *G. smeathmannii* (Clade 2), the BI analysis of GBSSI data suggest that section *Rheediopsis* is paraphyletic with two representatives of the section (*G. ovalifolia* and *G. staudtii*) in a clade (PP = 1.0) that is sister to a well supported (PP =0.97) and largely unresolved clade that contains *G. smeathmannii* and representatives of sections "*Rheedia*" and *Teracentrum*.

3.3 Combined analyses

According to an S-H test, the ML tree produced by an unconstrained analysis of the ITS dataset was not statistically different from that produced by an analysis constraining the dataset with the 75% MP bootstrap tree for the GBSSI dataset. Similarly the ML trees of the GBSSI dataset produced by unconstrained and constrained (by ITS 75% MP BS topology) analyses also were not significantly different. Thus the topologies obtained from analysis of the pruned ITS and GBSSI datasets are congruent, so the datasets were combined.

It was not possible to obtain GBSSI sequences for *Allanblackia floribunda* and *Pentaphalangium latissimum*. Nevertheless, because of their importance in evaluating certain taxonomic hypotheses and in understanding patterns of floral variation, they were included in the combined analysis. In the GBSSI portion of the data matrix their sequence was treated as missing data. The combined data sets had an aligned length of 1937 bp and included 52 accessions. The parsimony ratchet analysis yielded 3899 trees of 1867 steps

in length (CI excluding uninformative characters of 0.584 and a RI of 0.716). The Bayesian analysis implemented a mixed model, utilizing the appropriate models for each gene as indicated above (i.e., SYM+I+G for ITS and HKY+G for GBSSI). Because only one model can be specified in PAUP* when running ML analyses, MrModeltest was used to chose the GTR+I+G model for use in ML analyses of the combined dataset.

The topologies produced by the MP, ML, and BI analyses were congruent, with the BI and ML topologies being slightly more resolved than those produced by the MP analysis. All major clades found in the ITS or GBSSI analyses were also recovered in the combined analysis, and in general, posterior probability values and bootstrap percentages found in the combined analysis were comparable to those found in the independent analyses (Fig. 3). In all analyses Garcinieae is again supported as monophyletic (1.0/72/95) and is embedded in a paraphyletic Symphonieae. The two major lineages supported in the ITS analysis (i.e., Lineage A and Lineage B) are also supported (1.0/-/71 & 1.0/64/92, respectively). Within Lineages A and B the same major clades are supported as were found in the ITS analyses, and the same clade designations are used (i.e., Clades 1 - 9). Relative to the ITS analyses support for the paraphyly of section *Rheediopsis* increased, with MP and ML support for the sister relationship between *G. smeathmannii* and the *Rheedia*/*Teracentrum* clade increasing to levels of strong to moderate support (PP = 0.99 & LB = 76).

3.4 S-H tests and taxonomic and evolutionary hypotheses

For the individual and combined ITS/GBSSI data sets, the results of the portions of the S-H tests that evaluated topologies based on taxonomic hypotheses were similar. The results of the test utilizing the combined data set are presented in Table 1. For the ITS and combined data sets, topologies in which the segregate genera *Ochrocarpos*, *Rheedia*, and *Tripetalum* were forced to be monophyletic and outside of a monophyletic group containing the remaining *Garcinia* were significantly less likely ($P < 0.05$) than the most likely tree (Table 1). Additionally, the S-H test utilizing the ITS data set found that a topology supporting *Pentaphalangium* as distinct from *Garcinia* was significantly less likely ($P < 0.05$) than the best tree although the combined data set could not reject this possibility ($P < 0.109$). Because of poor resolution in the base of the GBSSI tree, none of the topologies supporting the segregate genera was statistically different from the unconstrained tree. For all data sets, topologies that enforced Garcinieae, Symphonieae, and each of Jones's (1980) sections individually to be monophyletic were not statistically different from the most likely tree. The tree resulting from an ML analysis constrained by Jones's (1980) scheme of evolutionary relationships was significantly different ($P < 0.05$) from the unconstrained tree (Table 1).

3.5 Morphological character reconstructions

Ancestral character states were reconstructed for three floral characters whose state changes appeared to correlate with deep nodes within the molecular phylogenies, but exhibited some ambiguity in their optimization. These were, 1) presence vs. absence

of fasciclodes, that is disks, appendages, and rings (see below), 2) presence vs. absence of a well developed pistillode, and 3) androecium fasciculate vs. non-fasciculate.

3.5.1 Fasciclodes (disks, appendages, and rings)

Under the likelihood reconstruction model, the presence of fasciclodes was found to be plesiomorphic when mapped onto the unconstrained ML phylogeny and on an ML phylogeny resulting from an analysis constraining Symphonieae as monophyletic (hereto referred to as the "constrained" ML phylogeny) and a single loss of fasciclodes was reconstructed as the most likely state for the ancestor of Lineage B (Fig. 4). Like the ML reconstructions, the parsimony model of reconstruction found the presence of fasciclodes to be plesiomorphic (with a loss in the ancestor of Lineage B) on the unconstrained ML phylogeny; however, reconstruction of this character was ambiguous on the constrained ML phylogeny (Fig. 4, inset).

3.5.2 Pistillodes

A taxon was scored as having a pistillode if it normally has an organ in the center of the flower with an obvious stigmatic area. On the unconstrained and constrained ML trees, the ML model of reconstruction found that the absence of a pistillode was the most likely state in the ancestor of the Garcinieae clade and at least five independent origins of pistillodes were reconstructed within Lineage B. Under a parsimony reconstruction model, the reconstruction of this character was ambiguous on the unconstrained ML topology with the state for the ancestor of Garcinieae and for deep nodes within Lineage B being equivocal. On the constrained ML topology, parsimony reconstruction found the

absence of a pistillode to be the ancestral condition for Garcinieae and for the ancestor of Lineage B and its descendant node. However reconstruction of this character at shallower levels within Lineage B was unclear with either four losses or one loss and three gains being equally parsimonious.

3.5.3 Fascicles

The ML model of reconstruction found three independent losses of stamen fascicles to be most likely (Fig. 4 – Clades 2, 5, & 7, white arrows), regardless of whether or not Symphonieae were constrained to be monophyletic. Reconstructions using a parsimony model were ambiguous with either three losses or two losses and a gain being equally parsimonious. Other morphological characters could be unambiguously assigned as synapomorphies for clades because they exhibited a single character state change within the ML phylogenies (Fig. 4 — black arrows).

4 Discussion

4.1 General

Separate analyses of the GBSSI and ITS datasets both yielded well resolved topologies with many well supported nodes at different levels throughout the phylogenies. These results are in general agreement with those of previously published (Bittrich et al., 2005; Gustafsson et al., 2002) and unpublished (Nazre, 1999; Sari, 2000) molecular phylogenetic studies. The study of Yapwattanaphun et al. (2004) did not provide vouchers for the sequences included and had few species in common with the

current study, so a comparison with the results presented here is not attempted. The taxon sampling strategy used in this study focused heavily on having multiple species that represented the range of floral morphological diversity of each of the sections recognized in the most recent of treatment of the genus (Jones, 1980). These sections were largely delimited using floral characters such as androecium fusion, pistillode presence or absence, and disk presence or absence. The results show that these characters vary at a higher level in *Garcinia*, characterize the major clades of *Garcinia*, and can be used to determine the phylogenetic position of a taxon.

4.2 Character evolution

Several floral characters correspond well with the phylogenies presented here and many clades are marked by synapomorphies (Fig. 4, black and white arrows), or at least by combinations of characters (Fig. 4, characters to right of shaded boxes). In particular, androecial characters, the presence/absence of receptacular disks and antesepalous appendages, and fusion of organs distinguish major clades. While floral variation is important, rhombic crystals in the mesophyll, highly branched exudate canals in the leaf, and stomata accessory cells with papillae are shared among species of some major clades.

4.2.1 Garcinieae

Species in the Garcinieae clade share many morphological features. *Allanblackia* and *Garcinia* s.l. are dioecious (vs. hermaphroditic in Symphonieae) and share capitate stigmas (vs. porose), colleters (vs. absent), and usually eperulate buds (vs. perulate), and

often have introrse anthers (vs. extrorse). Whether these characters are synapomorphies or symplesiomorphies is unclear, because a) Symphonieae may not be monophyletic and b) most of the characters vary in Clusieae. Thus, for example, if Symphonieae (which has perfect flowers) is monophyletic, dioecy could be a synapomorphy for Garcinieae; however, the sister group of the Garcinieae/Symphonieae clade (Clusieae) also has species with dioecious (rarely perfect) flowers, suggesting that dioecy is a synapomorphy for Clusioideae. Determining the polarity of characters states and the identification of synapomorphies will require these deeper level relationships to be resolved.

Within Garcinieae, members of *Garcinia* - Lineage A and *Allanblackia* have an irregular disk-like structure (hereafter "disk") in the center of the staminate flower (Fig 1, K, L, O, P, Q). The corresponding structures in the pistillate flowers are antesepalous, flap-like appendages (hereafter "lobes") that alternate with the staminodal phalanges in species that have phalangiate androecia. In the pistillate flowers of species that have free stamens, the structure corresponding to the disk in the staminate flowers is a ring-shaped structure (Fig. 1, N). The nature of these structures is unclear, they have been considered androecial and called "fasciclodes" (Jones, 1980; Robson, 1961, 1972; Stevens, 2006) and others considered them to be gynoecial (Moncur, 1988). Developmental and anatomical data and preliminary field observations suggest that these structures may be nectariferous in *Garcinia* (see Chapter 2).

In Clusiaceae and relatives, the presence of rings or structures in an antesepalous position are not confined Lineage A and *Allanblackia*. Like in *Garcinia*, in all of these groups these structures have been considered to be an outer whorl of modified stamens, also termed "fasciclodes" in other Clusiaceae and Hypericaceae (Robson, 1961, 1972;

Ronse De Craene and Smets, 1991; Stevens, 2006). There are nectariferous pads in Bonnetiaceae, which may turn out to be sister to Clusioideae (Davis et al., 2007).

If it is assumed these structures are homologous throughout Clusiaceae and its closely related families, determining their polarity is difficult given our present understanding of relationships. For example, ancestral character state reconstruction under a ML model finds the presence of staminodal structures to be pleisomorphic, regardless of whether or not Symphonieae is monophyletic (Fig. 4). However, under a parsimony model of reconstruction in topologies where Symphonieae is monophyletic, the reconstruction of this character is ambiguous for the ancestors of the Garcinieae/Symphonieae clade and for the ancestor the Garcinieae clade (Fig. 4, inset). However, a more detailed characterization of these structures will be important, especially in Symphonieae, in establishing their homology and pattern of evolution in Clusiaceae and its close relatives (see Chapter 2).

4.2.2 Garcinia

Garcinia s.l. have baccate fruits with a single ovule per carpel – a unique combination in Clusioideae and a single ovule per carpel is a synapomorphy for the genus. The optimization of ovule number becomes complicated if *Allanblackia* falls within *Garcinia*, as *Allanblackia* has many ovules in each carpel.

4.2.2.1 Lineage A

Members of Lineage A share rhombic (prismatic) crystals in the mesophyll (Vesque, 1889, 1893), a likely synapomorphy for this clade. All of the other species of *Garcinia* and all Symphonieae except *Pentadesma butyracea* have druses. Vesque (1889) was unable to find crystals in the leaves of the single species of *Allanblackia*, *A.*

floribunda, that he examined. In addition, species in this lineage also have globose to widely elliptic anther thecae. The flowers have receptacular nectaries and lack a well-developed pistillode, at most having a very rudimentary appendage of uncertain nature in the center of staminate flowers (Fig. 1 K-Q).

Within Lineage A, floral characters shared by members of Clade 1 include distally inflexed, phalangiate androecia, with filaments united most of their length, staminal disks and antesepalous appendages, branched styles, and petals that are ascending at anthesis (Fig. 1, K, L). The petals, phalanges, and receptacular disk of staminate flowers fall off of the flower leaving the calyx behind (P. Sweeney, pers. obs.). All representatives of this group examined by Jones (1980) had psilate pollen that was also 5- to 7-colporate – a unique combination within *Garcinia*.

Members of Clade 2 have distinctive sunken stomata with raised papilla-like protuberances arising from the accessory cells and partially covering the stomatal opening (Vesque, 1893: 288), an apparent synapomorphy. Species in this clade share psilate to rugulo-reticulate, tricolporate pollen with long ectoaperatures and endocolpi (Jones, 1980), and have cauline, fasciculate inflorescences. Synapomorphies for the *Rheedia* plus *G. livingstonei* clade are free stamens and non-fasciculate androecia (Fig. 1, P, Q). Marsaioli et al. (1998) reported that the pollen in the flowers of *Rheedia gardneriana* Planch. & Triana was mixed with floral oil; the broader distribution of this character is unknown.

Unresolved within Lineage A is *Garcinia conrauana*, which has a receptacular disk (staminate flowers) and antesepalous appendages (pistillate flowers), and egg-shaped anther thecae, like other species of Lineage A. It is unique in Lineage A by having fleshy,

club-shaped phalanges whose entire surface is covered by numerous (>150) stamens with short filaments (Fig 1, M).

4.2.2.2 Lineage B

Species in this clade have staminate and pistillate flowers that lack receptacular nectaries, which could be a synapomorphy depending on how deeper nodes in the phylogeny are resolved (Fig. 4). A pistillode is present in the staminate flowers of many species of Lineage B (e.g., Fig. 1, C, D, E, F, G, H); however, two of the major clades within Lineage B (Clades 2 and 4) usually lack a pistillode (e.g., Fig. 1, A, B). Within Lineage B, the seven major clades contain species with unique combinations of floral and or vegetative characters that vary little within their clades.

All members of Clade 3, except *G. pauciflora*, have sepals fused in bud (Fig. 1, H). Fused sepals is thus either a synapomorphy for all of Clade 3 (with a loss in *G. pauciflora*) or a synapomorphy for Clade 3 excluding *G. pauciflora* (if *G. pauciflora* is sister to the rest of the clade). Clade 3 also has staminate flowers with a fungiform pistillode and four to eight antepetalous fascicles (branched in some) covered with sessile to subsessile stamens (Fig. 1, H). Additionally species in this clade have obvious terminal bud scales (perulae), which are obscure or absent in most other species of *Garcinia* (Stevens, 2006).

Clade 4 species share usually four-merous flowers that have the stamens arranged into four, terete to strap-shaped, fascicles that are distally covered with sessile to subsessile, bithecate anthers. The staminate flowers have a fungiform pistillode. The fruits are two locular, have a woody pericarp, and are capped by a sessile, smooth, disk-like stigma.

Species in Clade 5 usually have fruits with furrows along the septal radii, a possible synapomorphy for the clade. Furthermore, members of Clade 5 have staminate flowers that lack a pistillode and that have stamens covering the slightly raised central portion of the flower (Fig. 1, A, B) – except in *G. atroviridis* (the first branching lineage of Clade 5; Fig. 1, C) where the stamens are arranged in a ring around a pistillode. Many species in Clade 5 have anthers in which each of the four sporangia opens separately, but *G. atroviridis*, *G. chapelieri*, and *G. asterandra* are conventionally bithecate (P. Sweeney, pers. obs.).

Garcinia mangostana and its relatives comprise Clade 6; however, there are no clear morphological synapomorphies for this clade. The taxa in this clade usually have an undivided four-lobed androecium that surrounds a fungiform pistillode (Fig. 1, D). The anthers are bithecate, elongate, and usually recurved.

Clade 7 has staminate flowers that lack a pistillode and that have non-fasciculate androecia similar to those in Clade 5 with 4- ca. 20 anthers (Jones, 1980; Whitmore, 1973a, b). The anthers have peltate connectives and apparently confluent apically-positioned thecae that have circumscissile dehiscence or multiple chambers (locelli) that dehisce via pores (Jones, 1980; Whitmore, 1973a, b).

Species in Clade 8 have distinctive staminate flowers in which the pistillode is surrounded by four antepetalous, strap-shaped fascicles (but a ring in *G. mannii*) with a single row of sessile, recurved, and often multilocellate anthers at the end of the fascicle (Fig. 1, E).

The last major clade of Lineage B, Clade 9, contains a collection of species that have not previously been united. These species have the synapomorphy of a distinctive

adaxial, exudate-containing canal pattern in the leaves, consisting of highly branched, anastomizing canals (Turner and Stevens, 1999; P. Sweeney, pers. obs.). All examined species have seeds with a lignified exotegmen (vs. exotegmen absent in other Garcinieae) and, where known, phanerocotylar germination (vs. cryptocotylar and epigeal in other Garcinieae) (Stevens, 2006). *P. latissimum*, *T. cymosum*, *G. warrenii*, and *G. prainiana* have stamen fascicles that are adnate to the petals (but only basally so in *G. prainiana*) (Fig. 1, F, G).

4.3 Previous classifications

The lack of molecular support for the monophyly of Symphonieae is surprising as members of this tribe share several morphological features that are potential synapomorphies including a short androgynophore and a distally branched style, the branches of which have distal apical pores that lead to a stigmatic cavity (Stevens, 2006). In the one species (*Symphonia globulifera*) for which detailed observations of the pollination mechanism are available, pollen is caught in a sticky droplet and sucked in through the pores, a truly remarkable mechanism (Bittrich and Amaral, 1996).

Within Garcinieae, if future data supports a sister relationship between *Allanblackia* and Lineage A, then *Allanblackia* will need to be united with *Garcinia*, which would require the transfer of about ten species.

4.3.1 Segregate genera

A broad circumscription of *Garcinia* is easily justified by molecular and morphological data (Jones, 1980; Stevens, 2006). Few nomenclatural changes would be required. Alternatively *Garcinia* could be restricted to Lineage B, where the type of the genus is found. Lineage A could be a separate genus; the name *Rheedia* L. is the earliest generic name used among species of Lineage A and thus would have priority. However, over 60 new combinations or names would be required, which seems undesirable, especially since the two would not be easily distinguishable (Backlund and Bremer, 1998). A better alternative would be to treat these lineages as subgenera (see below).

The taxonomic status and limits of the genus *Ochrocarpos* Thouars (1806) has been debated recently by Kostermans (1956; 1961) and De Wilde (1956). De Wilde (1956) suggested that *Ochrocarpos* in its entirety should be placed into *Mammea*. Kostermans (1956, 1961) largely agreed, but thought that *Ochrocarpos* should be maintained for species with phalangiate androecia and leaves lacking venation thought to be characteristic of true *Mammea*. Jones (1980) and Stevens (2005) both came to the same conclusion that *Ochrocarpos* was comprised of two unrelated groups of species, one related to *Garcinia* and the other to *Mammea*. The species of *Ochrocarpos* related to *Garcinia* have seeds that have an embryo comprised mostly of a swollen hypocotyl, a trait shared with other *Garcinia* and most Clusioideae (Brandza, 1908; Stevens, 2006), by staminate flowers with antepetalous stamen fascicles surrounding a fungiform pistillode, and by leaves with resin canals intersecting the secondary veins. The affinity of *Ochrocarpos* species with non-fasciculate androecia to *Mammea* has recently been confirmed by a combined phylogenetic analysis of molecular and morphological data (Notis, 2004). Kostermans (1956; 1961) and Stevens (2005) have provided new names in

Mammea for many taxa, while Sweeney and Rogers (in press) provide names in *Garcinia* for the Malagasy *Ochrocarpos* species with stamen fascicles.

The sampled representative of the segregate genus *Pentaphalangium*, *P. latissimum*, is nested in a clade with *Tripetalum cymosum* and some other species traditionally treated as *Garcinia*. *Pentaphalangium* was created by Warburg (1891) for the species *P. crassinerve*, which he distinguished from *Garcinia* because in its staminate flowers the phalanges were adnate to the petals for over half their length and because the pistil rudiment was excentric. Kostermans (1976) united *Pentaphalangium* with *Garcinia* noting that the excentric pistil rudiment was an abnormality and that the phalanges were never fused to the petals for more than half their length, and thus were not different from some other traditional *Garcinia* species with phalange-petal adnation (e.g., *G. warrenii* and *G. terpnophylla* Thwaites). Kostermans (1976) provided names in *Garcinia* for all *Pentaphalangium* species that needed them, although some of these were invalid because their epithets were already in use elsewhere in *Garcinia*.

The unification of the genus *Rheedia* with *Garcinia* is supported by this study. Earlier workers separated *Rheedia* from *Garcinia* because its flowers have free stamens and were purported to have two sepals instead of four (e.g., Planchon & Triana, 1860; Vesque, 1893; Engler, 1893, 1925). While arguing for the inclusion of *Rheedia* in *Garcinia*, Robson (1958) correctly pointed out that this distinction breaks down when one takes into account the total variation within the two genera, noting that the indefinite, sub-spiral perianth of the west African *G. pachyclada* N. Robson (section *Teracentrum*) was similar to that found in some Malagasy *Rheedia*. This circumscription has been adopted in several recent treatments (e.g., Adams, 1970, 1972; Jones, 1980; Kearns et al.,

1998; Hammel, 2001; Schatz, 2001; Stevens, 2006) – but see van den Berg (1979) and D'Arcy (1980) for recent treatments that maintain *Rheedia*. Many *Rheedia* species already have names in *Garcinia*, and recently Sweeney and Rogers (submitted) provide new combinations and names for all Malagasy *Rheedia* that lack valid names in *Garcinia*. Adams (1970), Hammel (1989), and Zappi (1993) provide names in *Garcinia* for several Central and South American *Rheedia*, but there are some that still need names in *Garcinia*.

The sole species of the genus *Tripetalum*, *T. cymosum*, is nested in a clade with *Pentaphalangium latissimum* and some other species traditionally treated as *Garcinia*. This species was transferred to *Garcinia* (as *G. cymosa* (K. Schum.) I. M. Turner & P. F. Stevens) by Turner and Stevens (1999) who argued that the characters used by Schumann (Schumann and Hollrung, 1889) to distinguish this species from *Garcinia* (i.e., three merous flowers and staminal phalanges fused to the petals) were insufficient for recognizing a separate genus. They noted that staminal phalanges fused to the petals were present in species of the segregate genus *Pentaphalangium* and in other *Garcinia* (see discussion under Lineage B). Turner and Stevens (1999) also noticed that *T. cymosum* has distinctive branched exudate-containing canals in their leaf blades, like species of the segregate genus *Pentaphalangium* and like some other *Garcinia* from the far east (see below, Lineage B - Clade 9).

4.3.2 Previous infrageneric classifications of Garcinia

The idea that species comprising Lineage A might be closely related has not been considered by recent authors. Pierre (1883) did notice that species from these groups,

were similar in having disks in their staminate flowers; however, this was not reflected in his classification. Vesque (1893) thought that species from these groups were evolutionarily connected, but he did not create a formal group for them. He hypothesized that species from his subgenus *Xanthochymus* (generally equivalent to Clade 1 – section *Xanthochymus*) gave rise to species in his subgenus *Rheediopsis*, i.e., sections *Rheediopsis* and *Teracentrum*, ("je conclus que les *Rheediopsis* dérivent du groupe nodal des *Xanthochymus*" Vesque, 1893: 290-291). While Vesque (1893) kept species of *Rheedia* separate from *Garcinia*, he observed (1893: 288) that species of *Rheedia* and those of *Garcinia* sections *Rheediopsis* and *Teracentrum* have similar stomata (see discussion of character evolution), and others have noted similarities among members of this clade (e.g., Engler, 1925; Robson, 1958; Jones, 1980), especially between sections *Rheedia* and *Teracentrum*, both of which have non-fasciculate androecia with free stamens. Engler (1925) observed that many species from Lineage A has similar egg-shaped thecae, and he grouped them together in his key to sections. Based on morphology most species in Lineage B would fall into Vesque's (1893) subgenus *Eugarcinia* (=*Garcinia*). He described species of this subgenus as having stomata more like those found in other Clusiaceae, that is, not sunken, lacking distinct subsidiary cells, and having narrow, elongate stomatal apertures. Other than Vesque (1893), others have not grouped members of this clade together.

4.3.2.1 Sectional classification sensu Jones (1980)

Many of Jones's (1980) sections are supported as monophyletic. Some appear to be para- or polyphyletic (i.e., *Brindonia*, *Garcinia*, *Discostigma*, *Rheedia*, and *Xanthochymus* – Figs. 2, 3), but monophyly cannot be rejected by S-H tests. For sections

Brindonia and *Garcinia*; however, polyphyly or paraphyly was apparent only in trees produced from the unpruned ITS data set where taxon sampling was denser (Fig. 2).

Section *Xanthochymus* sensu Jones (1980) is not monophyletic, but in all analyses a core group of species from section *Xanthochymus* forms a clade (Figs 2, 3; Clade 1). Previous workers have placed *G. prainiana* into section (or subgenus) *Xanthochymus* (Jones, 1980; Vesque, 1889, 1893), but the analyses presented here place this taxon within a subclade of Lineage B with which it shares many features (see below).

Representatives from sections *Rheedia*, *Rheediopsis*, and *Teracentrum* comprise Clade 2, but sections *Rheedia* and *Rheediposis* are paraphyletic. Thus all species of Clade 2 could be combined into a single section.

Species representing section *Paragarcinia* (Baillon) Vesque fall into Clade 3. *G. pauciflora* also falls in this clade, indicating that section *Paragarcinia* could be expanded to include *G. pauciflora*.

Two taxa representing section *Discostigma*, *G. eugeniifolia* and *G. rostrata* constitute Clade 4. Jones's (1980) section *Discostigma* contains many species with floral morphology like that found in *G. eugeniifolia* and *G. rostrata*; however, there are two groups of species placed in the section that differ in part by their androecial morphology (Jones, 1980). One group of species, including *G. balansae* Pierre, G. *lanessanii* Pierre, *G. terpnophylla* Thwaites and *G. warrenii*, differs by having their stamens fused to the petals, and the position of *G. warrenii* in the trees presented here suggests that this group may be better placed with species of Clade 9. The second group of species includes *G. dives* Pierre, *G. hunsteinii* Lauterb., *G. linii* C. E. Chang, *G. luzoniensis* Merrill, and *G. palawanensis* Elmer and is restricted to New Guinea, the Philippines and Taiwan (Jones,

1980). This latter group is reported to have peltate anthers like species of section *Hebradendron* (sensu Jones, 1980); nevertheless, Jones (1980) thought they were related to typical section *Discostigma* species because they share the same stamen arrangement and pollen apertures. Unfortunately, species representing the group containing *G. dives* were not available for this study.

Jones (1980) placed most species of Clade 5 into section *Brindonia*. Others have noted morphological similarities among these species (e.g., Pierre, 1883; Vesque, 1893; Engler, 1925). Two Malagasy species, *G. asterandra* and *G. chapelieri*, the latter placed in section *Garcinia* sensu Jones (1980) and the other unplaced, are also strongly supported as members of this clade. Their position in this clade is supported by their having staminate floral morphology (but two-chambered anthers) and fruits with superficial septal furrows like the other members of the clade.

Jones (1980) included all of the species of Clade 6 (Figs. 2, 3) as well as many others in section *Garcinia*, an admittedly heterogeneous group. With the exception of *G. opaca* King (which is reported to lack a pistillode), Whitmore (1973a, b) placed these species into his Group B. This study supports as monophyletic a core group of species from section *Garcinia* (sensu Jones, 1980), but some morphologically anomalous species like *G. cumingiana* Pierre, which lacks a pistillode, were not sampled in this study, so the future status of the group, at least when broadly circumscribed, remains uncertain.

Section *Hebradendron* is supported as monophyletic. This group was first treated as a distinct genus (*Hebradendron* Graham) and was later united with *Garcinia* by Planchon and Triana (1860). Jones (1980) cited the arrangement of the stamens into a single column and the peltate anthers as the most distinctive features of the group. Also

noting the distinctive anthers and stamen arrangement, Whitmore (1973a, b) included all of these species in his Group E.

Clade 9 contains a collection of species that have not previously been united. That some of these species might be closely related was only recently hypothesized by Turner and Stevens (1999) who noted that *G. hollrungii* Lauterb., *G. platyphylla* A. C. Sm., and *G. warrenii* as well as species placed into section *Mungotia* and into the segregate genera *Pentaphalangium* and *Tripetalum* share several morphological features (see above). Jones (1980) treated all of the species in Clade 9 as *Garcinia* but placed them into five different sections. Jones (1980) thought that the sections *Macrostigma* and *Tripetalum* might be related due to their sharing antepetalous stamen fascicles that are adnate to the petals for much of their length. *Garcinia amplexicaulis*, a New Caledonian endemic, was placed in section *Mungotia* (Engler, 1893, 1925; Jones, 1980) because it is described as having staminate flowers with stamens united into single central, fleshy stalk and bithecous anthers. This arrangement of stamens would be anomalous within Clade 9, but a close examination of staminate flowers from accessions at MO (*McPherson 1674*, *18536*) reveals that the center of the staminal column is naked and that the stamens are arranged into antepetalous lobes (P. Sweeney, pers. obs.). Other species not sampled in this study that have been placed into section *Mungotia* (e.g., *G. adinantha* A. C. Sm. & S. Darwin and *G. sessilis* (G. Forst.) Seem.) clearly have their stamens covering the apex of a central, fleshy stalk (Smith and Darwin, 1974; P. Sweeney, pers. obs.). It remains to be seen if these species will group with *G. amplexicaulis* in Clade 9 or if they will fall elsewhere. Vesque (1893) placed *G. prainiana* with species of section *Xanthochymus* (in his subgenus *Xanthochymus*) and this placement has been followed by subsequent

workers (e.g., Jones, 1980). However, the flowers of *G. prainiana* have a pistillode and lack receptacular disks and antepetalous appendages, unlike those found in other section *Xanthochymus* species, or in other species in Lineage A. Its flowers, with staminal phalanges adnate to the petals, and branching adaxial exudate-containing canal pattern agree with the molecular data and support its placement here.

The phylogenetic relationships found in this study provide a framework for a future infrageneric classification of *Garcinia*. This study identifies several highly supported clades that generally correspond to sections recognized by Jones (1980) and that are characterized by synapomorphies or combinations of characters. Furthermore, the two major lineages of *Garcinia* suggest a convenient partition of the genus at the subgeneric level. Formal recognition of subgenera and a revised sectional classification will be presented in a future publication.

4.4 Diversity

It is difficult to point to a single feature –a "key adaptation"– that may have caused the diversity of floral form found in *Garcinia*. Some have speculated that the pattern of diversity in the closely related *Clusia* could have been spurred by the evolution of resin secretion in the flowers (Armbuster, 1984; Bittrich and Amaral, 1997; Gustafsson and Bittrich, 2003; Gustafsson et al., 2007), but resin production has not been reported in *Garcinia*. Gustafsson and Bittrich (2003) noted that the extensive floral diversity within *Clusia* was rather similar to that within *Garcinia* and they suggested that a predisposition to be "labile" at a developmental genetic level may be connected with the diversity within

two (see also Bittrich and Amaral, 1997). This hypothesis becomes more interesting when one considers the pattern of diversity and kinds of variation present in *Garcinia* and *Clusia*. Each genus is basically dioecious and has a great range of floral morphology, and each has major clades that are characterized by unique combinations of floral characters, in particular those of the androecium. The kinds of changes that occur among major clades are similar, with variation including the amount of staminal fusion, anther shape, number of loculi per anther, presence/absence of locelli, the presence/absence of pistillodes, and fusion of perianth parts. These similarities suggest that underlying genetic mechanisms responsible for the similar pattern of variation within the two groups might be the same (Gustafsson and Bittrich, 2003; Vavilov, 1922). To further explore this hypothesis, future work needs to focus on determining the developmental-genetic basis for the variation within *Garcinia*, *Clusia*, and their close relatives. Furthermore while possibly explaining the pattern and scope of diversity present, this hypothesis still begs the question of what outside factors interfaced with this "lability" and drove floral diversification within the group.

Little is known of the pollination biology in *Garcinia*. Richards (1990b) reported that *Trigona* bees visited several different species of *Garcinia* (here representing two clades of Lineage B, one with pistillodes in the staminate flowers and the other without). The bees foraged for nectar (produced on the stigma of pistillate and staminate flowers) in species with pistillodes in their staminate flowers, and for pollen in species without nectar and pistillodes (Richards, 1990b). The pollen in the flowers of *Rheedia gardneriana* Planch. & Triana is mixed with floral oil (Marsaioli et al., 1998), a phenomenon that has also been observed in *Clusia* and *Tovomita* where fragrant oils act

as an attractant for bees (Bittrich and Amaral, 1997; Nogueira et al., 1998). Further studies on the pollination biology of *Garcinia* species are sorely needed.

The correlation between the presence of a pistillode and the absence of the receptacular disk might be interesting from a pollination biology standpoint. In flowers of *G. dulcis* (from Lineage A), a mucilaginous substance is produced from the center of the flower (P. Sweeney, pers. obs.), the disks of *G. macrophylla*, *G. ovalifolia*, *G. smeathmannii* (from Lineage A) produce a sweet-tasting, watery exudate (P. Sweeney, pers. obs.), and the extrastaminal "disk" in *Symphonia* has been described as nectariferous (Stevens, 2006). If production of nectar from receptacular disks and appendages in Lineage A and Symphonieae is widespread, it would suggest a scenario where the receptacular disk/appendages and pistillode may serve the same functions, perhaps explaining why their presence is largely mutually exclusive.

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References

Adams, C.D., 1970. Miscellaneous additions and revisions to the flowering plants of Jamaica. Phytologia 20, 309–314.

Adams, C.D., 1972. Flowering plants of Jamaica. The University Press, Glasgow, Scotland, UK.

Allem, A.C., 2004. Optimization theory in plant evolution: an overview of long-term evolutionary prospects in the angiosperms. Bot Rev 69, 225-251.

Altekar, G., 2004. Parallel Metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics 20, 407-415.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J Mol Biol 215, 403-410.

Armbuster, W.S., 1984. The role of resin in angiosperm pollination: ecological and chemical considerations. Am J Bot 71, 1149-1160.

Ashton, P.S., 1988. Systematics and ecology of rain forest trees. Taxon 37, 622-629.

Backlund, A., Bremer, B., 1998. To be or not to be. Principles of classification and monotypic plant families. Taxon 47, 391-400.

Baum, D.A., Sytsma, K.J., Hoch, P.C., 1994. A phylogenetic analysis of *Epilobium* (Onagraceae) based on nuclear ribosomal DNA sequences. Syst Bot 19, 363-388.

Bentham, G., 1862. Guttiferae. In: Bentham, G., Hooker, J.D. (Eds.), Genera Plantarum. Reeve & Co., London, UK, pp. 167-177.

Bittrich, V., Amaral, M.C.E., 1996. Flower morphology and pollination biology of some *Clusia* species from the Gran Sabana (Venezuela). Kew Bull 51, 681-694.

Bittrich, V., Amaral, M.C.E., 1997. Floral biology of some *Clusia* species from central Amazonia. Kew Bull 52, 617-635.

Bittrich, V., Gustafsson, M.H.G., Stevens, P.F., Sweeney, P.W., 2005. Phylogeny and character evolution in Clusiaceae and their immediate relatives. XVII International Botanical Congress, Abstracts. IBC, Vienna, AT, p. 219.

Brandza, G., 1908. La germination des Hypéricacées et des Guttifères. Ann Sci Nat, Bot 9 Série 8, 221-300.

D'Arcy, W.G., 1980. Flora of Panama. Part VI. Family 123. Ann Mo Bot Gard 67, 969-1043.

Davis, C.C., Latvis, M., Nickrent, D.L., Wurdack, K.J., Baum, D.A., 2007. Floral gigantism in Rafflesiaceae. Science 315, 1812.

De Wilde, J.J.F.E., 1956. A delimitation of *Mammea* L. Acta Bot Neerl 5, 171–178.

Doust, A.N., Drinnan, A.N., 2004. Floral development and molecular phylogeny support the generic status of *Tasmannia* (Winteraceae). Am J Bot 91, 321-331.

Doyle, J., Doyle, J.L., 1987. Genomic plant DNA preparation from fresh tissue–CTAB method. Phytochemical Bull 19.

Endress, P.K., 1994. Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge, UK.

Endress, P.K., 2006. Angiosperm floral evolution: morphological developmental framework. Adv Bot Res 44, 1-61.

Endress, P.K., Matthews, M.L., 2006. First steps toward a floral structural characterization of the major rosid subclades. Pl Syst Evol 260, 223-251.

Engler, A., 1893. Guttiferae In: Engler, A., Prantl, K. (Eds.), Die natürlichen Pflanzenfamilien. W. Engelmann, Leipzig, DE, pp. 194–242.

Engler, A., 1925. Guttiferae In: Engler, A., Prantl, K. (Eds.), Die natürlichen Pflanzenfamilien. W. Engelmann, Leipzig, DE, pp. 154–237.

Felsenstein, J., 2004. Inferring phylogenies. Sinauer Associates, Sunderland, Mass.

Goldman, N., Anderson, J.P., Rodrigo, A.G., 2000. Likelihood-based tests of topologies in phylogenetics. Syst Bio 49, 652-670.

Gustafsson, M.H.G., Bittrich, V., 2003. Evolution of morphological diversity and resin secretion in flowers of *Clusia* (Clusiaceae): insights from ITS sequence variation. Nord J Bot 22, 183–203.

Gustafsson, M.H.G., Bittrich, V., Stevens, P.F., 2002. Phylogeny of Clusiaceae based on *rbcL* sequences. Int J Pl Sci 163, 1045–1054.

Gustafsson, M.H.G., Winter, K., Bittrich, V., 2007. Diversity, phylogeny, and classification of *Clusia*. In: Lüttge, U. (Ed.), Clusia: a woody Neotropical genus of remarkable plasticity and diversity. Ecological Studies. Springer-Verlag, Berlin, pp. 95-116.

Hammel, B., 1989. New combinations and taxonomies in Clusiaceae. Ann Mo Bot Gard 76, 927-929.

Hammel, B., 2001. *Garcinia*. In: Stevens, W.D., Ulloa Ulloa, C., Pool, A., Montiel, O.M. (Eds.), Flora de Nicaragua. Missouri Botanical Garden Press, Saint Louis, Missouri, USA, p. 626.

Hecker, K.H., Roux, K.H., 1996. High and low annealing temperatures increase both specificity and yield in touchdown and stepdown PCR. Biotechniques 20, 478-485.

Heymsfield, S.B., Allison, D.B., Vasselli, J.R., Pietrobelli, A., Greenfield, D., Nunez, C., 1998. *Garcinia cambogia* (Hydroxycitric Acid) as a potential antiobesity agent: A randomized controlled trial. J Am Med Assoc 280, 1596-1600.

Ho, C.K., Huang, Y.L., Chen, C.C., 2002. Garcinone E, a xanthone derivative, has potent cytotoxic effect against hepatocellular carcinoma cell lines. Planta Med 68, 975-979.

Holder, M., Lewis, P.O., 2003. Phylogeny estimation: traditional and Bayesian approaches. Nature Reviews: Genetics 4, 275-284.

Jaramillo, M.A., Manos, P.S., Zimmer, E.A., 2004. Phylogenetic relationships of the perianthless Piperales: reconstructing the evolution of floral development. Int J Pl Sci 165, 403-416.

Jones, S.W., 1980. Morphology and major taxonomy of *Garcinia* (Guttiferae). University of Leicester, Leicester, UK.

Kearns, D.E., Berry, P.E., Stevens, P.F., Cuello, N.L., Pipoly, J.J., Robson, N.K.B., Holst, B.K., Kubitzki, K., Weitzman, A.L., 1998. Clusiaceae. In: Berry, P.E., Holst, B.K., Yatskievych, K. (Eds.), Flora of the Venezuelan Guayana. Missouri Botanical Garden Press, St. Louis, Missouri, USA, pp. 248–329.

Kolaczkowski, B., Thornton, J.W., 2004. Performance of maximum parsimony and likelihood phylogenetics when evolution is heterogeneous. Nature 431, 980-984.

Kostermans, A.J.G.H., 1956. Additional notes on Mimosaceae. The genera *Mammea* L. and *Ochrocarpos* Thou. Djawatan Kehutanan Indonesia Bagian Planologi Kehutanan, Djalen Perniagaan 44, 9–15.

Kostermans, A.J.G.H., 1961. A monograph of the Asiatic and Pacific species of *Mammea* L. (Guttiferae). Communication of the Forest Research Institute, Indonesia 72, 1–63.

Kostermans, A.J.G.H., 1976. Notes on the Clusiaceae of Sri Lanka and reduction of *Pentaphalangium* Warb. Ceylon J Sci Bot 12, 55-71.

Lee, H.S., Ashton, P.S., Yamakura, T., Tan, S., Davies, S.J., Itoh, A., Chai, E.O.K., Ohkubo, T., LaFrankie, J.V., 2002. The 52-hectare forest research plot at Lambir Hills, Sarwak, Malaysia: tree distribution maps, diameter tables and species documentation. Forest Department Sarawak & The Arnold Arboretum-CTFS Asia Program.

Leins, P., Erbar, C., 1991. Fascicled androecia in Dilleniidae and some remarks on the *Garcinia* androecium. Bot Acta 104, 336-344.

Lewis, P.O., Holder, M.T., Holsinger, K.E., 2005. Polytomies and Bayesian phylogenetic inference. Syst Bio 54, 241-253.

Lodhi, M.A., Ye, G.N., Weeden, N.F., Reisch, B.I., 1994. A simple and efficient method for DNA extraction from grapevine cultivars, *Vitis* species and *Ampelopsis*. Pl Mol Bio Reporter 12, 6–13.

Mackeen, M.M., Ali, A.M., Lajis, N.H., Kawazu, K., Hassan, Z., Amran, M., Habsah, M., Mooi, L.Y., Mohamed, S.M., 2000. Antimicrobial, antioxidant, antitumour-

promoting and cytotoxic activities of different plant part extracts of *Garcinia atroviridis* Griff. ex T. Anders. J Ethnopharmacol 72, 395-402.

Maddison, W.P., Maddison, D.R., 2006. Mesquite: A modular system for evolutionary analysis.

Malcomber, S.T., 2002. Phylogeny of *Gaertnera* Lam. (Rubiaceae) based on multiple DNA markers: Evidence of a rapid radiation in a widespread, morphologically diverse genus. Evolution 56, 42-57.

Marsaioli, A.J., Porto, A.L.M., Gonçalves, R.A.C., de Oliveira, C.M.A., Manfio, G.P., Bittrich, V., 1998. The ecosystem of microorganisms, bees, and *Clusia* floral resin and oils, from the chemistry point of view. http://www.iupac.org/symposia/proceedings/phuket97/marsaioli.html.

McMahon, M., Hufford, L., 2005. Evolution and development in the amorphoid clade (Amorpheae: Papilionoideae: Leguminosae): petal loss and dedifferentiation. Int J Pl Sci 166, 383-396.

Moncur, M.W., 1988. Floral development of tropical and subtropical fruit and nut species: an atlas of scanning electron micrographs. Division of Water and Land Resources, Commonwealth Scientific and Industrial Research Organisation Australia, Melbourne, AU.

Murray, M.G., Thompson, W.F., 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8, 4321-4326.

Nazre, M., 1999. Life history studies of *Garcinia* (Clusiaceae) species in the Pasoh 50 ha plot. National University of Malaysia, Kuala Lumpur, MY.

Nixon, K., 1999. The parsimony ratchet, a new method for rapid parsimony analysis.

Cladistics 15, 407-414.

Nogueira, P.C.L., Marsaioli, A.J., Amaral, M., Bittrich, V., 1998. The fragrant floral oils of *Tovomita* species. Phytochemistry 49, 1009-1012.

Notis, C., 2004. Phylogeny and character evolution of Kielmeyeroideae (Clusiaceae) based on molecular and morphological data. University of Florida, Gainesville, Florida, USA.

Nylander, J.A.A., 2004. MrModeltest 2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Perrier de la Bâthie, H., 1948. Revision des Guttifères de Madagascar et des Comores. Mém Mus Natl Hist Nat 24, 75–110.

Perrier de la Bâthie, H., 1951. Guttifères In: Humbert, H. (Ed.), Flore de Madagascar et des Comores (plantes vasculaires). Firmin-Didot, Paris, FR, pp. 1–186.

Petit-Thouars, A.D., 1806. Genera Nova Madagascariensia. Académie des Sciences, Paris, FR.

Pierre, J.B.L., 1883. Flore Forestière de la Cochinchine, fascicle 5. O. Doin, Paris, FR.

Planchon, J.E., Triana, J., 1860. Mémoire sur la famille des Guttifères. Ann Sci Nat, Bot 4 Série 14, 226–367.

Richards, A.J., 1990a. Studies in *Garcinia*, dioecious tropical forest trees: agamospermy. Bot J Linn Soc 103, 233-250.

Richards, A.J., 1990b. Studies in *Garcinia*, dioecious tropical forest trees: the phenology, pollination biology and fertilization of *G. hombroniana* Pierre. Bot J Linn Soc 103, 251-261.

Robson, N.K.B., 1958. New and little known species from the Flora Zambesiaca area, VI. Bol Soc Brot 32, 151–173.

Robson, N.K.B., 1961. Guttiferae. In: Exel, A.W., Will, H. (Eds.), Flora Zambesiaca. Crown Agents for Overseas Governments and Administrations, London, UK, pp. 371-375.

Robson, N.K.B., 1972. Evolutionary recall in *Hypericum* (Guttiferae)? Trans & Proc Bot Soc Edinburgh 41, 365-338.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572-1574.

Ronse De Craene, L.P., Smets, E., 1991. Androecium and floral nectaries of *Harungana madagascariensis* (Clusiaceae). Pl Syst Evo 178, 179-194.

Ronse De Craene, L.P., Soltis, P.S., Soltis, D.E., 2003. Evolution of floral structures in basal angiosperms. Int J Pl Sci 164, S329-S363.

Sari, R., 2000. Review of *Garcinia* (Clusiaceae) based on molecular systematics. James Cook University, Cairns, Australia.

Schatz, G.E., 2001. Generic tree flora of Madagascar. Cromwell Press, Wiltshire, UK.

Schumann, K., Hollrung, M., 1889. Die flora von Kaiser Wilhelmsland. Asher & Co., Berlin, DE.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Molec Biol Evol 16, 1114-1116.

Shimodaira, H., 2002. An approximately unbiased test of phylogenetic tree selection. Syst Bio 51, 492-508.

Sikes, D.S., Lewis, P.O., 2001. PAUPRat: PAUP* implementation of the parsimony

ratchet. Beta software. Version 1.

Simmons, M.P., Pickett, K.M., Miya, M., 2004. How meaningful are Bayesian support values? Molec Biol Evol 21, 188-199.

Simmons, M.P., Zhang, L.B., Webb, C.T., Reeves, A., Miller, J.A., 2006. The relative performance of Bayesian and parsimony approaches when sampling characters evolving under homogeneous and heterogeneous sets of parameters. Cladistics 22, 171-185.

Smith, A.C., Darwin, S.P., 1974. Studies of Pacific Island plants, XXVIII. Guttiferae of the Fijian region. J Arnold Arbor 55, 215-263.

Smyth, D.R., 2005. Morphogenesis of flowers – our evolving view. Plant Cell 17, 330-341.

Stevens, P.F., 2005. Combinations in *Mammea* (Clusiaceae) with the description of a remarkable species, *M. zeereae*, from Madagascar. Harv Paper Bot 9, 429–434.

Stevens, P.F., 2006. Clusiaceae. In: Kubitzki, K. (Ed.), The families and genera of vascular plants. Springer, Berlin, DE, pp. 48-66.

Suzuki, Y., Glazko, G.V., Nei, M., 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. Proc Natl Acad Sci 99, 16138-16143.

Sweeney, P.W., Bradford, J.C., Lowry, P.R., 2004. Phylogenetic position of the New Caledonian endemic genus *Hooglandia* (Cunoniaceae) as determined by maximum parsimony analysis of chloroplast DNA. Ann Mo Bot Gard 91, 266-274.

Swofford, D.L., 2001. PAUP*: Phylogenetic analysis using parsimony (* and other methods), version 4.0 b8. Sinauer, Sunderland, Massachusetts, USA.

Thomas, D.W., Kenfack, D., Chuyong, G.B., Moses, S.N., Losos, E.C., Condit, R.S., Songwe, N., 2003. Tree species of Southwestern Cameroon: Tree distribution maps, diameter tables, and species documentation of the 50-hectare Korup Forest Dynamics Plot. Center for Tropical Forest Science of the Smithsonian Tropical Research Institute; Bioresources Development and Conservation Programme-Cameroon, Washington, D.C., USA.

Thomas, S.C., 1997. Brief communication: geographic parthenogenesis in a tropical forest tree. Am J Bot 84, 1012-1012.

Turner, I.M., Stevens, P.F., 1999. The transfer of *Tripetalum cymosum* K. Schum. (Guttiferae) to *Garcinia*. Gard Bull Singapore 51, 175-177.

van den Berg, M.E., 1979. Revisão das espécies brasileiras do gênero *Rheedia* L. (Guttiferae). Acta Amazonica 9, 43-74.

Vavilov, N.I., 1922. The law of homologous series in variation. J Genet 12, 67-87.

Vesque, J., 1889. Epharmosis: Pars secunda. Genitalia foliaque Garciniearum et Calophyllearum. Vincennes, Paris, FR.

Vesque, J., 1893. Guttiferae In: de Candolle, A.L.P.P., de Candolle, A.C.P. (Eds.), Monographiae Phanerogamarum. G. Masson, Paris, FR, pp. 1–669.

Warburg, O., 1891. Beiträge zur Kenntnis der paupanischen Flora. Bot Jahrb Syst 13, 230-445.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M., Gelfand, D., Sninsky, J., White, T.J. (Eds.), PCR protocols: A guide to methods and applications. Academic Press, San Diego, California, USA, pp. 315-322.

Whitmore, T.C., 1973a. *Garcinia* In: Whitmore, T.C. (Ed.), Tree flora of Malaya: a manual for foresters. Longman, London, UK, pp. 196–225.

Whitmore, T.C., 1973b. Guttiferae In: K. M. Kochummen and T. C. Whitmore: Notes on the systematy of Malayan phanerogams, XVIII-XXII. Gard Bull Singapore 26, 269-286.

Whitmore, T.C., 1998. An introduction to tropical rain forests. Oxford University Press, New York, New York, USA.

Yapwattanaphun, C., Subhadrabandhu, S., Honsho, C., Yonemori, K., 2004. Phylogenetic relationship of mangosteen (*Garcinia mangostana*) and several wild relatives (*Garcinia spp*.) revealed by ITS sequence data. J Amer Soc Hort Sci 129, 368-373.

Zappi, D.C., 1993. A new combination in *Garcinia* (Guttiferae). Kew Bull 48, 22.

Table 1. Results of Shimodaira–Hasegawa tests of topological differences. The best tree is from the result of an unconstrained analysis of the combined GBSSI/ITS data set. The constraint trees are the result of topologically constrained analyses of the combined dataset using the indicated topology as a constraint. All trees were analyzed under the GTR+I+G model of nucleotide substitution. Trees that are significantly different from the best tree are those with P values < 0.05 (marked with an asterisks).

Tree	-ln Likelihood	Difference from best tree	P value
Likelihood tree	12468.48757	(best)	
Tribal constraint topologies			
Garcinieae	12468.48757	0	1.0
Symphonieae	12482.49045	14.00288	0.749
Segregate genera constraint topologies			
Garcinia sensu lato	12470.88296	2.39539	0.984
Ochrocarpos	12628.96953	160.48196	0.002*
Rheedia	12619.25737	150.7698	0.003*
Tripetalum	12552.12847	83.6409	0.045*
Pentaphalangium	12530.57525	62.08768	0.109
Sectional (sensu Jones, 1980) constraint topologies			
Tagmanthera	12468.48757	0	1.0
Discostigma	12519.17918	50.69161	0.230
Hebradendron	12468.48757	0	1.0
Brindonia	12468.48757	0	1.0
Xanthochymus	12539.44691	70.95935	0.071
Garcinia	12468.48757	0	1.0
Rheediopsis	12474.4032	5.91563	0.930
Paragarcinia	12469.38464	0.89707	.993
Rheedia	12487.50074	19.01317	0.670
Jones (1980) hypothesis	12898.04362	429.55605	0.000*

Appendix. GenBank accession numbers, voucher information, and sectional placement (sensu Jones, 1980) for taxa used in this study. An em dash (—) indicates the gene region was not sampled. If there are multiple sequences (i.e., clones) for the same gene from a particular specimen they are separated by a space or when they consist of a consecutively numbered set, the lower and upper accession of the set are provided separated by an en dash (–). Voucher specimens are deposited in the following institutions: AAU = University of Aarhus, FTG = Fairchild Tropical Botanic Garden, KEP = Forest Research Institute Malaysia, MBC = Montgomery Botanic Center, MO = Missouri Botanical Garden, NY = New York Botanical Garden, SING = Singapore Botanic Garden.

***Taxon* [*sectional placement*]**— GenBank accessions: GBSSI, ITS; Source; Voucher specimen.

Allanblackia floribunda Oliver — —, EU128370–EU128371; Wild; *C. Ewango s.n., 21-VI-04*, Democratic Republic of Congo, MO.

Clusia flava Jacq.— EU128337, —; Cult. Climatron MO 951080 (Mexico, *Brunner 2440*); *P. Sweeney 1455*, garden, MO.

Garcinia afzelii Engl. [*Tagmanthera*]— EU128260, EU128429–EU128430; Wild; *P. Sweeney 1427*, Cameroon, Southwest Province, MO.

G. aff. afzelii Oliver [Tagmanthera]— EU128259 EU128261, EU128424–EU128427 EU128431; Wild; *P. Sweeney 1441*, Cameroon, Southwest Province, MO.

G. amplexicaulis Vieill. ex Pierre [*Mungotia*]— EU128280–EU128281, EU128479–EU128480; Wild; *G. McPherson 19127*, New Caledonia, Province du Nord, MO.

G. asterandra Jum. & H. Perrier [unplaced]— —, EU128478; Wild; *H. Schmidt 4314*, Madagascar, Antsiranana, MO.

G. atroviridis Griff. ex T. Anders. [*Brindonia*]— —, EU128374; Cult. SING; *P. Sweeney 1002*, garden, MO.

G. bancana Miq. [*Brindonia*]— EU128244–EU128245, —; Wild; *P. Sweeney* 1104, Malaysia, Selangor, MO.

G. cantleyana Whitmore ***var. grandifolia*** Whitmore [*Hebradendron*]— EU128264–EU128267, EU128477; Wild; *P. Sweeney 1060*, Malaysia, Pahang, MO.

G. cataractalis Whitmore [*Hebradendron*]— EU128240–EU128241, EU128476; Cult. KEP (Malaysia); *P. Sweeney 1050*, garden, MO.

G. celebica L. [*Garcinia*]— EU128242–EU128243, EU128475; Cult. Bogor Botanic Garden VI.A.16 (Indonesia, Java); *P. Sweeney 1028*, garden, MO.

G. chapelieri (Planch. & Triana) H.Perrier [*Garcinia*]— —, EU128474; Wild; *P. Sweeney 1256*, Madagascar, Tamatave, MO.

G. conrauana Engl. [*Tetraphalangium*]— EU128306, EU128470–EU128473; Wild; *S. Moses 961*, Cameroon, Southwest Province, MO.

G. dulcis (Roxb.) Kurz [*Xanthochymus*]— EU128310–EU128312, EU128468; Cult. SING*; P. Sweeney 993*, garden, MO.

G. dumosa King [*Hebradendron*]— EU128268, EU128463–EU128467; Wild; P. *Sweeney 1047*, Malaysia, Selangor, MO.

G. eugeniifolia Wall. ex T. Anderson [*Discostigma*]— EU128272–EU128273, EU128462; Wild; *P. Sweeney 985*, Singapore, MO.

G. fruticosa Lauterb. [*Brindonia*]— EU128246, EU128461; Cult. Bogor Botanic Garden VI.C.118a (South Papua); *P. Sweeney 1025*, garden, MO.

G. gnetoides Hutchinson & Dalziel [*Xanthochymus*]— EU128307, EU128460; Wild; *S. Moses 951*, Cameroon, Southwest Province, MO.

G. griffithii T. Anders. [*Brindonia*]— —, EU128458– EU128459; Wild; *P. Sweeney 982*, Singapore, MO.

G. hombroniana Pierre [*Garcinia*]— —, EU128454–EU128457; Cult. FTG 87627A; *P. Sweeney 1458*, garden, MO[a].

G. hombroniana Pierre [*Garcinia*] — EU128247, —; Wild; *P. Sweeney 1086*, Malaysia, Pahang, MO.

G. kola Heckel ["*G. kola* (2)", *Xanthochymus*]— EU128308, EU128453; Wild; *S. Moses 955*, Cameroon, Southwest Province, MO.

G. kola Heckel ["*G. kola* (1)", *Xanthochymus*]— EU128309, EU128452; Wild; *P. Sweeney 1443*, Cameroon, Southwest Province, MO.

G. lateriflora Blume [*Hebradendron*]— EU128278–EU128279, —; Cult. Bogor Botanic Garden VI.A.17 (Java); *P. Sweeney 1012*, garden, MO.

G. livingstonei T. Anders. [*Teracentrum*]— EU128298, EU128450; Cult. Bogor Botanic Garden VI.A.30 (Tropical Africa); *P. Sweeney 1007*, garden, MO.

G. lucida Vesque [*Xanthochymus*]— EU128299–EU128300, EU128449; Wild; *S. Moses 979*, Cameroon, Southwest Province, MO.

G. malaccensis Hook. f. [*Garcinia*]— EU128248, EU128437; Cult. (South Sumatra); *P. Sweeney 1035*, garden, MO.

G. mangostana L. [*Garcinia*]— EU128249, EU128432– EU128436; Cult. Bukit Timah, Singapore; *P. Sweeney 987*, garden, MO.

G. mannii Oliver [*Tagmanthera*]— EU128262, EU128428; Wild; *D. Kenfack 1651*, Cameroon, Southwest Province, MO.

G. nervosa Miq. [*Xanthochymus*]— EU128313, —; Wild; *P. Sweeney 1076*, Malaysia, Selangor, MO.

G. nigrolineata Planch. ex T. Anders. [*Brindonia*]— EU128250, EU128422–EU128423; Wild; *P. Sweeney 1049*, Malaysia, Negeri Sembilan, MO.

G. opaca King ***var. dumosa*** Whitmore [*Garcinia*]— EU128251–EU128253, EU128421; Wild; *P. Sweeney 1127*, Malaysia, Negeri Sembilan, MO.

G. aff. ovalifolia Oliver [*Rheediopsis*]— —, EU128393–EU128396; Wild; *G. McPherson 17952*, Gabon, Estuaire, MO.

G. ovalifolia Oliver ["*G. ovalifolia* (2)", *Rheediopsis*]— EU128301, EU128417–EU128420; Wild; *S. Moses 960*, Cameroon, Southwest Province, MO.

G. ovalifolia Oliver ["*G. ovalifolia* (1)", *Rheediopsis*]— EU128302, EU128413–EU128416; Wild; *P. Sweeney 1409C*, Cameroon, Southwest Province, MO.

G. pauciflora Baker [unplaced]— EU128294–EU128295, EU128411; Wild; *P. Sweeney 1236*, Madagascar, Tamatave, MO.

G. porrecta Laness.[*Garcinia*]— EU128254–EU128256, EU128410; Cult. Bogor Botanic Garden VI.C.153 (Sumatra); *P. Sweeney 1015*, garden, MO.

G. prainiana King [*Xanthochymus*]— EU128285–EU128286, EU128409; Cult. KEP*; P. Sweeney 1077*, garden, MO.

G. punctata Oliver [*Tagmanthera*]— EU128289–EU128292, EU128391; Wild; *G. Walters 583*, Gabon, Estuaire, MO.

G. rigida Miq. [*Garcinia*]— EU128257–EU128258, EU128408; Cult. Bogor Botanic Garden XXIII.A.221 (North Sulawesi); *P. Sweeney 1019*, garden, MO.

G. rostrata Hassk. ex Hook. f. [*Discostigma*]— —, EU128407; Wild; *P. Sweeney 1071*, Malaysia, Pahang, MO.

G. rostrata Hassk. ex Hook. f. [*Discostigma*]— EU128274–EU128277, —; Wild; *P. Sweeney 1082*, Malaysia, Pahang, MO.

G. rubriflora Boerl., non *G. rubriflora* Engl. [unplaced]— —, EU128406; Cult. Bogor Botanic Garden VI.C.236 (Indonesia, Buru); *P. Sweeney 1024*, garden, MO.

G. scortechinii King [*Hebradendron*]— EU128269–EU128270, EU128401–EU128405; Wild; *P. Sweeney 994*, Singapore, MO.

G. sizygiifolia Pierre [*Brindonia*]— —, EU128400; Cult. Bogor Botanic Garden VI.C.19 (Indonesia, Sarawak); *P. Sweeney 1017*, garden, MO.

G. smeathmannii (Planch. & Triana) Oliver [*Rheediopsis*]— EU128303–EU128304, EU128398–EU128399; Wild; *S. Moses 954*, Cameroon, Southwest Province, MO.

G. spicata Hook. f. [*Xanthochymus*]— EU128314–EU128317, EU128389– EU128390; Cult. Bogor Botanic Garden VI.C.123 (Sri Lanka); *P. Sweeney 1020*, garden, MO.

G. staudtii Engl. [*Rheediopsis*]— EU128305, EU128385–EU128388; Wild; *P. Sweeney 1445*, Cameroon, Southwest Province, MO.

G. subelliptica Merr. [*Xanthochymus*]— EU128318–EU128319, EU128381– EU128384; Wild; *K. F. Chung s.n.*, Taiwan, MO[a].

G. uniflora King [*Hebradendron*]— EU128263, —; Wild; *P. Sweeney 1137*, Malaysia, Pahang, MO.

G. uniflora King [*Hebradendron*]— —, EU128379; Cult. Bogor Botanic Garden VI.C.374 (North Sulawese), *P. Sweeney 1018*, garden, MO.

G. urophylla Scort. ex King [*Hebradendron*]— EU128271, EU128378; Wild; P. *Sweeney 1081*, Malaysia, Pahang, MO.

G. verrucosa Jum. & H. Perrier [*Xanthochymus*]— EU128320–EU128321, EU128376; Wild; *P. Sweeney 1286*, Madagascar, Tamatave, MO.

G. warrenii F. Muell. [*Discostigma*]— EU128287–EU128288, EU128375; Cult. SING 19971504 (Australia, Queensland); *P. Sweeney 997*, garden, MO.

G. xanthochymus Hook. f. [*Xanthochymus*]— EU128322–EU128325, —; Wild; Cult. SING*; P. Sweeney 992*, garden, MO, MO.

Lorostemon coelhoi Paula— —, EU128366–EU128367; Wild; *B. Vicentini s.n.*, Brazil, Amazonas, MO.

Montrouziera sphaeroidea Pancher. ex Planch. & Triana— EU128335, EU128368; Wild; *Cameron 981*, New Caledonia, NY.

Moronobea coccinea Aubl.— EU128336, EU128364–EU128365; Wild; *M. Costa 425*; Brazil, Amazonas, MO.

O. decipiens Baill. [*Paragarcinia*]— —, EU128392; Wild; *P. Sweeney 1410*, Madagascar, Nosy Mangabe, MO.

O. multiflorus O. Hoffmann [*Paragarcinia*]— —, EU128372–EU128373; Wild; *K. Abdul—Salim 132*, Madagascar, Antsiranana, MO.

O. parvifolius S. Elliot [*Paragarcinia*]— EU128293, EU128397 EU128412; Wild; *K. Abdul—Salim 138*, Madagascar, Antsiranana, MO.

O. tsaratananae H. Perrier [*Paragarcinia*]— EU128296–EU128297, EU128380; Wild; *P. Sweeney 1232*, Madagascar, Analamazaotra, MO.

Pentadesma butyracea Sabine— EU128334, EU128369; Cult. Bogor Botanic Garden VI.C.246a (Tropical Africa); *P. Sweeney 1005*, garden, MO.

Pentaphalangium latissimum Lauterb. [*Macrostigma*]— —, EU128451; Cult. Bogor Botanic Garden VI.C.58 (South Papua); *P. Sweeney 1026*, garden, MO.

Rheedia commersonii Planch. & Triana [*Rheedia*]— —, EU128438; Wild; *P. Sweeney 1252*, Madagascar, Tamatave, MO.

R. commersonii Planch. & Triana [*Rheedia*]— EU128328–EU128329, —; Wild; *P. Sweeney 1257*, Madagascar, Tamatave, MO.

R. intermedia Pittier [*Rheedia*]— EU128326, —; Cult. MBC 87622.

R. intermedia Pittier [*Rheedia*]— —, EU128443–EU128446; Wild; *G. Davidse 35685*, Belize, Toledo, MO.

R. macrophylla (C. Mart.) Planch. & Triana ["*R. macrophylla (1)*", *Rheedia*]— —, EU128439–EU128440; Cult. Bogor Botanic Garden VI.A.39 (South America); *P. Sweeney 1010*, garden, MO.

R. macrophylla (C. Mart.) Planch. & Triana ["*R. macrophylla (2)*", *Rheedia*]— EU128327, EU128442 EU128446–EU128448; Cult. MBC 87621; *P. Sweeney 1456*, garden, MO.

R. megaphylla Planch. & Triana [*Rheedia*]— EU128330–EU128331, —; Wild; *P. Sweeney 1242*, Madagascar, Tamatave, MO.

R. urschii H. Perrier [*Rheedia*]— —, EU128377; Wild; *P. Sweeney 1205*, Madagascar, Tamatave, MO.

Symphonia globulifera L.f.— EU128332–EU128333, —; Wild; *M. Gustafsson 502*; Ecuador, Orellana, AAU.

Tripetalum cymosum K. Schum. [*Tripetalum*]— EU128282–EU128284, EU128469; Cult. SING; *P. Sweeney 1000*, garden, MO.

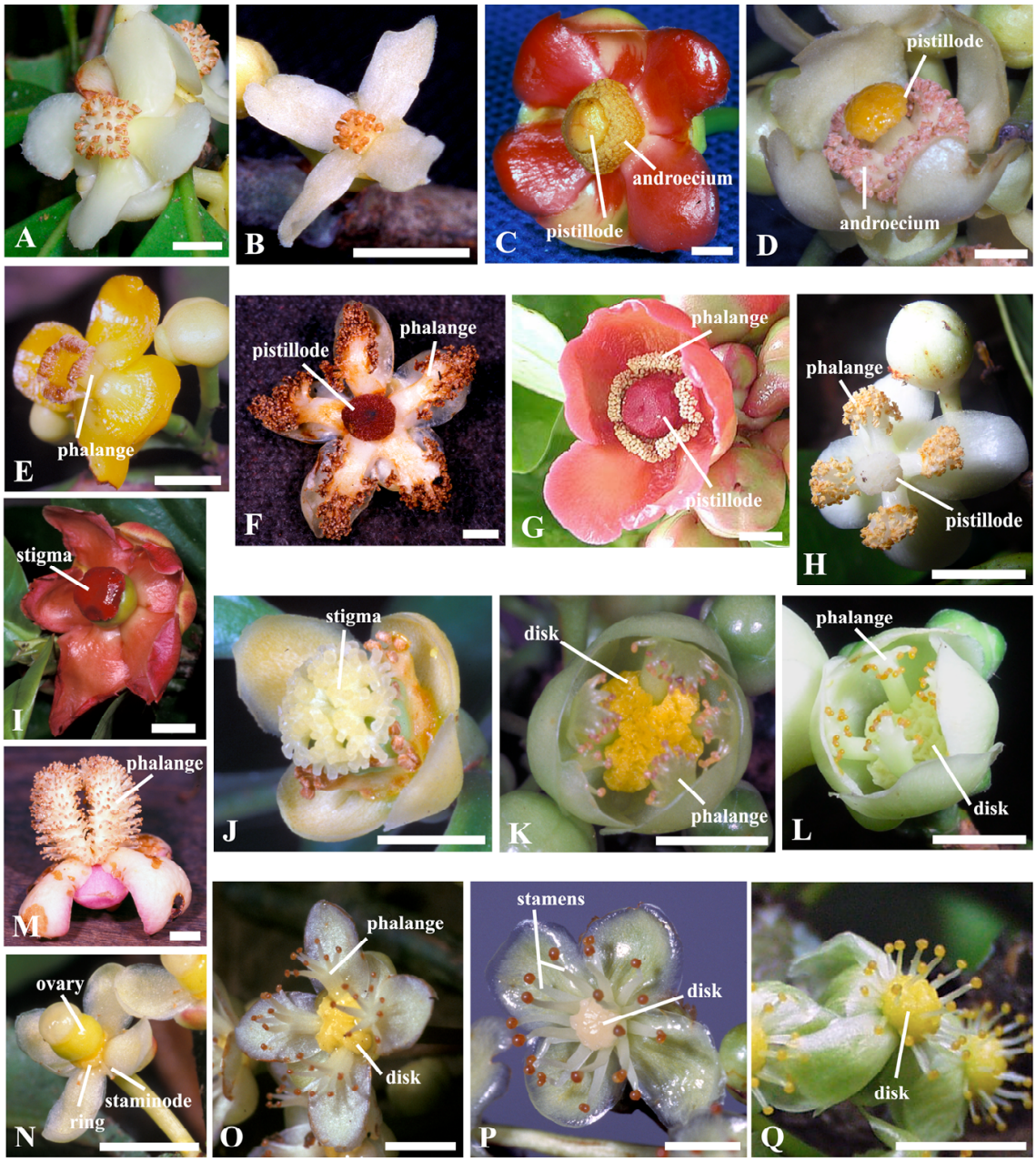
[a] Voucher: fixed material in 70% ethanol

Fig. 1. Images of flowers from representative species of *Garcinia* and its segregate genera. Some features discussed in the text are labeled and scale bars are 10.0 mm. Unless otherwise indicated authors for names are provided in the Appendix and images were taken by the author. A. *Garcinia aff. asterandra*, staminate (Photo: Fortunat Rakotoarivony). B. *G. parvifolia*, staminate. C. *G. atroviridis*, staminate. D. *G. aff. hombroniana*, staminate. E. *G. afzelii*, staminate. F. *Pentaphalangium latissimum*, staminate. G. *G. prainiana*, staminate (© Top Tropicals LLC). H. *Ochrocarpos aff. parvifolius*, staminate (Photo: P. Ranirison, © Conservatoire et Jardin botaniques de la Ville de Genève). I. *G. prainiana*, pistillate. J. *G. cowa* Roxb. ex DC., pistillate. K. *G. xanthochymus*, staminate. L. *G. verrucosa*, staminate (Photo: P. Ranirison, © Conservatoire et Jardin botaniques de la Ville de Genève). M. *G. conrauana*, staminate. N. *Rheedia aff. aphanophlebia* (Baker) H. Perrier, pistillate. O. *G. smeathmannii*, staminate. P. *R. macrophylla*, staminate. Q. *G. livingstonei*, staminate (Photo: H. Brisse).

Fig. 2. 50% majority rule consensus of Bayesian trees of *Garcinia* and relatives inferred from Bayesian analysis of the unpruned ITS data set. Bayesian posterior probabilities are given above branches and maximum parsimony (on left) and maximum likelihood (on right) bootstrap percentages are given below. Sectional names of Jones (1980) are provided to the right of OTUs, or in cases where an entire clade belongs to a section the placement is provided for the clade. Thin vertical lines to the left of OTUs connect clones from a single accession.

Fig. 3. Maximum likelihood tree inferred from combined analysis of ITS and GBSSI data sets. Thick solid lines indicate strong support (≥ 0.95 Bayes & ≥ 85% MP or ML) from at least two analyses while thick dashed lines indicate support from one analysis – all other branches received less than strong support. The sectional placement sensu Jones (1980) is provided next to each OTU, or in cases where an entire clade belongs to a section the placement is provided for the entire clade. Geographic distributions are given for each OTU or clades where applicable, summarized as Africa (AFR), Asia (from India to eastern Pacific, ASIA), Central America (CA), Madagascar (MAD), and South America (SA).

Figure 4. Maximum likelihood phylogenies resulting from the analysis of the combined data set showing ancestral character state reconstructions for the presence vs. absence of fasciclodes and the position of other synapomorphies and character combinations shared by species of major clades. The tree in the inset is the most likely ML tree produced by constraining Symphonieae to be monophyletic and shows the reconstruction of fasciclode occurrence under a parsimony model. The other, larger phylogram resulted from an unconstrained ML analysis (same as that shown in Fig. 3) and shows ancestral reconstruction of fasciclode occurrence under the ML model. Lineages A and B are indicated by vertical white bars and the major Clades (1-9) discussed in the text are indicated by labeled, shaded boxes.


A
B
androecium
pistillode
C
pistillode
androecium
D
E
phalange
pistillode
phalange
F
phalange
pistillode
G
phalange
pistillode
H
stigma
I
stigma
J
disk
phalange
K
phalange
disk
L
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ovary
staminode
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N
phalange
disk
O
stamens
disk
P
disk
Q

Figure 1.

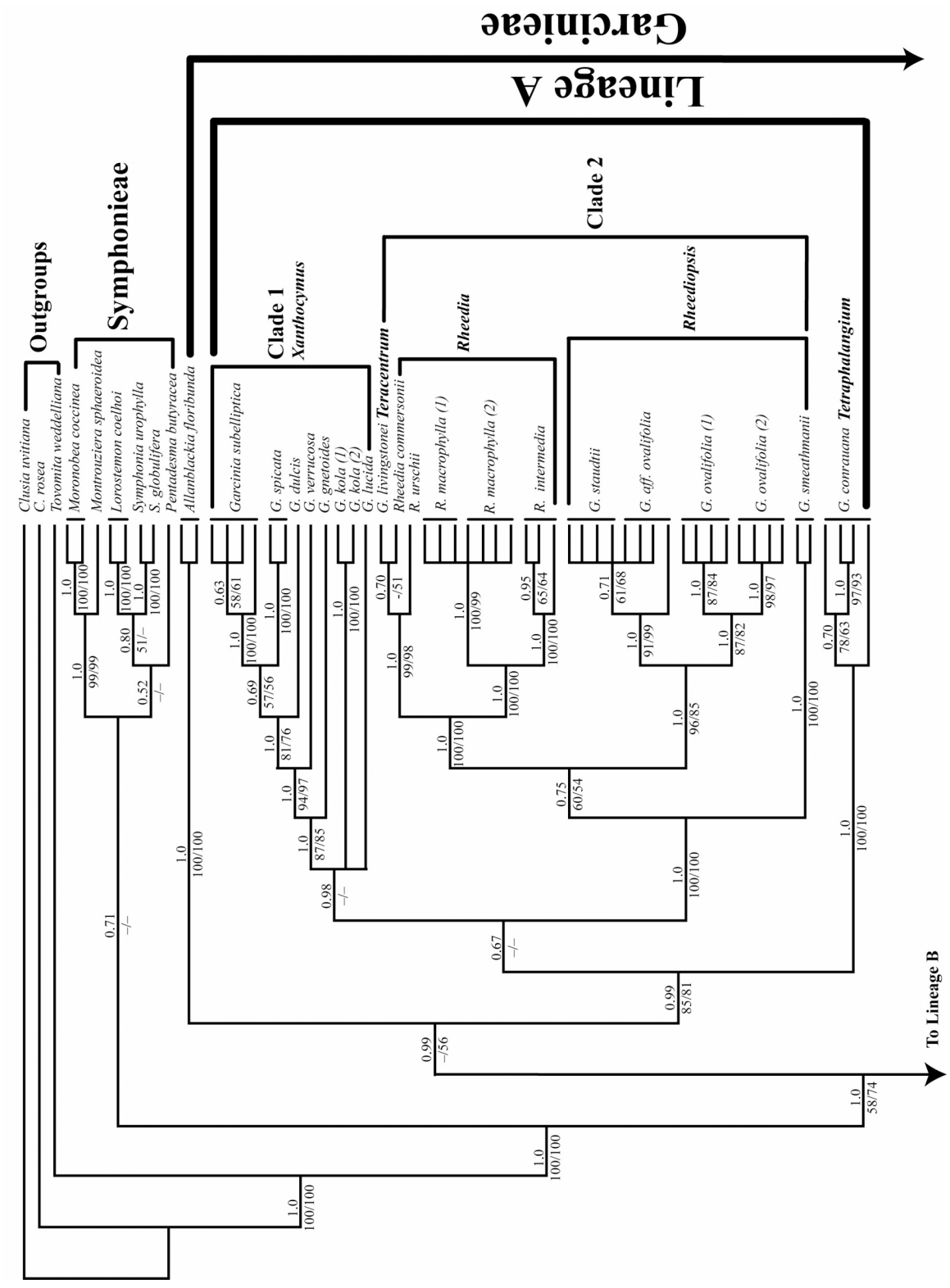

Figure 2, part 1.

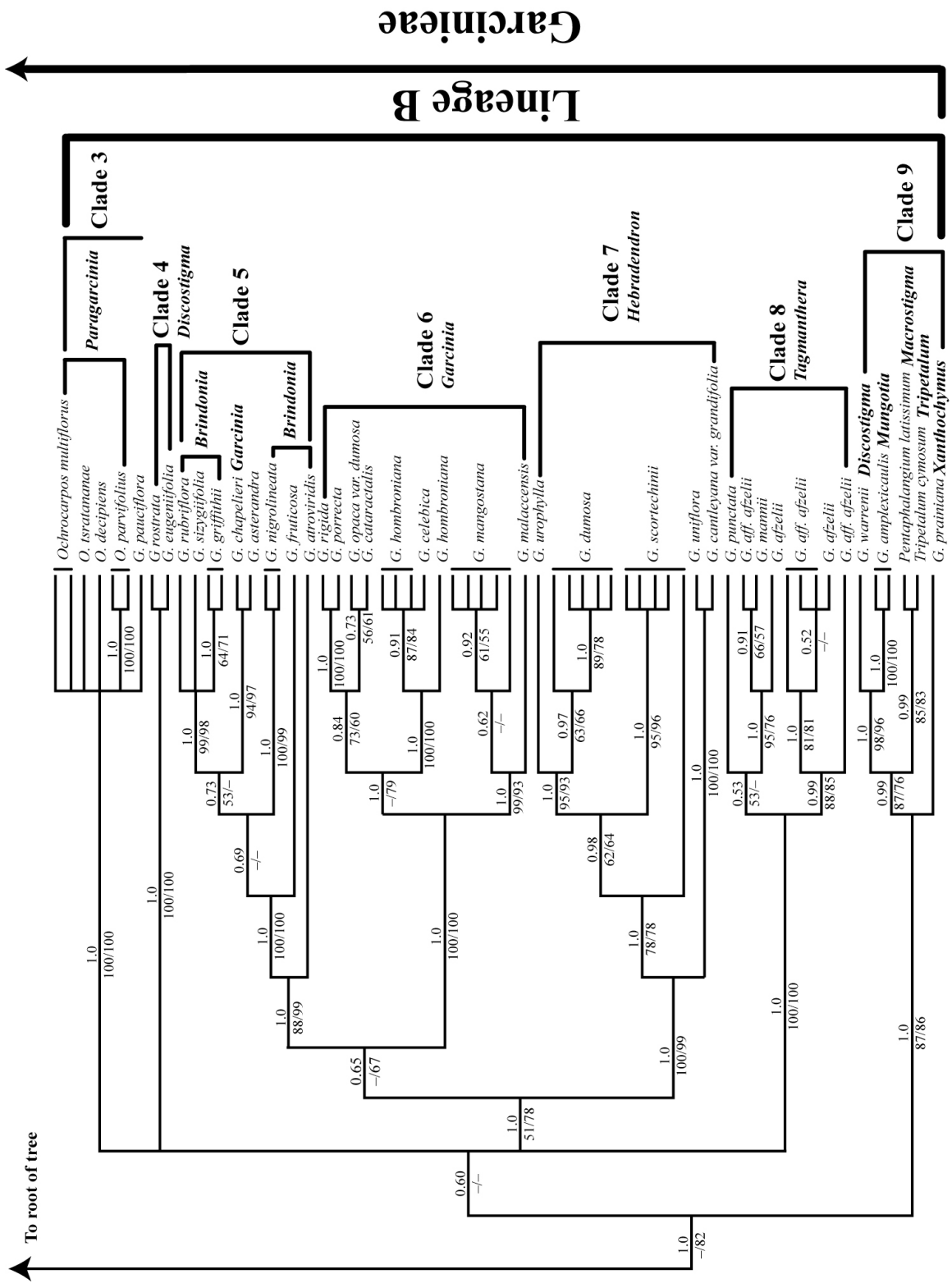

Figure 2, part 2.

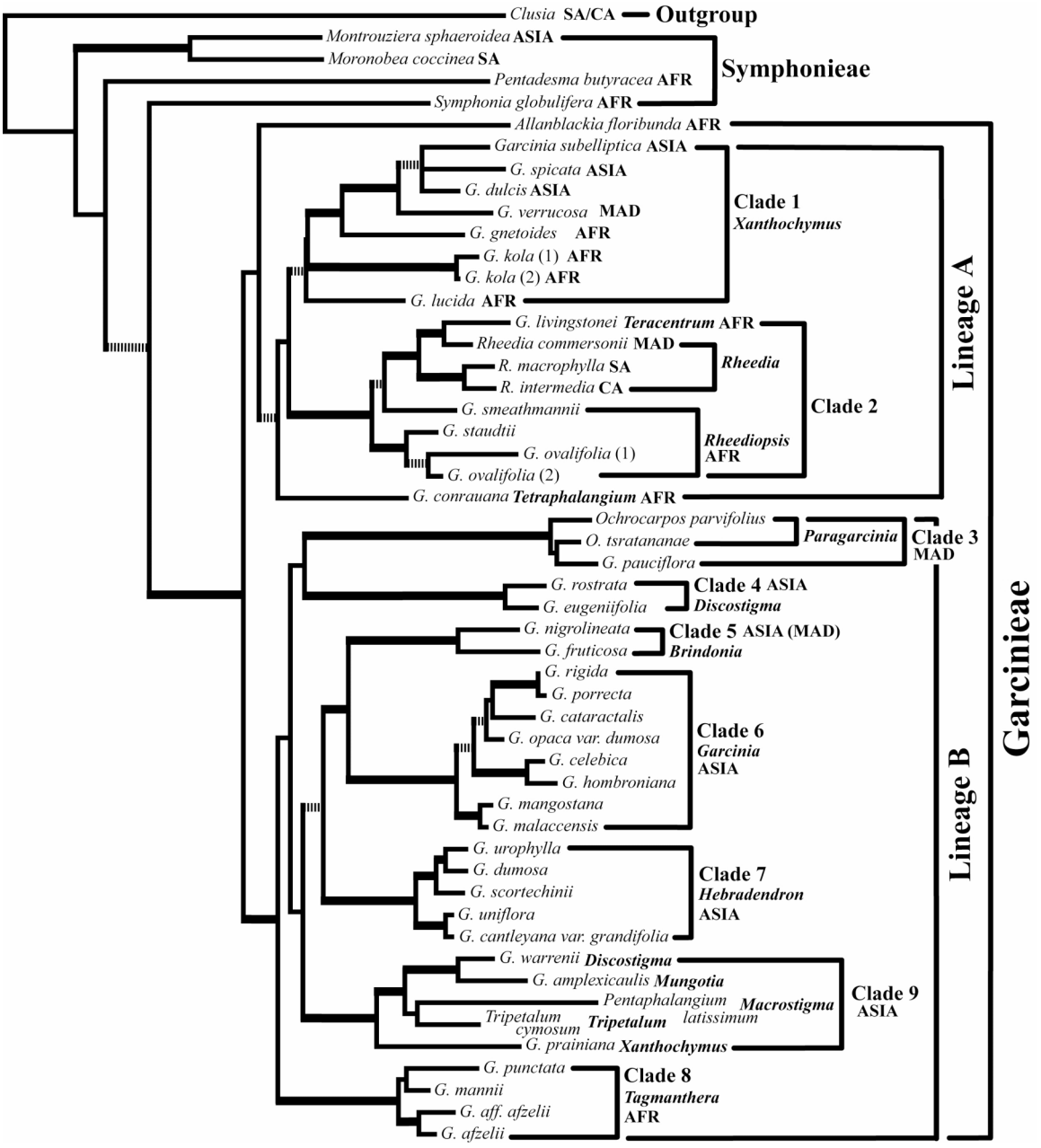
Clusia SA/CA
Outgroup
Montrouziera sphaeroidea ASIA
Moronobea coccinea SA
Pentadesma butyracea AFR
Symphonieae
Symphonia globulifera AFR
Allanblackia floribunda AFR
Garcinia subelliptica ASIA
G. spicata ASIA
G. dulcis ASIA
G. verrucosa MAD
G. gnetoides AFR
G. kola (1) AFR
G. kola (2) AFR
G. lucida AFR
Clade 1
Xanthochymus
G. livingstonei Teracentrum AFR
Rheedia commersonii MAD
R. macrophylla SA
R. intermedia CA
Rheedia
G. smeathmannii
G. staudtii
G. ovalifolia (1)
G. ovalifolia (2)
Rheediopsis
AFR
Clade 2
Lineage A
G. conrauana Tetraphalangium AFR
Ochrocarpos parvifolius
O. tsratananae
G. pauciflora
Paragarcinia
Clade 3
MAD
G. rostrata
G. eugeniifolia
Clade 4 ASIA
Discostigma
G. nigrolineata
G. fruticosa
Clade 5 ASIA (MAD)
Brindonia
G. rigida
G. porrecta
G. cataractalis
G. opaca var. dumosa
G. celebica
G. hombroniana
G. mangostana
G. malaccensis
Clade 6
Garcinia
ASIA
G. urophylla
G. dumosa
G. scortechinii
G. uniflora
G. cantleyana var. grandifolia
Clade 7
Hebradendron
ASIA
G. warrenii Discostigma
G. amplexicaulis Mungotia
Pentaphalangium latissimum
Macrostigma
Tripetalum cymosum
Tripetalum
G. prainiana Xanthochymus
Clade 9
ASIA
G. punctata
G. mannii
G. aff. afzelii
G. afzelii
Clade 8
Tagmanthera
AFR
Lineage B
Garcinieae

Figure 3.

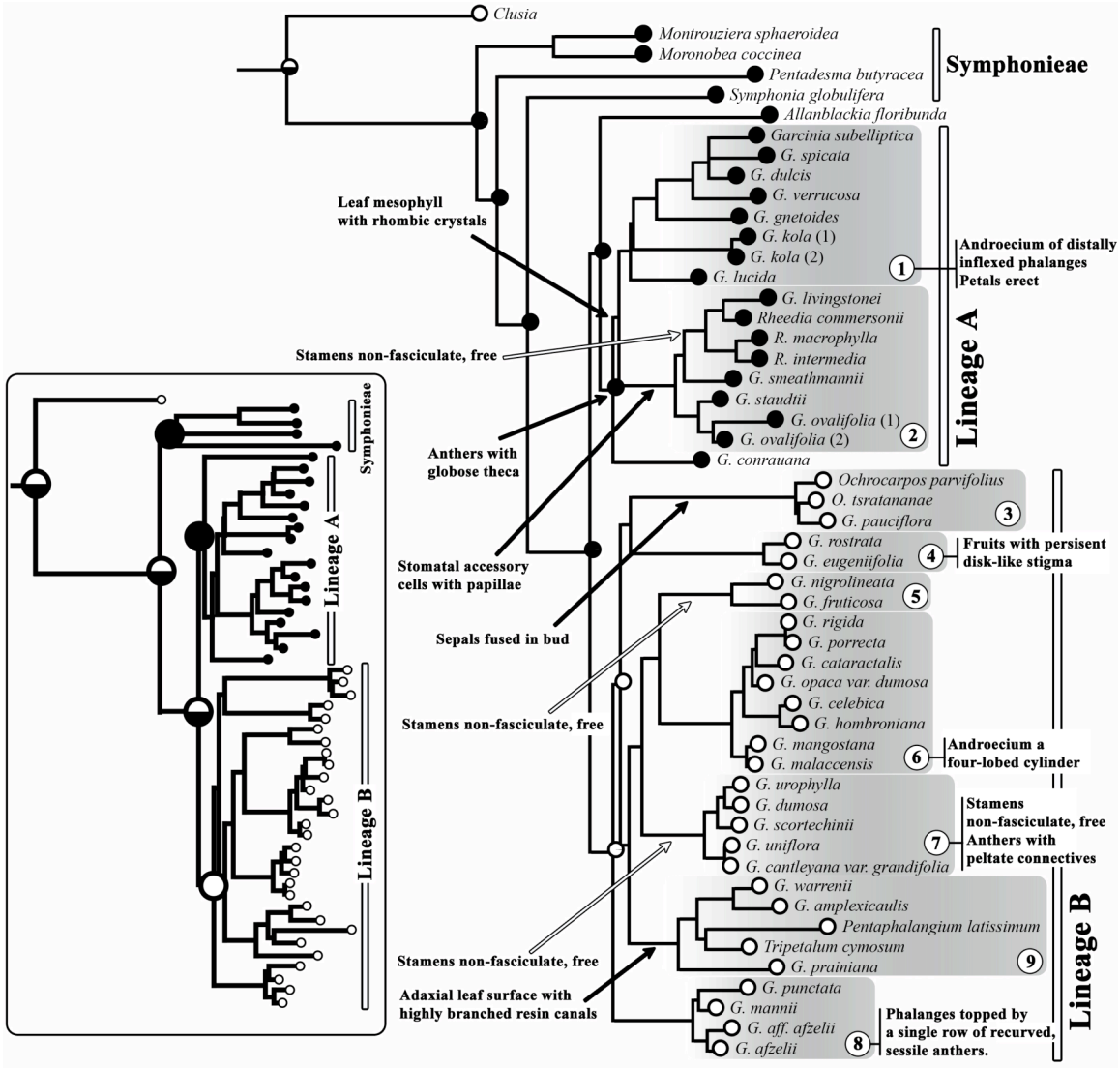
Clusia
Montrouziera sphaeroidea
Moronobea coccinea
Pentadesma butyracea
Symphonia globulifera
Allanblackia floribunda
Symphonieae
Garcinia subelliptica
G. spicata
G. dulcis
G. verrucosa
G. gnetoides
G. kola (1)
G. kola (2)
G. lucida
1
Androecium of distally inflexed phalanges
Petals erect
Leaf mesophyll with rhombic crystals
G. livingstonei
Rheedia commersonii
R. macrophylla
R. intermedia
G. smeathmannii
G. staudtii
G. ovalifolia (1)
G. ovalifolia (2)
2
Lineage A
Stamens non-fasciculate, free
G. conrauana
Anthers with globose theca
Ochrocarpos parvifolius
O. tsratananae
G. pauciflora
3
G. rostrata
G. eugeniifolia
4
Fruits with persisent disk-like stigma
Stomatal accessory cells with papillae
G. nigrolineata
G. fruticosa
5
Sepals fused in bud
G. rigida
G. porrecta
G. cataractalis
G. opaca var. dumosa
G. celebica
G. hombroniana
Stamens non-fasciculate, free
G. mangostana
G. malaccensis
6
Androecium a four-lobed cylinder
G. urophylla
G. dumosa
G. scortechinii
G. uniflora
G. cantleyana var. grandifolia
7
Stamens non-fasciculate, free
Anthers with peltate connectives
G. warrenii
G. amplexicaulis
Pentaphalangium latissimum
Tripetalum cymosum
G. prainiana
9
Stamens non-fasciculate, free
G. punctata
G. mannii
G. aff. afzelii
G. afzelii
Adaxial leaf surface with highly branched resin canals
8
Phalanges topped by a single row of recurved, sessile anthers.
Lineage B
Symphonieae
Lineage A
Lineage B

Figure 4.

Chapter 2

Floral development and anatomy in *Garcinia.*

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Patrick W. Sweeney

Abstract

Molecular phylogenetic studies of *Garcinia* have identified a large clade whose component species can be identified by flowers that have nectariferous antesepalous appendages and intrastaminal rings and disks. The position of these structures in mature flowers does not support the current hypothesis that these structures represent an outer whorl of stamens. To better understand the nature of these structures in *Garcinia*, floral development and anatomy were studied in six *Garcinia* species. An outer whorl, staminodal origin for the disks and appendages is not supported by timing of development or position. Disks and appendages are not apparent until late in development and the disks arise in the center of flower. Anatomical data are equivocal, disks are supplied by traces that arise from the floral stele and appendages receive traces from the floral stele and from stamen trunk bundles. These data also reject a gynoecial origin for these structures, and suggest that these structures are instead intrastaminal receptacular nectaries. Other interesting features of floral development include open carpel development and the lack of a general developmental floral plan in the genus.

Keywords: *Garcinia*, Clusiaceae, anatomy, development, diplostemony, nectary

Introduction

The genus *Garcinia* L. exhibits some of the most extreme diversity of floral form, particularly in the androecium, as is found anywhere in angiosperms (Leins and Erbar 1991; 1997; Sweeney, submitted). Androecia can have their stamens free or united to different degrees and in various ways and the anthers vary in shape and number of loculi. Variation also occurs in the number of parts within whorls, in the degree of fusion between parts within a whorl and between organs of different whorls, in whether pistillodes and staminodes are present or absent, and in whether floral organs of uncertain nature such as antesepalous (i.e., in front of the sepals) appendages or intrastaminal disks are present or absent (Fig. 1).

Garcinia comprises more than 250 species of dioecious small shrubs to medium-sized trees that are a common component of lowland tropical forests, and is probably best known by the fruit of mangosteen (*G. mangostana* L.). A molecular phylogenetic study (Sweeney, submitted) supports a broad circumscription of the genus, the species falling into one of two major clades that can be distinguished by their floral morphologies (Fig. 2). One clade, Lineage A, has species with staminate flowers that lack a well-developed pistillode and that have a fleshy structure (herein called "disk"; Fig. 1A, 1E, 1I) in the center of the flower; pistillate flowers have either fleshy antesepalous appendages alternating with clusters of staminodes united at their base (Fig. 1C, 1G) or ring-shaped structures (herein called "rings") between the staminodes and ovary (Fig. 1J). Some authors have described these structures in *Garcinia* as nectaries (Leins and Erbar 1991), and in some species (e.g., *G. macrophylla* Mart., *G. smeathmannii*, *G. staudtii*) they secrete a watery, sweet-tasting substance (P. Sweeney, pers. obs.). The other major

Garcinia clade, Lineage B, has species that often have a well-developed pistillode in staminate flowers but that lack the disks, appendages, and rings found in Lineage A (Fig 1K–1P).

All of the disks, antesepalous appendages, and rings in *Garcinia* are often interpreted as an outer antesepalous whorl of stamens, because having two alternating whorls of stamens with the outer whorl opposite the sepals is thought to be the ancestral condition for Clusiaceae (Robson 1961, 1972; Jones 1980; Stevens 2006). In staminate flowers of Lineage A (Fig. 1), there is a fertile whorl of stamens that may be free (Fig. 1I) or bundled (Fig. 1A, 1B, 1E, 1F); in the latter case they are called phalanges. This whorl surrounds an often lobed disk in the center of the flower. Because the disk lobes are opposite the sepals, the disk has been interpreted as the outer staminal whorl and thus as androecial (Robson 1961, 1972; Bamps et al. 1978; Jones 1980). In pistillate flowers of Lineage A (Fig. 1), some species have what are thought to be an inner whorl of stamen-like (but sterile) antepetalous stamen bundles ("staminodes") that alternate with antesepalous structures (herein called "appendages") (Fig. 1C, 1D) that have been interpreted as an outer whorl of highly reduced sterile, stamen bundles (“fasciclodes”) (Robson 1961, 1972; Jones 1980). In other species, the inner whorl is thought to be represented by the fleshy ring (Fig. 1J), whereas the outer whorl consists of free staminodes that surround or are embedded in the ring, (Robson 1961, 1972; Jones 1980). In both types of flowers, these whorls surround the ovary.

The position of the disks, antesepalous appendages, and rings argues against their being an outer whorl of stamens. The disks in the staminate flowers of Lineage A are inside what is interpreted to be the inner whorl of stamens. The pistillate flowers of some

species in this clade (i.e., former *Rheedia* species and *G. livingstonei* and relatives; Fig. 2) have a ring, instead of antesepalous appendages, between the ovary and the free, staminodes, an unusual position for an outer staminal whorl. It is possible that the androecium of *Garcinia* is obdiplostemonous (i.e., where the antepetalous stamens appear to be the outer whorl), as in some Malpighiales such as Malpighiaceae, although there is no evidence of a phylogenetic relationship between the two.

Instead of being staminodes, the disks, appendages, and rings in *Garcinia* may be nectaries that are associated with some other organ of the flower or with the receptacle (cf. intrastaminal receptacular nectaries, Schmid 1988). Nectariferous floral structures occur in several genera of Clusiaceae and closely related families (Fig. 3, the "Clusiaceae alliance", Kubitzki 2006). *Allanblackia* has a floral morphology similar to that of *Garcinia* Lineage A, with a nectariferous disk (Hutchinson et al. 1954) in the staminate flowers and antesepalous appendages in pistillate ones. *Symphonia*, which is closely related and possibly sister to *Garcinia* plus *Allanblackia*, has a nectariferous, lobed ring outside the staminal column (Gill et al. 1998; Abdul-Salim 2002; Stevens 2006). Within Bonnetiaceae, the possible sister group of Clusiaceae (Davis et al. 2007), *Archytaea* Mart. and *Ploiarium* Korth. have antesepalous "nectariferous tissue pads" alternating with antepetalous stamen fascicles (Dickison and Weitzman 1998). *Harungana madagascariensis* Poir. (in Hypericaceae, outside Clusiaceae/Bonnetiaceae) has antesepalous nectariferous appendages alternating with antepetalous stamen phalanges (Ronse De Craene and Smets 1991).

As in *Garcinia*, the nectariferous structures in the species of the Clusiaceae alliance discussed above, as well as disks and antesepalous appendages in other species

(all species of Symphonieae and most Hypericaceae) (Fig. 3), have been interpreted as an outer whorl of stamens (Robson, 1972). Developmental and anatomical data support this in some species. Development and anatomy support that the nectar-secreting antesepalous appendages in flowers of *Harungana madagascariensis* are staminodal and represent the outer stamen whorl (Ronse De Craene and Smets 1991). The antesepalous nectariferous tissue pads of *Archytaea* and *Ploiarium* are supplied by double bundles, like the fertile stamen fascicles, and consequently are interpreted to be staminodal (Dickison and Weitzman 1998).

Thus in the Clusiaceae alliance, in some groups (e.g., Bonnetiaceae and *Harungana madagascariensis*) there is evidence that nectaries are staminodal, while in *Garcinia* structures that appear to be functioning as nectaries may be non-staminodal, possibly associated with other organs or with the receptacle. Nectaries, which are most often defined by their function, have evolved multiple times within eudicots (Lee et al. 2005b; Bernardello 2007) and are present in many different families of Malpighiales (Bernardello 2007). Furthermore, nectaries are variable in their structure and their location, being associated with floral organs or with the receptacle (Schmid 1988; Smets and Cresens 1988) and it is not uncommon for their location to vary within a family or even within a genus (Bernardello 2007).

To explore the hypothesis that the disks, appendages, and rings in *Garcinia* represent an outer whorl of modified stamens, developmental and anatomical data were collected from six species of *Garcinia*. If the disks, appendages, and rings in *Garcinia* represent an outer whorl of stamens then they should share developmental (e.g., timing and position of initiation) and anatomical features (e.g., vascularization) with the outer

whorl of stamens in other Malpighiales and eudicots. Alternatively, these structures might not be an outer whorl of stamens and instead could be nectaries derived from other floral organs or associated with the receptacle. As this is the first study to provide detailed developmental data for multiple species from across the *Garcinia* phylogeny, additional features of anatomy and development are documented and discussed as well.

Material and Methods

Anatomical, developmental, and morphological studies were carried out on the taxa listed in Table 1. For anatomical and developmental studies, buds and flowers were field collected into 70% EtOH and later dissected under a stereomicroscope. For developmental studies, dissected specimens were dehydrated in an ethanol series (Ruzin 1999), critical-point dried in a Structure Probe Incorporated (SPI) SPI-DRY™ Jumbo or a Tousimis Samdry®-795 critical point drier, and then sputter coated with gold particles using a Polaron E5000 or a Tousimis Samsputter®-2a. The dried and coated specimens were viewed under an Amray AMR1000 or a Hitachi S-2600 H scanning electron microscope (SEM) where they were photographed on Polaroid™ Type 55 film and then scanned into a digital format or digital images were captured directly. For anatomical studies, dissected buds and open flowers were dehydrated, infiltrated with Histoclear™, and then embedded in paraffin following the protocols in Ruzin (1999). Sections that were 8–15 μm in thickness were produced using a rotary microtome. Sections were mounted on slides and then were stained with Safranin O/Fast Green FCF (Ruzin 1999).

Results

General features of anthetic flowers

The flowers are unisexual, four- or five-merous, and polyandrous (Fig. 1). The sepals and petals are imbricate in aestivation. The androecium consists of phalanges equal in number and opposite to the petals or it consists of a cylinder covered abaxially with stamens (in *G. atroviridis*). In some pistillate flowers the androecium consists of clusters of free staminodes that are opposite the petals (in *G. xanthochymus*). Stamens are conventionally bithecate (but multilocellate in *G. afzelii*), each theca dehiscing by a single longitudinal slit. Anthers are supported by filaments or are sessile (in *G. afzelii*). Functional ovaries are two- to five-locular and superior. Placentation is axile, and each locule contains one ovule.

General features of floral development

Few features are shared among all species and the major differences among the anthetic flowers are visible early in development. In four-merous flowers, the outer two sepals develop first and enclose the rest of the developing flower, and the inner two sepals develop simultaneously and before the petals and androecium (e.g. Figs. 9B, 10A, 11A). In five-merous flowers the sepals are initiated in a spiral sequence (e.g. Figs. 5A, 5B, 7A–7C). In all species examined, an angular meristem is present after all of the sepals have initiated and petals are initiated in the angles of the meristem (e.g. Figs 4A, 5D, 7C, 9B, 10A, 11A). In species without a ring-mound stamen primordium, primary stamen primordia are initiated at the same time, are equal in number to, and are opposite the petals (e.g. Figs. 4C, 5G, 9C, 10B, 11B). In four-merous flowers, the petals and phalanges initiate simultaneously (Figs. 9C, 10B, 11B). In five-merous flowers the petal and androecial whorls are initiated in a spiral sequence (Figs. 4C, 5B, 5F, 7B, 7F).

Functional and rudimentary gynoecia, when present, are initiated shortly after or at approximately the same time as the corolla and androecium.

Garcinia xanthochymus

Gross morphology shared by staminate and pistillate flowers. The perianth is five-merous and the calyx and corolla are quincuncial in aestivation (Fig. 1A–1D). In mature flowers the petals are erect with overlapping edges and the corolla forms a bowl-shaped structure (Fig. 1A). The androecium consists of five groups of stamens (staminodes in pistillate flowers) opposite the petals (Fig. 1A–1D). Anthers are introrse with two globose thecae that dehisce via a small slit.

Developmental morphology shared by staminate and pistillate flowers. After differentiation and expansion of the sepals the apical meristem is pentagonal in shape (Figs. 4A, 5D). Petal primordia are initiated in a clockwise or counter-clockwise spiral sequence in the angles of the meristem (Figs. 4B–4D, 5F, 5G). At about the same time that the petals are initiated, primary stamen (staminode in pistillate flowers) primordia are apparent opposite the petal primordia (Figs. 4B–4D, 5F, 5G). It is not clear if the petal and stamen (staminode in pistillate flowers) primordia arise simultaneously from the receptacle or if they are differentiated from a common petal-stamen (staminode) primordium by formation of a slit. The primary stamen (staminode in pistillate flowers) primordia first expand into a hemispherical bulge (Figs. 4D, 4E, 5G, 5H) and then later initiate individual secondary stamen (staminode in pistillate flowers) primordia along their periphery (Figs. 4E–4G, 5H).

Gross morphology of staminate flowers. The androecium consists of five, antepetalous phalanges (Fig. 1A, 1B). The ends of the phalanges arch over the center of

the flower and are covered by ca. 12 stamens with short filaments (Fig. 1A). A fleshy, five-lobed, pitted disk occupies the center of the flower with the lobes positioned between the phalanges and opposite the sepals (Fig. 1A, 1B). A stomate occupies the bottom of each disk pit. In center of the disk of some flowers there is a slender projection that is occasionally capped by a smooth flattened structure.

Developmental morphology of staminate flowers. Calyx development was not observed in staminate flowers of this species. Within each primary stamen primordium up to ca. 12 individual secondary primordia initiate in a centrifugal direction (Fig. 4F, 4H) and these develop into individual stamens. The anther differentiates first followed by elongation of the filament (Fig. 4H–4K). The base of the phalange begins to elongate after development of individual stamen primordia (Fig. 4L). After the petal and primary stamen primordia have begun to expand, a low bulge is formed in the center of the flower (Fig. 4F, FH, 4J). Later the bulge elongates to form a slender appendage (Fig. 4L, 4M). The central disk begins to expand after differentiation and development of the petal and phalange primordia are well underway (Fig. 4J, 4L).

Gross morphology of pistillate flowers. The rudimentary androecium consists of five groups of staminodes that are opposite the petals (Fig. 1C, 1D). The staminodes can all be free or variously united into phalanges. There are fewer staminodes in each phalange (ca. 3–4) than there are stamens in staminate flowers, and individual staminodes have longer filaments. The staminodes have antherodes with two globose thecae that have small slits. The gynoecium is five locular and is capped by a five-branched stigma, the distal ends of which are covered with papillae. Antesepalous appendages alternate

with the phalanges. The appendages are covered with pits that have a stomata-like pore at their base.

Developmental morphology of pistillate flowers. Five sepals are initiated in a clockwise or counter-clockwise spiral sequence (Fig. 5A–5D). As the petal and primary staminode primordia differentiate, or soon after, the center of the flower begins to swell to form what is presumed to be the gynoecium (Fig. 5G, 5H). Stages between this and later ones where all organs are well formed were not available. In later developmental stages the filaments of each staminode elongate while elongation of the stalk of the phalange is less pronounced (Fig. 5I–5L). Antesepalous appendages are not apparent until late in development and these expand until the flowers reach anthesis (Fig. 5I–5L). The highly pitted and textured morphology of antesepalous appendages appears near anthesis (Fig. 5L). Stomata are present in the bottom of the pits (Fig. 5M–5O).

Anatomy of pistillate flowers. The pedicel has a stele comprised of separate bundles (Fig. 6A). As the sepals arise in a spiral sequence, between four and six traces branch off to supply each sepal (Fig. 6B), and these bundles further branch. After traces are given off to all of the sepals, the stele is roughly pentagonal in shape (Fig. 6C). Next a trace branches off to supply the petals (Fig. 6D – black arrows), and then two traces flanking each petal trace branch off to supply the staminodes (Fig. 6D – white arrows). Shortly after the staminodal traces depart, some bundles in the floral stele branch and send traces to the antesepalous appendages (Fig. 6E – gray arrows), and these traces then further divide. The staminodal bundles also give off other traces that supply the antesepalous appendages (Fig. 6F – gray arrows), and these then branch as they enter the appendages; the staminodal bundles continue on to supply the staminodes (Fig. 6G–6I –

white arrows in 6G, 6H). The floral stele continues upward and at a point where the individual staminodes begin to differentiate from the receptacle, traces arise from the stele that move toward the center of the flower to form a central ring of vascular bundles (Fig. 6H–6K). The bundles in the central ring are the ventral bundles of the carpels, and the other bundles of the stele comprise the medial and dorsal bundles (Fig. 6K). At the point when the ventral bundles form a central ring, the outline of five carpels is visible (Fig. 6K). Still higher up, five locules, each with a single ovule, are visible (Fig. 6L, 6M). The traces branch off from the ventral bundles to supply the ovules; placentation is axile (Fig. 6M). Toward the apex of the ovary, the flanks of the carpels are not completely fused and a pentagonal area, possibly a compitum, occupies the center of the ovary (Fig. 6N).

Garcinia nervosa

Only staminate flowers were available for this species.

Gross morphology. The morphology of mature flowers is similar to that described above for *G. xanthochymus.* The perianth is five-merous and the calyx and corolla are quincuncial in aestivation. The petals are erect with overlapping edges and the corolla forms a bowl-shaped structure. The androecium consists of five phalanges, and each is opposite a petal. The ends of the phalanges arch over the center of the flower and are covered by ca. 12 stamens. A fleshy, five-lobed, pitted disk occupies the center of the flower with the lobes protruding between the phalanges. A stomate-like pore occupies the bottom of each disk pit. Occasionally an elongated appendage arises from the center of the disk.

Developmental morphology. The sepals are initiated in a clockwise or counter-clockwise spiral sequence (Fig 7A–7C) and expand to cover the rest of the developing flower (Fig. 7D). After differentiation of the sepals the apical meristem is pentagonal in shape (Fig. 7C, 7E) and development proceeds in a manner similar to that of the staminate flowers of *G. xanthochymus*. Petal primordia develop in a clockwise or counter-clockwise spiral sequence in the angles of the meristem. The petal primordia expand over the phalange primordia (Fig. 7F–7I) and eventually cover the center of the flower (Fig. 7J). Primary stamen primordia are initiated opposite the petal primordia. The phalange primordia expand into a hemispherical shape (Fig. 7F–7G) and begin to form along their periphery protuberances that differentiate into secondary stamen primordia, which become stamens (Fig. 7F–7I). Individual stamen primordia develop in a centrifugal direction (Fig. 7H) with the anthers differentiating first.

Anatomy. The pedicel has a pith and cortex of large celled parenchyma and a stele comprised of separate bundles (Fig. 8A). As the sepals arise in a spiral sequence, traces branch off to supply each sepal (Fig. 8B–8D – gray arrows) and these then branch to supply the sepal as it broadens (Fig. 8E). After traces are given off to all of the sepals, the vasculature of the stele is confined to five areas (Fig. 8E, 8F – black arrows; the grey arrow points to a trace supplying the last formed sepal). Next, common petal-phalange bundles are given off in quick succession (Fig 8G, 8H – black arrows), and traces branch off to the disk (Fig. 8G, 8H – black and white striped arrows). The bundles running to the disk ramify (Fig. 8H–8J) and terminate before reaching the area that has cells with densely staining cytoplasm. The common petal-phalange bundles branch with one trace supplying the petals (black arrows Fig. 8I, 8J) and the other the phalanges (white arrows -

Fig. 8I, 8J). The petal bundles branch into several traces that spread out to supply the petals (Fig. 8I–8L). The phalange bundle branches repeatedly to yield traces that supply the filament of each individual stamen within the phalange (Fig. 8J–8N – white arrows in 8J, 8K). The cells of the disk contain dense, darkly staining granular cytoplasm (Fig. 8I-N). Towards the apex of the flower, in the center of the disk in the base of the elongated appendage, the cells are less darkly staining than those of the disk, and no vascular tissue was visible in this area (Fig. 8M, 8N).

Garcinia smeathmannii

Only staminate flowers were available for this species.

Gross morphology. The perianth is four-merous and the calyx and corolla are opposite-decussate in aestivation (Fig. 1E, 1F). The petals are spreading at anthesis (Fig. 1E). The androecium is phalangiate with a staminal phalange opposite each petal (Fig. 1E, 1F). Each phalange includes ca. 9–10 stamens that are free for approximately half the length of the phalange (Fig. 1E). A four-lobed disk occupies the center of the flower, and the lobes are positioned between the phalanges (Fig. 1E, 1F).

Developmental morphology. The outer two sepals develop first and enclose the rest of the developing flower (Fig 9A). After initiation of the inner two sepals the apical meristem is approximately square in shape (Fig. 9B). Four petal primordia initiate at about the same time in the corners of the meristem (Fig. 9C), and expand to cover the developing stamens (Fig. 9D–9L). Four primary stamen primordia develop concurrently with the petals (Fig. 9C–9L). Soon after the primary stamen primordia initiate, secondary stamen primordia, which will become the individual stamens, appear in a centrifugal direction on their surface (Fig. 9D–9F). On each primary stamen primordium, anthers

differentiate first (Fig. 9I–9L). The central disk is not apparent in early stages of development (Fig. 9J).

Garcinia staudtii

Only pistillate flowers were available for this species.

Gross morphology. The perianth is four-merous and the calyx and corolla are opposite-decussate in aestivation (Fig. 1G, 1H). The petals are spreading at anthesis (Fig. 1G). The androecium is phalangiate with a staminodal phalange opposite each petal (Fig. 1G, 1H). Within each phalange there are usually two to three staminodes with short filaments, but there can be fewer staminodes and they can be completely free. The gynoecium consists of a two-locular ovary capped by a sessile, two-lobed stigma (Fig. 1G, 1H). Antesepalous appendages alternate with the phalanges.

Developmental morphology. The outer two of the four sepals develop first and enclose the rest of the developing flower. The inner two sepals then develop simultaneously and expand to partially cover the rest of the developing flower (Fig. 10A–10E). After the inner sepals have differentiated, the apical meristem is approximately square in shape (Fig. 10A). Four petal primordia initiate approximately simultaneously in the corners of the meristem and expand to partially cover the center of the flower (Fig. 10B, 10F–10K). Four primary staminode primordia develop concurrently with the petals (Fig. 10B, 10D, 10F–10H, 10J) and first expand into a flattened hemispherical bulge (Fig. 10B, 10F). Individual staminode primordia develop along the periphery of the phalange primordia with the central staminode developing first (Fig. 10G, 10H, 10J). At the same time or shortly after the petals and phalanges have initiated, but before the individual staminode primordia initiate, the gynoecium is initiated and develops first as a swollen

area in the center of the flower (Fig. 10F). After the individual staminode primordia appear, a gynoecial ridge, placenta, and developing ovules are apparent in the center of the gynoecium (Fig. 10I, 10J). The ovules are exposed during early development (Fig. 10I). The gynoecial ridge expands upward to form the ovary wall (Fig. 10I–10K). The four antesepalous appendages are not apparent until late in development (Fig. 10L). Stomata are present on the antesepalous appendages (Fig. 10N).

Garcinia afzelii

Gross morphology shared by staminate and pistillate flowers. The perianth is four-merous and the calyx and corolla are opposite-decussate in aestivation (Fig. 1K–1N). The petals are spreading at anthesis. The center of the flower is occupied by a globose functional ovary or mushroom-shaped pistillode (Fig. 1K–1N).

Developmental morphology shared by staminate and pistillate flowers. The outer two sepals develop first and enclose the rest of the developing flower. The inner two sepals develop approximately simultaneously (Fig 11A, 11B) and expand to partially cover the rest of the developing flower. After the inner two sepals have differentiated the apical meristem is approximately square in shape (Fig. 11A). The four petal primordia develop approximately simultaneously from the corners of the meristem and expand to cover the center of the flower (Figs. 11B–11G, 12A–12D). The gynoecium initiates at the same time as the petals; it is discernable early in development as a lobed swelling in the center of the flower (Figs. 11B, 11C, 12A, 12B) and further develops as four congenitally fused carpel primordia (Figs. 11D, 11E, 12E). The carpel walls expand upward and their margins fuse to enclose the central area of the flower (Figs. 11F, 12F).

Gross morphology of staminate flowers. A strap-shaped staminal phalange is positioned opposite each petal (Fig. 1K, 1L). The distal end of the phalange is covered by a row of up to ca. 8 sessile recurved anthers (Fig. 1K). The anthers are multilocellate with each theca having two rows of locelli – that is each microsporangium is separated into multiple chambers. The center of the flower is occupied by a mushroom-shaped pistillode (Fig. 1K, 1L).

Developmental morphology of staminate flowers. Four primary phalange primordia develop concurrent with and opposite the petals (Fig. 11B–11O) and first expand into a flattened hemisphere shape (Fig. 11B–11D). The phalanges soon develop individual stamen primordia along their outer edge (Fig. 11H, 11I). The individual anthers are initiated with a recurved orientation that is retained until anthesis (Fig. 11I–11N). Late in development the locelli appear as two rows of bumps on the surface of the anthers (Fig. 11L–11O – white arrows in 11N). The bases of the phalanges elongate only late in development (Figs. 11I–11N). The pistillode initiates at the same time as the petals and phalanges and is discernable early in development as a lobed swelling in the center of the flower (Fig. 11B, 11C). The pistillode develops at the same time as the petals and phalanges and develops as four congenitally fused carpel primordia (Fig. 11D, 11E).

Gross morphology of pistillate flowers. Staminodes and staminodal phalanges are absent (Fig. 1M, 1N). The gynoecium consists of a four-locular, globose ovary that is capped by a sessile, capitate stigma (Fig. 1M, 1N).

Developmental morphology of pistillate flowers. The gynoecium initiates at the same time or shortly after the petals and is first visible as a swelling in the center of the

flower (Fig. 12A). No trace of an androecium is discernable at any stage of development. Four carpel primordia are first discernable as four protuberances (Fig. 12B) that later differentiate into four hemispherical lobes (Fig. 12E). These lobes expand and fuse to form the stigma while the lower portion of the gynoecium develops into a four locular ovary (Fig. 12F–12H).

G. atroviridis

Only staminate flowers were available for this study.

Gross morphology. Mature flowers are four merous and the perianth is opposite-decussate in aestivation (Fig. 1O, 1P). The androecium consists of a cylinder that is covered on its abaxial surface by numerous (> 200) bithecous anthers (Fig. 1O, 1P). The center of the flower is occupied by a globe-shaped pistillode that is capped by a disk-shaped stigma (Fig. 1O, 1P). Up to eleven rudimentary ovules were observed in some pistillodes, while others had none.

Developmental morphology. Early in development four petals are visible as lobes positioned around the androecium, which is an undifferentiated ring (Fig. 13A). The petals expand as individual stamens develop on the ring-mound primary primordium (Fig. 13B–13D). Individual stamen primordia first appear on the ring-mound primordium as protuberances and are initiated in a centrifugal direction (Fig. 13B–13F). The pistillode is visible early in development as an undifferentiated swollen area in the center of the flower (Fig. 13A). The swollen area develops individual hemispherical carpel primordia and these are fused together to into a ring (Fig. 13D). In later stages, the carpel walls expand and enclose the central area of the flower (Fig. 13E, 13F).

Discussion

Disks, appendages, and rings

Disks, appendages, and rings as nectaries. Schmid (1988: 187) defines nectaries in functional and anatomical terms as a "...localized, often multicellular glandular structure that occurs on vegetative or reproductive organs and that regularly secretes nectar, a sweet solution containing mainly sugars and generally serving as a reward for pollinators...". Gross morphology and field observations suggest that the disks, appendages, and rings in *Garcinia* are nectariferous. A sweet tasting, watery exudate has been observed on the disks of staminate flowers of *G. macrophylla*, *G. smeathmannii*, and *G. staudtii* (P. Sweeney, pers. obs.). The presence of stomata, a common feature of nectaries, on the disks and appendages of the species examined here, as well as their bright yellow color, perhaps a visual attractant and/or a by product of starch hydrolysis (Horner et al. 2007), is further evidence that they may function as nectaries. Nectaries cannot be defined anatomically (Nepi 2007), but the disks and appendages examined in this study have stomata and are comprised largely of densely staining cytoplasm (cf. "nectary parenchyma", Nepi, 2007), both characteristics that are exhibited in many nectaries. Based on the developmental and anatomical evidence presented here the nectaries in *Garcinia* can be classified as receptacular nectaries (Schmid 1988).

Staminal origin of disk and appendages. The timing and position of development of the disks and appendages in the flowers examined in this study do not support the hypothesis that these structures represent an outer whorl of stamens in *Garcinia*. In the staminate flowers of *G. nervosa, G. smeathmannii*, and *G. xanthochymus*, the disk is not apparent until late in development (Figs. 4, 7, 9) long after the petals and phalanges have initiated and have begun to expand. Likewise, the antesepalous appendages in the

pistillate flowers of *G. xanthochymus* and *G. staudtii*, are not visible until late in development (Figs. 5, 10). In regards to position of development, the disk arises in center of flower in staminate flowers of *G. xanthochymus* and *G. smeathmannii*, not outside the inner whorl of stamens, and in the spaces between the phalanges. If these structures were staminodal and represented the outer whorl of stamens they would be expected to arise before (or perhaps with) (Endress 1994; Hufford 1996b) and outside the whorl of stamens.

Ronse De Craene and Smets (1991) observed that the antesepalous appendages of *Harungana madagascariensis* also arise late in development. In development, these structures in some respects resembled certain stages in the development of the stamen phalanges. They are vascularized by traces that arise from the stele like those of the sepals, petals, and stamens and before (positionally) those of the antepetalous stamen phalanges. In suggesting that these appendages are staminodal, Ronse De Craene and Smets (1991) de-emphasized developmental timing and instead emphasized the above-mentioned features of development and anatomy. The antesepalous structures in *Archytaea* and *Ploiarium* receive their vascularization like the antepetalous stamen bundles, that is via a trace that arises directly from the stele and then forks to form two bundles, leading to the conclusion that the glands are staminodal (Dickison and Weitzman 1998). In the pistillate flowers of *G. xanthochymus*, the antesepalous appendages receive vascular traces from both the main floral stele and from traces that branch off from the bundles that supply the staminodes. In the staminate flowers of *G. nervosa*, the disk is supplied by traces that arise directly from the stele after the departure of the staminode traces. The vascularization pattern found in *Garcinia* is not particularly

helpful in evaluating the hypothesis that these structures are an outer whorl of stamens. The nectaries are supplied, in part, by traces that arise directly from the stele, as are all of the floral organs, and they do not share any unique features with the vasculature of the stamens. It has been shown in some species that disk shaped intrastaminal receptacular nectaries (sensu Schmid 1988) also receive their vascularization directly from the stele (e.g., Link 1992); these have never been suggested to be staminodal.

Gynoecial origin of disks and appendages. The position of the disks in the staminate flowers of species from Lineage A supports the view that they could be pistillodal (e.g., Moncur 1988). This is not supported by the timing of development, however. Disks arise late, unlike the gynoecium in pistillate flowers and the pistillodes in the flowers of *G. afzelii* and *G. atroviridis*, all of which initiate early, simultaneous with or shortly after the petals and androecium. Additionally, the pistillodes in *G. afzelii* and *G. atroviridis* are morphologically similar during development to the gynoecium in pistillate flowers, with carpels being clearly visible at certain stages. Finally pistillate flowers have structures corresponding to the disk in staminate flowers, which complicates any interpretation that the disk as a pistillode.

Receptacular nectaries. The evidence presented here supports the view that disks and appendages in *Garcinia* are independent structures arising directly from the receptacle (receptacular nectaries Schmid 1988; "nectaria persistentia" Smets and Cresens 1988; Smets et al. 2003), rather than modified floral organs. Elsewhere in the Malpighiales, receptacular nectaries in the form of an annulus or disk are common in many families (Bernardello 2007), and in many cases these are intrastaminal (e.g., Euphorbiaceae, Humiriaceae, Irvingiaceae, Ixonanthaceae, Rhizophoraceae, Salicaceae).

The intrastaminal ring in species of *Ceriops*, *Kandelia*, and *Rhizophora* (Rhizophoraceae) arises late in development after all other floral organs are partly developed (Juncosa and Tomlinson 1987). In *Ceriops* species the ring is lobed and protrudes between the stamens. *Irvingia gabonensis* Baill. and *Klainedoxa gabonensis* Pierre (Irvingiaceae) have large, slightly lobed intrastaminal rings with stomata at the bottom of a narrow duct (Link 1992).

Homology. A large part of this study is concerned with homology assessment. Homology can be equated with synapomorphy (Patterson 1982; Stevens 1984; de Pinna 1991). In this context, homology assessments can be viewed as a two-step process. There is an initial step of establishing morphological correspondence between structures (homologous at the "primary level", de Pinna 1991; cf. "structural" homology, Hufford 1996a; Hufford 2001) using some criteria—e.g., Remane's positional, structural, and transitional principal criteria (Remane 1952; translated and summarized in Riedl 1978). If structures are considered homologous at the primary level, then further testing of this initial hypotheses within a phylogenetic framework can establish if they share these similarities due to inheritance from a common ancestor (i.e., homologous at the "secondary level", de Pinna 1991; cf. "phylogenetic" homology, Hufford 1996a; Hufford 2001). This study does not support the hypotheses that the nectaries in *Garcinia* are homologous at the primary level with an outer whorl of stamens. However, within *Garcinia*, the nectaries share many morphological correspondences such as gross morphological similarity (yellow in color, presence of stomata), similar time of developmental initiation (after the other floral organs have initiated and have begun to develop), and similar position (intrastaminal), suggesting that within this group they are

homologous at the primary level. Furthermore, phylogenetic analyses of molecular sequence data (Sweeney, submitted) place all taxa having nectaries into a monophyletic group (Lineage A, Figs. 2, 3) suggesting that these structures are homologous at the secondary level of homology and thus were inherited from a common ancestor.

Disk, appendages, and rings within Lineage A. Within Lineage A, the staminate flowers all have disks in the center of the flower (Fig. 1A, 1E, 1I). The pistillate flowers of *G. conrauana*, all species of Clade 1, and some species of Clade 2 (Fig. 2) have antesepalous appendages (Fig. 1C, 1D, 1G, 1F). However, the pistillate flowers of taxa in a derived clade within Clade 2 (Sweeney submitted) have a ring between the staminodes and gynoecium (Fig. 1J). The position of these rings and some aspects of their gross morphology (they have stomata and are often yellow like the appendages) suggests that they are homologous to the antesepalous appendages found elsewhere in Lineage A. This is further supported by a developmental study of *Garcinia madruno* (Kunth) Hammel which has staminate flowers with free stamens and a disk (Moncur 1988); the disk was apparent only late in development like the floral disk of the taxa with phalanges examined in this study.

Disks, appendages, and rings among Clusiaceae and relatives. In the Clusiaceae alliance (Fig. 3), disks, antesepalous appendages, and rings are widespread and have all been interpreted as representing an outer whorl of stamens (Robson 1961, 1972). Conversely, this study indicates that the antesepalous structures in Clusiaceae and relatives are not homologous. In *Harungana madagascariensis* (Hypericaceae), evidence points to a staminal origin for the antesepalous structures (Ronse De Craene and Smets 1991), and most genera of Hypericaceae have similar antesepalous structures that have

been considered to be staminodal (as "fasciclodes") (Robson 1961, 1972; Stevens 2006). The anatomical evidence presented above suggests that the nectariferous tissue pads in Bonnetiaceae, in *Archytaea* and *Ploiarium*, are also of staminal origin (Dickison and Weitzman, 1998).

In Symphonieae, which all have hermaphroditic flowers, the situation is less clear, as there are extrastaminal and intrastaminal structures (rings and appendages) in the flower. Some Symphonieae, i.e. species of *Montrouziera* and *Pentadesma*, are similar to pistillate flowers of Lineage A in having antesepalous structures, and flowers of *Platonia insignis* Mart. have a lobed, intrastaminal disk adaxial to the androecium. However in some other Symphonieae, the possible equivalent structures are outside, not inside, the staminal whorl. For example, *Moronobea riparia* Planch. & Triana and *M. jenmani* Engl. (Vesque 1893 and pers. obs.) have a disk beneath the ovary and androecium (the "androgynophore" in Stevens 2006). However in *M. coccinea* Aubl., the lobed disk is obscure in flower, but there is a prominent lobed structure beneath the young fruits. The lobed disk in these species are often assumed to be derived from the stamens, but there are no developmental or anatomical studies of flowers from species in this group.

In summary, current evidence suggests that in the Clusiaceae alliance there are at least two types of floral nectaries, some associated with staminodes and others associated with the receptacle.

Other Aspects of Development

General features of development. A striking feature of the flowers examined here is that differences found in the mature flowers are evident from early developmental stages and there does not seem to be a basic floral ground plan that is shared among all of

the species. For example, among species the mature flowers differ in merosity, organ presence/absence, and degree of congenital stamen fusion. These differences exist from the beginning of development. It has been suggested that in general major developmental differences should be found among taxa separated at deep phylogenetic levels and that developmental differences between closely related species (e.g., within a genus) should appear late (e.g., Tucker 1984; Tucker 1997; cf. von Baer's Law, Ronse De Craene and Smets 2001). This might suggest that *Garcinia* and the major clades within the genus have been independently evolving for a long time. There is a fossil assigned to Clusiaceae, morphologically similar to *Clusia* and *Garcinia*, from the Turonian (89 million years ago) (Crepet and Nixon 1998), and it has been estimated that Clusiaceae diverged over 90 million years before present (Davis et al. 2005). However, there is no reason to assume that ontogenies must be conservative among closely related taxa (Hufford 1995, 1996b, 2001) and the data presented here may show that major developmental repatternings (see Hufford 1996b) can occur among closely related species at early developmental stages.

A parallel situation occurs in the related genus *Clusia*, which exhibits floral diversity, in the androecium in particular, similar to that in *Garcinia* (Bittrich and Amaral 1996; Gustafsson 2000; Sweeney, submitted). Differences among *Clusia* species are also exhibited from early stages of development (Gustafsson 2000). Future studies documenting the developmental genetic basis of floral morphology within *Garcinia*, *Clusia*, and other taxa in the Clusiaceae alliance are needed to further understand whether these parallels extend to the genetic level.

Pistillodes in Garcinia. Pistillodes in staminate flowers of *Garcinia* from Lineage B have been generally recognized by their similarity to the gynoecium in the pistillate flowers. In some species (e.g., *G. atroviridis*), the pistillode is well developed, being similar in shape to the gynoecium of the pistillate flower and even having rudimentary ovules (P. Sweeney, pers. obs.). In other species, the pistillodes are more rudimentary, comprised of a narrow stalk that is capped by a stigma-like structure that resembles that in a fertile gynoecium. Their developmental timing is the same as that of the gynoecium in pistillate flowers and individual carpels are apparent in early stages of development. Because the disks in the staminate flowers of Lineage A are morphologically different from the gynoecium in the pistillate flowers, they have rarely been considered pistillodes (but see Moncur, 1988), and the developmental data presented here supports the view that they are not pistillodal. In some flowers of *G. xanthochymus* an elongated appendage was present in the center of disks, sometimes with a smooth, flattened structure at the end of the appendage. This structure looks like a pistillode. It elongates before the disk fully expands and its cells stain differently from those of the disk (Fig. 8M, 8N), suggesting it is not part of the disk. However, the stigmas in the pistillate flowers of *G. xanthochymus* have five stylodia that are terminated by papillate stigmatic areas, a morphology quite unlike that of the plate-shaped structure of the elongated appendage.

Androecium development and anatomy. The androecia in *Garcinia* and in other species of the Clusiaceae alliance have been discussed in regards to general hypotheses of androecial evolution in angiosperms (e.g., Corner 1946; Kawano 1965; Robson 1972; Stebbins 1974; Ronse De Craene and Smets 1987, 1991; Leins and Erbar 1991, 1997). Subsequent phylogenetic analyses have not supported some of these hypotheses (Hufford

1998). For example, a fasciculate androecium is not the plesiomorphic state among angiosperms (telome theory, Wilson 1937). Other evolutionary patterns are emerging. Among core eudicots (Soltis et al. 2005) it is common to have twice as many stamens as petals with the stamens borne in two alternating whorls – the outer opposite the sepals and the inner opposite the petals (Stevens 2001 onwards; Soltis et al. 2005), and many groups have secondarily evolved polyandrous androecia (Endress 1994; Soltis et al. 2005). In these groups polyandry occurs by the development of primary primordia, quite often antepetalous, that later produce numerous secondary primordia that develop into individual stamens (Endress 1994; Stevens 2001 onwards; Soltis et al. 2005) with stamen development commonly proceeding in a centrifugal direction (Corner 1946; Weberling 1992; Endress 1994). The development of the phalangiate and ring-shaped androecia in the species examined in this study, as well as that in other Clusiaceae and close relatives (Payer 1857; Sattler 1973; Ronse De Craene and Smets 1991; Bittrich and Amaral 1996, 1997; Hochwallner and Weber 2006; but see Gustafsson 2000 for an exception), follows this developmental pattern. In many angiosperm flowers that have primary and secondary androecium primordia, the androecial units that arise from the primary primordia (e.g., fascicles or phalanges) are supplied from the stele by a single bundle ("stamen trunk bundle") that later branches to supply the individual stamens (Endress 1994). This pattern is observed in *Garcinia xanthochymus* and *G. nervosa* where the phalanges are supplied by a single trace that later branches to supply each of the stamens.

Gynoecium development. *G. staudtii* exhibits open carpel development; the ovules are initiated and begin to develop before the inner space of the ovary is enclosed, (Fig. 10I). Open carpel development in species with syncarpous gynoecia is rare

(Endress 1994; Tucker and Kantz 2001). Open carpel development is not observed in pistillate flowers of *G. afzelii* (this study), nor has it been observed in other Clusiaceae and relatives examined (Payer 1857; Sattler 1973; Ronse De Craene and Smets 1991; Hochwallner and Weber 2006). In a review of this condition in angiosperms, Tucker and Kantz (2001) found that it evolved in only a few families (20) and species (69). The distribution of this condition in *Garcinia* should be further explored to determine if it is more widespread in the genus and if it is a synapomorphy for a clade.

Dioecy in Garcinia. In the *Garcinia* species examined here the flowers are unisexual by abortion (Type I flowers) or they are unisexual from their inception (Type II flowers) – the two types of unisexual flowers that have been recognized (Mitchell and Diggle 2005). If the elongated appendages seen in some staminate flowers of *G. xanthochymus* and *G. nervosa* are interpreted to be pistillodes, then within each of these species the staminate flowers are Type I or Type II, a rare phenomenon in dioecious species (Mitchell and Diggle 2005). Additionally and if mature flower morphology is taken into account, within all but one of the *Garcinia* species examined the flower type differed between staminate and pistillate flowers, a condition found in only 9% of the species examined by Mitchell and Diggle (2005). To further explore the developmental transitions involved in the evolution of dioecy in *Garcinia*, the distribution of Type I and Type II flowers needs to be thoroughly documented with developmental studies of key species and species level phylogenies for major clades within the genus.

Conclusion

Morphology and field observations suggest that the disks, antesepalous appendages, and rings present in *Garcinia* are nectaries. The developmental evidence

presented in this study does not support the hypothesis that these nectaries in *Garcinia* represent an outer whorl of stamens and instead suggest that these structures are associated with the receptacle (cf. intrastaminal receptacular nectaries Schmid 1988; Bernardello 2007), as occur elsewhere in Malpighiales. This result, along with evidence from other studies, suggests that floral nectaries occupy different locations in the flowers of species in the Clusiaceae alliance (Fig. 3) suggesting they have had multiple evolutionary origins in the clade. Indeed, floral nectaries occupying different positions are widely distributed within unrelated groups of Malpighiales (Bernardello 2007) and thus their presence appears to be evolutionarily labile.

Future work should focus on more fully resolving relationships within the Clusiaceae alliance and on conducting developmental and anatomical studies of flowers of a representative sample of species from across the clade, focusing in particular on groups in which these structures have not been closely examined (e.g., Symphonieae). Field studies are also needed to determine if the disks, appendages, and rings are nectariferous in species where they have not been described as such. Developmental genetic studies would be helpful in further exploring the evolution of the nectaries in *Garcinia* and in the Clusiaceae alliance. The developmental genetics of nectaries has been most studied in *Arabidopsis thaliana* where the gene *crabs claw* (*CRC*) has been shown to be a key gene for nectary development (Bowman 1999; Baum et al. 2001; Lee et al. 2005a). The expression of *CRC* is conserved in eudicot nectaries and is required for nectary development in rosids and asterids – despite the varied morphologies and positions of the nectaries in this these clades (Lee et al. 2005b). It has been proposed that variation in nectary position in eudicots may have evolved by changes in genes

controlling expression of *CRC* (Lee et al. 2005b). Such a scenario could explain the variability in nectary morphology and position in the Clusiaceae alliance. Expression data of *CRC* in a representative sample of species would help test this hypothesis. Additionally, expression data may also be helpful in further exploring the nature of the disks, appendages, and rings in species that have not been described as being nectariferous.

This study also documents within *Garcinia* a number of interesting features of floral development and evolution, including open carpel development (Tucker and Kantz 2001) and different unisexual flower types (Types I and II, Mitchell and Diggle 2005) within a species occurring within flowers of the same gender. Additionally this study finds that the different floral morphologies from a disparate sampling of *Garcinia* species is evident from the beginning of flower development and that there is no obvious general floral plan in the genus.

Literature Cited

Abdul-Salim, K 2002 Systematics and biology of *Symphonia* L. f. (Clusiaceae). Ph.D. dissertation. Harvard University, Cambridge, Massachusetts, USA.

Bamps, P, N Robson, B Verdcourt 1978 Guttiferae. Pages 1-34 *in* RM Polhill ed. Flora of Tropical East Africa. Crown Agents for Overseas Governments and Administrations, London, UK.

Baum, SF, Y Eshed, JL Bowman 2001 The *Arabidopsis* nectary is an ABC-independent floral structure. *Development* 128: 4657-4667.

Bernardello, G 2007 A systematic survey of floral nectaries. Pages 19–128 *in* SW Nicolson, M Nepi, E Pacini eds. Nectaries and nectar. Springer, Dordrecht, NL.

Bittrich, V, MCE Amaral 1996 Flower morphology and pollination biology of some *Clusia* species from the Gran Sabana (Venezuela). *Kew Bull* 51: 681-694.

_____ 1997 Floral biology of some *Clusia* species from central Amazonia. *Kew Bull* 52: 617-635.

Bittrich, V, MHG Gustafsson, PF Stevens, PW Sweeney 2005 Phylogeny and character evolution in Clusiaceae and their immediate relatives. Pages 219 XVII International Botanical Congress, Abstracts. IBC, Vienna, AT.

Bowman, JL 1999 CRABS CLAW, a gene that regulates carpel and nectary development in *Arabidopsis*, encodes a novel protein with zinc finger and helix-loop-helix domains. *Development* 126: 2387-2396.

Corner, EJH 1946 Centrifugal stamens. *J Arnold Arbor* 27: 423-437.

Crepet, WL, KC Nixon 1998 Fossil Clusiaceae from the late Cretaceous (Turonian) of New Jersey and implications regarding the history of bee pollination. *Amer J Bot* 85: 1122-1122.

Davis, CC, M Latvis, DL Nickrent, KJ Wurdack, DA Baum 2007 Floral gigantism in Rafflesiaceae. *Science* 315: 1812.

Davis, CC, CO Webb, KJ Wurdack, CA Jaramillo, MJ Donoghue 2005 Explosive radiation of Malpighiales supports a mid-Cretaceous origin of tropical rainforests. *Am Nat* 165: E36-E65.

de Pinna, MCC 1991 Concepts and tests of homology in the cladistic paradigm. *Cladistics* 7: 367–394.

Dickison, WC, AL Weitzman 1998 Floral morphology and anatomy of Bonnetiaceae. *J Torrey Bot Soc* 125: 268-286.

Endress, PK 1994 Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge, UK.

Gill, GE, RT Fowler, SA Mori 1998 Pollination Biology of *Symphonia globulifera* (Clusiaceae) in Central French Guiana. *Biotropica* 22: 310-315.

Gustafsson, MHG 2000 Floral morphology and relationships of *Clusia gundlachii* with a discussion of floral organ identity and diversity in the genus *Clusia*. *Int J Pl Sci* 161: 43-53.

Hochwallner, H, A Weber 2006 Flower development and anatomy of *Clusia valerioi*, a Central American species of Clusiaceae offering floral resin. *Flora* 201: 407-418.

Horner, HT, RA Healy, G Ren, D Fritz, A Klyne, C Seames, RW Thornburg 2007 Amyloplast to chromoplast conversion in developing ornamental tobacco floral nectaries provides

sugar for nectar and antioxidants for protection. *Am J Bot* 91, 12-24.

Hufford, L 1995 Patterns of ontogenetic evolution in perianth diversification of *Besseya* (Scrophulariaceae). *Amer J Bot* 82: 655-680.

_____ 1996a The morphology and evolution of male reproductive structures of Gnetales. *Int J Pl Sci* 157: 95-112.

_____ 1996b Ontogenetic evolution, clade diversification, and homoplasy. Pages 271–301 *in* MJ Sanderson, L Hufford eds. Homoplasy: the recurrence of similarity in evolution. Academic Press, San Diego, California, USA.

_____ 1998 Early development of androecia in polystemonous Hydrangeaceae. *Amer J Bot* 85: 1057-1067.

_____ 2001 Ontogenetic sequences: homology, evolution, and the patterning of clade diversity. Pages 27–58 *in* ML Zelditch ed. Beyond heterochrony: the evolution of development. Wiley-Liss, New York, New York, USA.

Hutchinson, J, JM Dalziel, RWJ Keay 1954 Guttiferae. Pages 290-295 Flora of West Tropical Africa. Vol. 1. Crown Agents for Overseas Governments and Administrations, London, UK.

Jones, SW 1980 Morphology and major taxonomy of *Garcinia* (Guttiferae). Ph.D. dissertation. University of Leicester, Leicester, UK.

Juncosa, AM, PB Tomlinson 1987 Floral development in mangrove Rhizophoraceae. *Amer J Bot* 74: 1263-1279.

Kawano, S 1965 Anatomical studies on the androecia of some members of the Guttiferae-Moronoboideae. *Bot Mag (Tokyo)* 78: 97-108.

Kubitzki, K 2006 The families and genera of vascular plants. Vol. 9. Springer, Berlin, DE.

Lee, JY, SF Baum, J Alvarez, A Patel, DH Chitwood, JL Bowman 2005a Activation of CRABS CLAW in the Nectaries and Carpels of Arabidopsis. *Plant Cell* 17: 25-36.

Lee, JY, SF Baum, SH Oh, CZ Jiang, JC Chen, JL Bowman 2005b Recruitment of CRABS CLAW to promote nectary development within the eudicot clade. *Development* 132: 5021-5032.

Leins, P, C Erbar 1991 Fascicled androecia in Dilleniidae and some remarks on the *Garcinia* androecium. *Bot Acta* 104: 336-344.

_____ 1997 Floral developmental studies: some old and new questions. *Int J Pl Sci* 158: 3-12.

Link, DA 1992 The floral nectaries in the Irvingiaceae. *Pl Syst Evol* 180: 236-242.

Mitchell, CH, PK Diggle 2005 The evolution of unisexual flowers: morphological and functional convergence results from diverse developmental transitions. *Amer J Bot* 92: 1068-1076.

Moncur, MW 1988 Floral development of tropical and subtropical fruit and nut species: an atlas of scanning electron micrographs. Division of Water and Land Resources, Commonwealth Scientific and Industrial Research Organisation Australia, Melbourne, AU.

Nepi, M 2007 Nectary structure and ultrastructure. Pages 129-166 *in* SW Nicolson, M Nepi, E Pacini eds. Nectaries and nectar. Springer, Dordrecht, NL.

Patterson, C 1982 Morphological characters and homology. Pages 21-74 *in* KA Joysey, AE Friday eds. Problems of phylogenetic reconstruction. Vol. 21. Academic Press, New York, New York, USA.

Payer, JB 1857 Traité d'organogénie végétale comparée de la fleur. Masson, Paris, FR.

Remane, A 1952 Die Grundlagen des natürlichen Systems der vergleichenden Anatomie und der Phylogenetik. Geest & Portig, Leipzig, DE.

Riedl, R 1978 Order in living organisms. John Wiley & Sons, New York, New York, USA.

Robson, NKB 1961 Guttiferae. Pages 371-375 *in* AW Exel, H Will eds. Flora Zambesiaca. Vol. 1. Crown Agents for Overseas Governments and Administrations, London, UK.

_____ 1972 Evolutionary recall in *Hypericum* (Guttiferae)? *Trans & Proc Bot Soc Edinburgh* 41: 365-338.

Ronse De Craene, LP, E Smets 1987 The distribution and the systematic relevance of the androecial characters oligomery and polymery in the Magnoliophytina. *Nordic J Bot* 7: 239-253.

_____ 1991 Androecium and floral nectaries of *Harungana madagascariensis* (Clusiaceae). *Pl Syst Evol* 178: 179-194.

_____ 2001 Staminodes: their morphological and evolutionary significance. *Bot Rev* 67: 351–402.

Ruzin, SE 1999 Plant microtechnique and microscopy. Oxford University Press, Oxford, UK.

Sattler, R 1973 Organogenesis of flowers: a photographic text-atlas. University of Toronto Press, Toronto, CA.

Schmid, R 1988 Reproductive versus extra-reproductive nectaries: historical perspective and terminological recommendations. *Bot Rev* 54: 179-232.

Smets, EF, EM Cresens 1988 Types of floral nectaries and the concepts 'character' and 'character-state'—a reconsideration. *Acta Bot Neerl* 31: 121-128.

Smets, EF, P Jansen, P Caris, P Lens 2003 Distribution and evolution of floral nectaries in angiosperms: a review. *Palmarum Hortus Francofurtensis* 7: 103.

Soltis, DE, PS Soltis, PK Endress, MW Chase 2005 Phylogeny and evolution of angiosperms. Sinauer, Sunderland, Massachusetts, USA.

Stebbins, GL 1974 Flowering plants: evolution above the species level. Belknap Press of Harvard University Press, Cambridge, Massachusetts, USA.

Stevens, PF 1984 Homology and phylogeny: morphology and systematics. *Syst Bot* 9: 395-409.

_____ 2001 onwards Angiosperm Phylogeny Website. Version 8, June 2007 [and more or less continuously updated since]. Website http://www.mobot.org/MOBOT/research/APweb/.

_____ 2006 Clusiaceae. Pages 48-66 *in* K Kubitzki ed. The families and genera of vascular plants. Vol. 9. Springer, Berlin, DE.

Tucker, SC 1984 Origin of symmetry in flowers. Pages 351-369 *in* RA White, WC Dickison eds. Contemporary problems in plant anatomy. Academic Press, New York, New York, USA.

_____ 1997 Floral evolution, development, and convergence: the hierarchical-significance hypothesis. *Int J Pl Sci* 158: 143-161.

Tucker, SC, KE Kantz 2001 Open carpels with ovules in Fabaceae. *Int J Pl Sci* 162: 1065-1073.

Vesque, J 1893 Guttiferae. Pages 1–669 *in* ALPP de Candolle, ACP de Candolle eds. Monographiae Phanerogamarum. Vol. 8. G. Masson, Paris, FR.

Weberling, F 1992 Morphology of flowers and inflorescences. Cambridge University Press, Cambridge, UK.

Wilson, CL 1937 The phylogeny of the stamen. *Amer J Bot* 24: 686-699.

Table 1

Studied taxa with voucher, collection information, position in *Garcinia* phylogeny (Sweeney, submitted), and sectional placement (sensu Jones, 1980).

Taxon (gender)	**Voucher information**	**Origin of sample**	**Lineage, Clade (Section)**
Garcinia afzelii Engl. (staminate)	P. Sweeney 1427	Cameroon	Lineage B, Clade 8 (Tagmanthera)
G. afzelii Engl. (pistillate)	P. Sweeney 1441	Cameroon	Lineage B, Clade 8 (Tagmanthera)
G. atroviridis Griff. ex T. Anders. (staminate)	P. Sweeney 1095	Rimba Ilmu Botanic Garden, Malaysia	Lineage B, Clade 5 (Brindonia)
G. nervosa Miq. (staminate)	P. Sweeney 1080	Malaysia	Lineage A, Clade 1 (Xanthochymus)
G. smeathmannii (Planch. & Triana) Oliver (staminate)	P. Sweeney 1432	Cameroon	Lineage A, Clade 2 (Rheediopsis)
G. staudtii Engl. (pistillate)	P. Sweeney 1447	Cameroon	Lineage A, Clade 2 (Rheediopsis)
G. xanthochymus Hook. f. (pistillate)	P. Sweeney 1457	Singapore Botanic Gardens, Singapore	Lineage A, Clade 1 (Xanthochymus)
G. xanthochymus Hook. f. (staminate)	P. Sweeney 1459	Fairchild Tropical Garden, U.S.A.	Lineage A, Clade 1 (Xanthochymus)

Figure 1. Mature flowers and floral diagrams for *Garcinia* species examined in this study. *Garcinia xanthochymus*. A. Mature staminate flower. B. Floral diagram for staminate flower. C. Pre-anthetic pistillate flower (sepals and petals removed). D. Floral diagram for pistillate flower. *Garcinia smeathmannii*. E. Mature staminate flower. F. Floral diagram. *Garcinia staudtii*. G. Mature pistillate flower. H. Floral diagram. *Garcinia macrophylla* Mart. I. Mature staminate flower. *Garcinia aff. aphanophlebia* Baker. J. Mature pistillate flower. *Garcinia afzelii*. K. Mature staminate flower. L. Floral diagram for staminate flower. M. Mature pistillate flower. N. Floral diagram for pistillate flower. *Garcinia atroviridis*. O. Mature staminate flower. P. Floral diagram. ap = antesepalous appendage, an = androecium, d = disk, o = ovary, p = petal, ph = phalange, pt = pistillode, st = stigma.

Figure 2. Phylogeny showing relationships of *Garcinia* and close relatives. Relationships and lineage and clade designations from Sweeney (submitted). Solid lines indicate strong support for clades in at least two analyses (≥ 0.95 Bayes & ≥ 85% MP or ML) while thick dashed lines indicate support from one analysis.

Figure 3. Hypothesized relationships of Clusiaceae and close relatives (the "Clusiaceae alliance") showing the distribution of disk, appendages, and rings. Relationships portrayed are based on Bittrich et al. (2005), Davis et al. (2007), and Sweeney (submitted). Solid lines indicate strong support for clades in at least two analyses, thick dashed lines indicate support from one analysis, and an asterisk (*) indicates that a node has weak support (See Fig. 2).

Figure 4. *Garcinia xanthochymus* staminate flower development. A. Pentagonal shaped apical meristem (sepals removed). B, C. Apical meristem showing initiation of petal and

phalange primordia (sequence of petal initiation shown in C). D–H. Developing petal and phalange primordia. I. Developing phalanges (side view, petals removed). J. Developing phalanges and disk (side view, petals and one phalange in foreground removed). K. Developing phalanges. L. Developing phalanges, disk, and central appendage (side view). M. Flower pre-anthesis showing central disk surrounded by three phalanges (side view, petals and two phalanges in foreground removed). Scale bars: 100 μm in A–I, 500 μm in J and K, 1 mm in L and M. a = individual stamen primordium, ea = elongated appendage.

Figure 5. *Garcinia xanthochymus* pistillate flower development. A–C. Spiral initiation and development of sepals (sequence of initiation shown in B, C). D. Pentagonal shaped apical meristem with three outer sepals removed. E. Pentagonal shaped apical meristem (sepals removed). F. Apical meristem showing initiation of petal and phalange primordia (side view). G, H. Developing petals, phalanges, and gynoecium. I. Late developing flower showing phalanges, antesepalous appendages, ovary, and stigma (side view, sepals and petals removed). J. Close-up view of phalanges and antesepalous appendage. K. Late developing flower showing phalanges, antesepalous appendages, ovary, and stigma (side view). L. Pre-anthesis flower showing phalanges, antesepalous appendages (indicated by white arrows), and ovary (side view, sepals and petals removed). M. Close-up view of antesepalous appendage. N, O. Close-up views of pit and pore. Scale bars: 10 μm in O, 100 μm in N, 200 μm in A–H, 500 μm in J and M, 1 mm in I and K, and 2 mm in L.

Figure 6. *Garcinia xanthochymus*, serial sections of a pistillate flower bud from pedicel to a region of the flower just beneath the stigma. See text for detailed explanation. Scale bars: 0.5 mm in K & M and 1.0 mm in all other images. ap = antesepalous appendage,

dmb = dorsal and medial vascular bundles of carpel, P = petal, S = sepal, white arrows indicate bundles supplying the staminodes, black arrows indicate bundles supplying the petals, and gray arrows indicate traces supplying the antesepalous appendages.

Figure 7. *Garcinia nervosa* staminate flower development. A, B. Apical meristem showing spiral initiation of sepals (sequence of initiation shown in B). C. Pentagonal shaped apical meristem surrounded by developing sepals (first initiated sepal removed). D. Sepals covering remainder of developing flower. E. Pentagonal shaped apical meristem (sepals removed). F–I. Developing petal and phalange primordia (sequence of petal initiation shown in F). J. Petals showing imbricate aestivation. Scale bars: 200 μm in A–C and E–G, 500 μm H–J, and 1 mm in D.

Figure 8. *Garcinia nervosa*, serial sections of flower from pedicel to the distal region of the disk. See text for detailed explanation. D., F, and H. are close-up views of C., E., and G., respectively. Scale bars: 0.5 mm in A and 1.0 mm in B–N. dskb = vascular bundles supplying disk, ea = elongated appendage, white arrows indicate bundles supplying phalanges, black arrows indicate bundles supplying the petals (except in F where they show the five areas of the vascular stele), gray arrows indicate bundles supplying sepals, and black and white arrows indicate bundles supplying the disk.

Figure 9. *Garcinia smeathmannii* staminate flower development. A. Early flower bud with one outer sepal removed to reveal the remainder of the developing flower. B. Initiation of two inner sepals that bound approximately square shaped apical meristem (outer sepals removed). C. Two inner sepals and initiation of petal and phalange primordia from apical meristem. D. Early developing petal and phalange primordia (sepals removed). E. Same as previous but with one inner sepal remaining. F. Developing

sepals (inner pair), petals, and phalanges (outer sepals removed). G–I. Same as previous but with all sepals removed. J. Developing flower showing area in center of flower (one phalange and two petals removed). K, L. Late developing flower showing expansion of petals over androecium (sepals removed). Scale bars: 100 μm in B–J, 250 μm in A, K, and L.

Figure 10. *Garcinia staudtii* pistillate flower development. A. Two early developing sepals and approximately square shaped apical meristem (outer sepals removed). B. Two early developing sepals and initiation of petal and phalange primordia. C–E. Expansion of inner sepals to cover rest of developing flower (outer sepals removed). F. Developing petal and phalange primordia and initiation of gynoecium (sepals removed). G–H. Developing petals, phalanges, and gynoecium and initiation of individual staminodes on phalanges. I. Developing flower showing petals and development of gynoecial ridge, placenta, and ovules. J. Developing petals, phalanges, and gynoecium (petal in foreground and on right removed). K. Same as previous with petals remaining (sepals removed). L. Late developing flower showing developing staminodes, antesepalous appendages, and gynoecium (petals and sepals removed). M. Pre-anthesis flower showing phalanges, antesepalous appendages, and gynoecium (petals and sepals removed). N. Close-up view of antesepalous appendage showing stomata-like pores (indicated by white arrows). Scale bars: 100 μm in A–C, F–K and N, 200 μm in D and E, and 1 mm in L and M. g = gynoecium, ov = ovule.

Figure 11. *Garcinia afzelii* staminate flower development. A. Apical meristem showing initiation of inner pair of sepals. B–C. Early developing petals, phalanges, and gynoecium. D. Developing petals, phalanges, and gynoecium showing four hemispherical

shaped carpel primordia. E–G. Expansion of petals to cover remainder of developing flower and growth of carpel primordia to enclose the inner cavity of pistillode. H, I. Early development of anthers (one petal removed). J–O. Development of phalanges and pistillode (sepals and petals removed). Scale bars: 100 μm in A, 200 μm in B–F and K, 500 μm in G–J and L, 1 mm in M, and 2 mm in N and O. c = carpel, t = theca.

Figure 12. *Garcinia afzelii* pistillate flower development. A. Early developing meristem showing developing petals and gynoecium (sepals removed). B. Developing petals and gynoecium showing initiation of four carpel primordia. C, D. Developing petals enclosing developing gynoecium. E. Developing gynoecium showing four hemispherical shaped carpel primordia and gynoecial cavity (petals removed). F. Developing gynoecium showing expansion of carpel primordia to enclose gynoecial cavity. G, H. Late developing ovary showing developing stigma. Scale bars: 100 μm in A and B, 250 μm in E–G, 500 μm in C and D, 1 mm in H.

Figure 13. *Garcinia atroviridis* staminate flower development. A. Early petals, ring primordium, and pistillode (sepals removed). B, C. Developing ring primordium showing initiation of sepals along inner periphery (sepals removed and petal in foreground removed in B.). D. Developing petals and pistillode showing developing carpels fused into a crenellated ring. E, F. Developing androecium showing centrifugal initiation of stamens and development of anthers and growth of gynoecial ridge to enclose central cavity of gynoecium (petals removed). Scale bars: 100 μm in A–D and 200 μm in E and F.

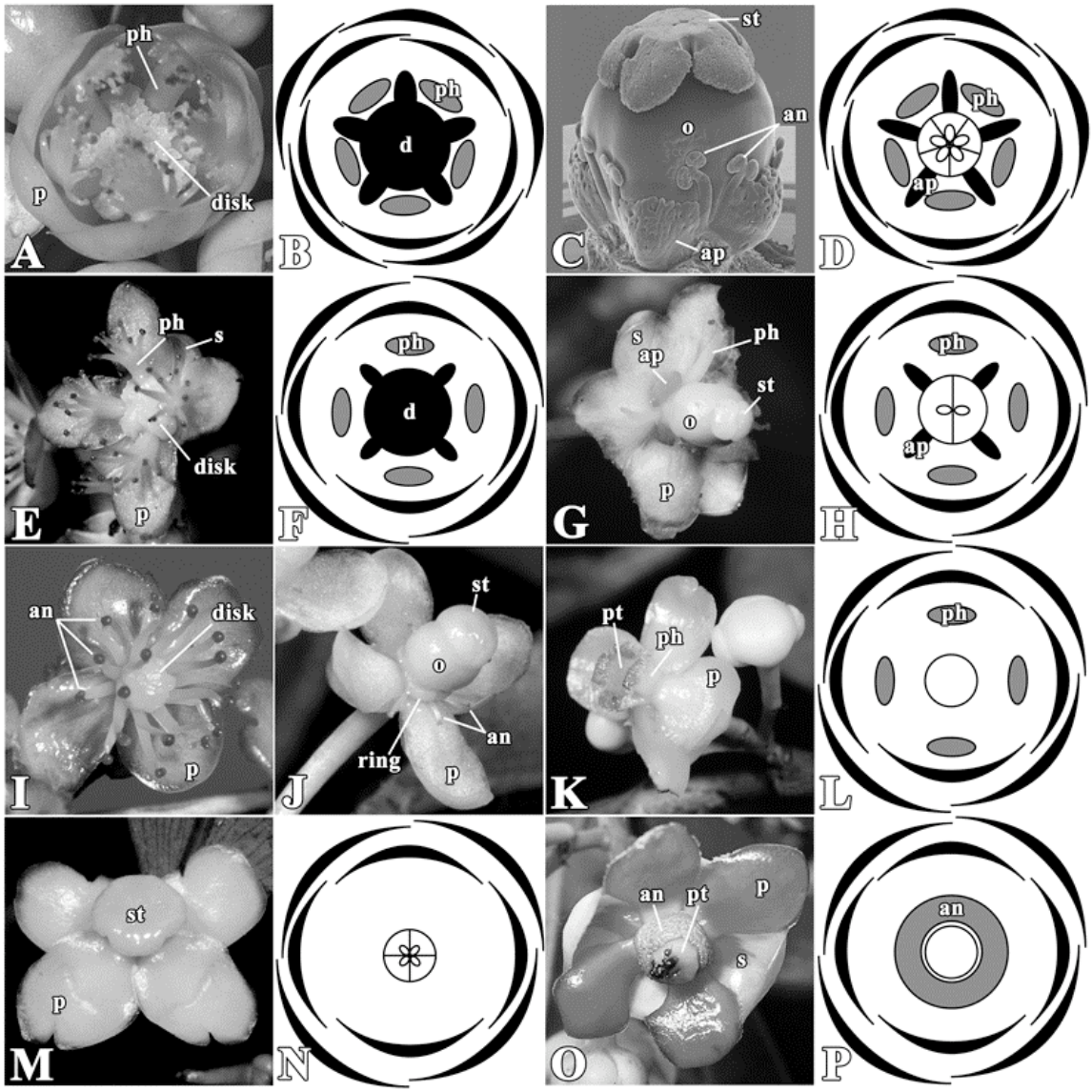
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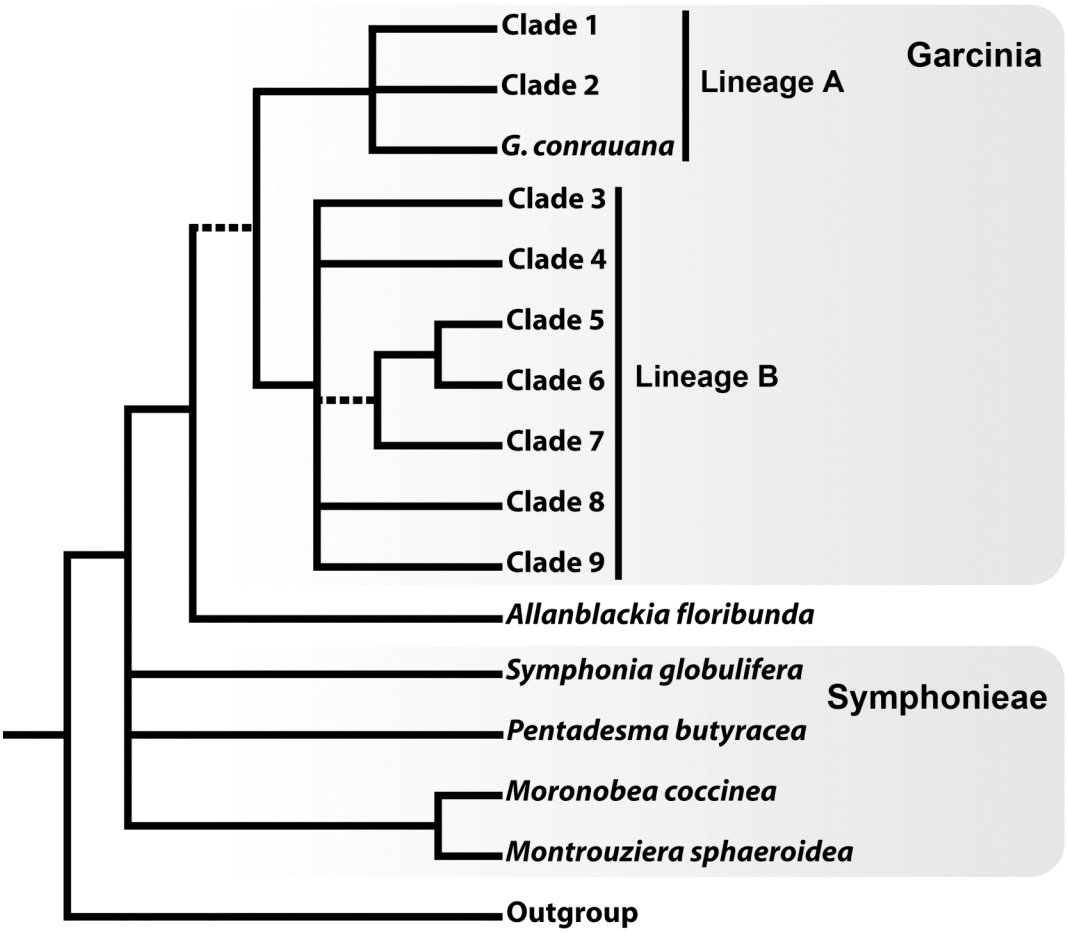
Figure 1.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Clade 8
Clade 9
Lineage B
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Pentadesma butyracea
Moronobea coccinea
Montrouziera sphaeroidea
Symphonieae
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Figure 2.

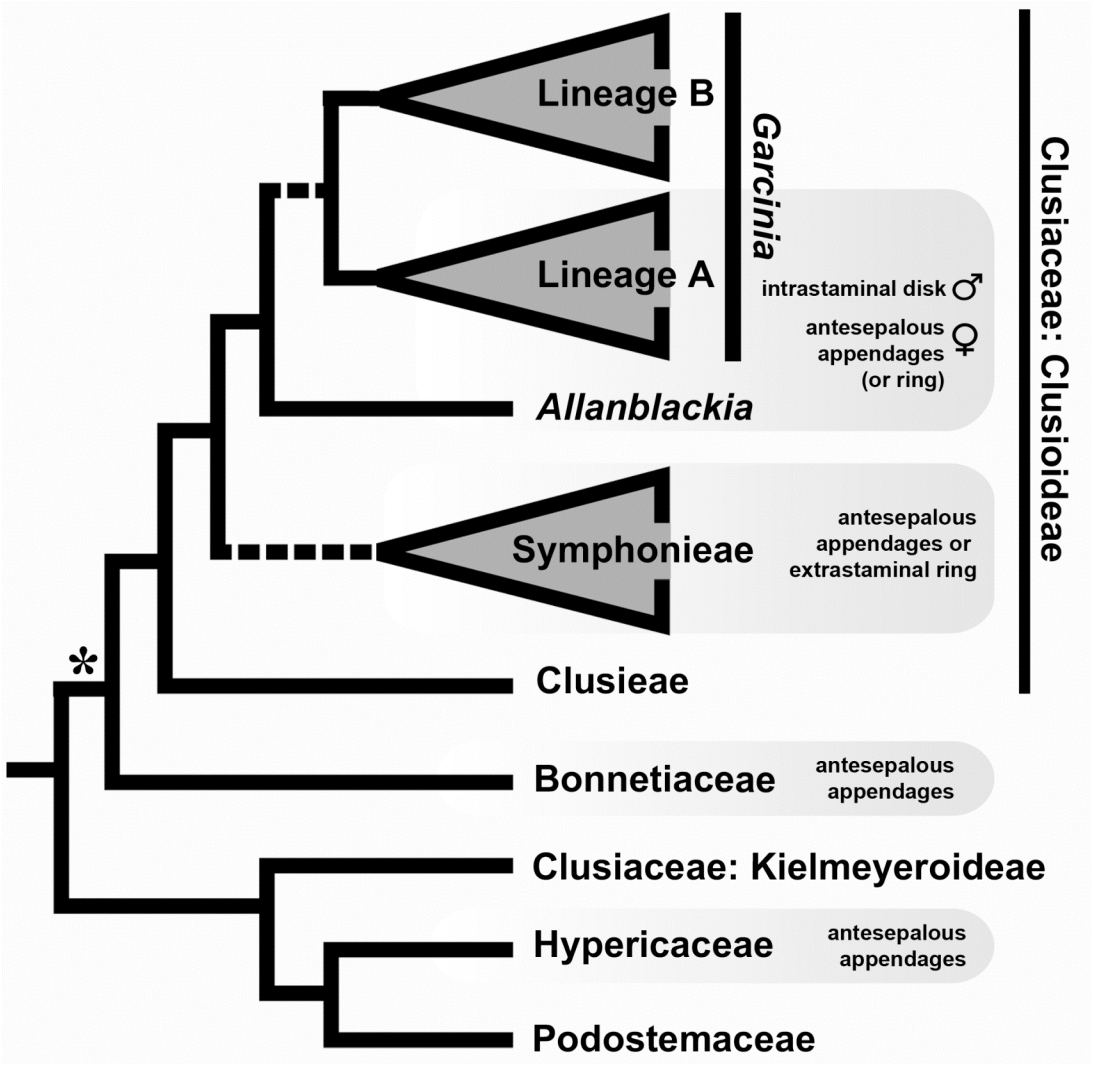
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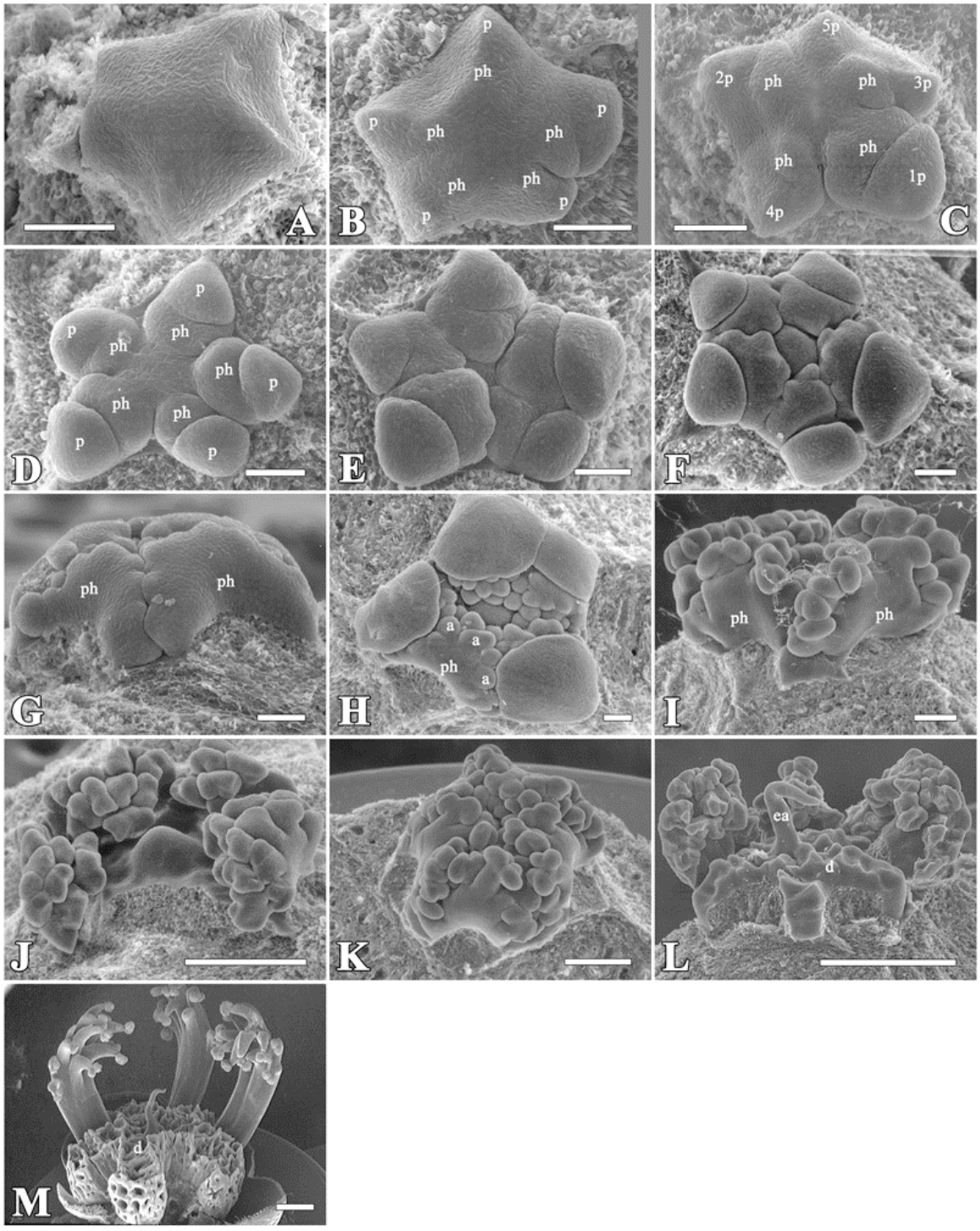
Figure 3.



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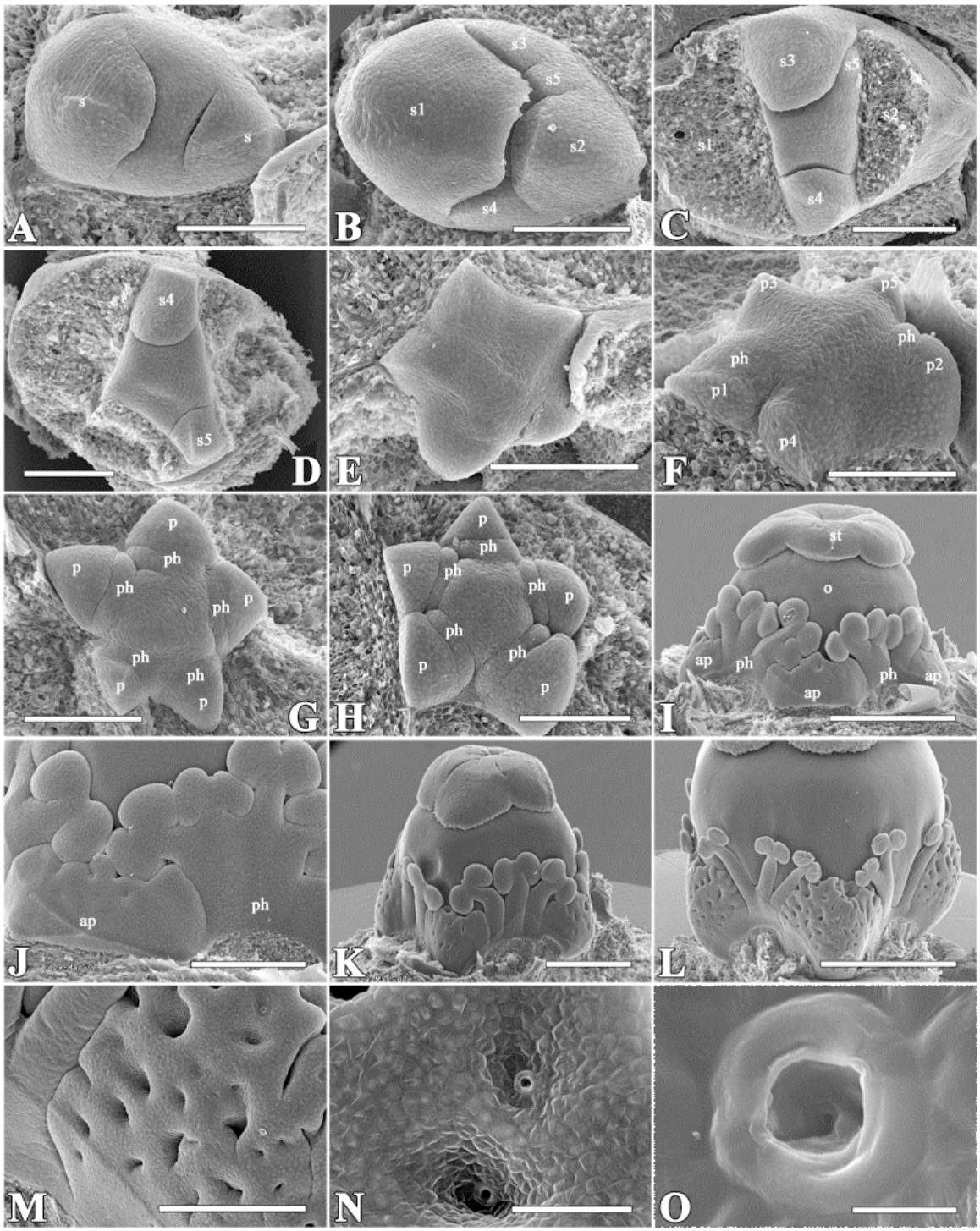
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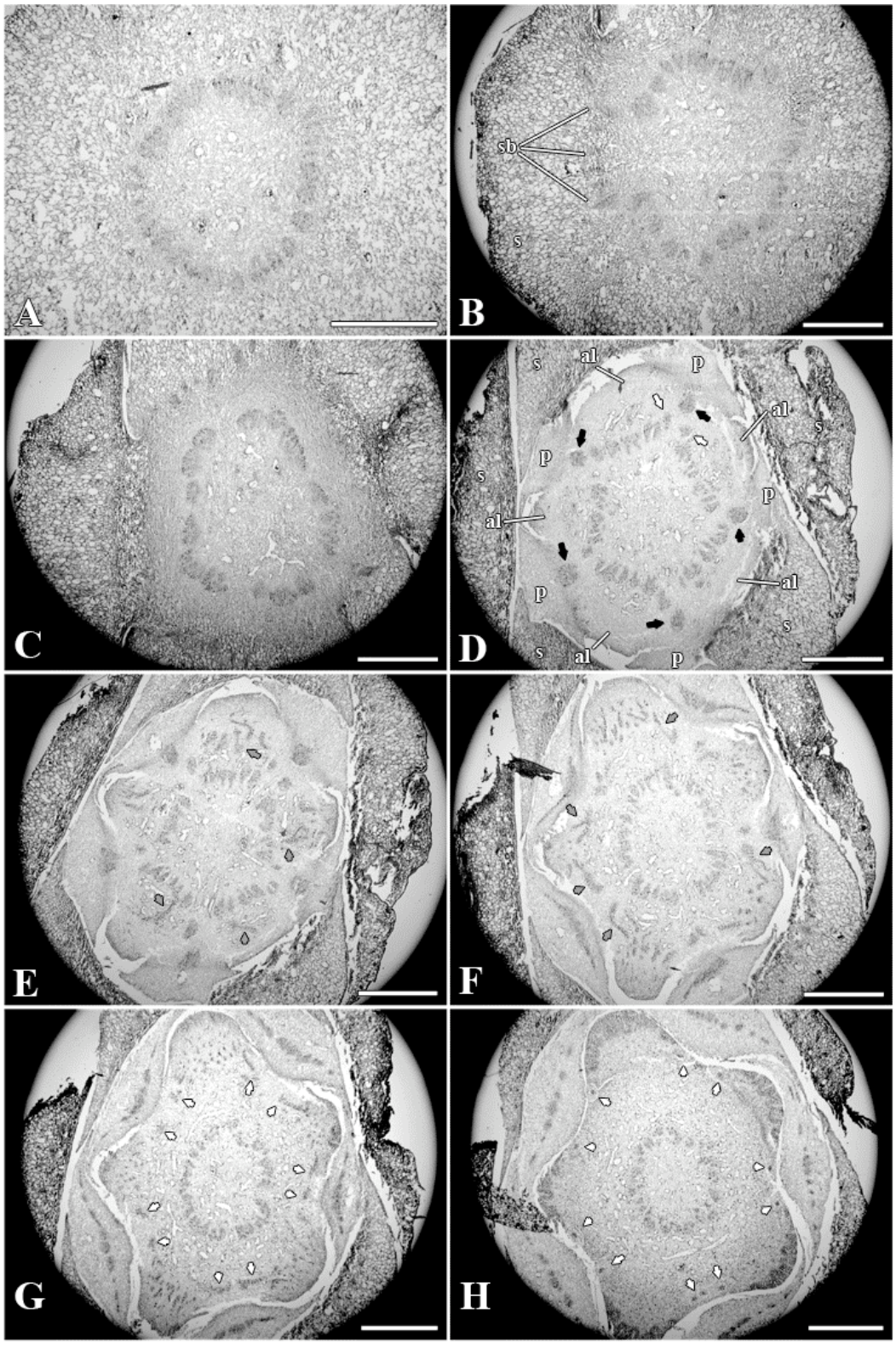

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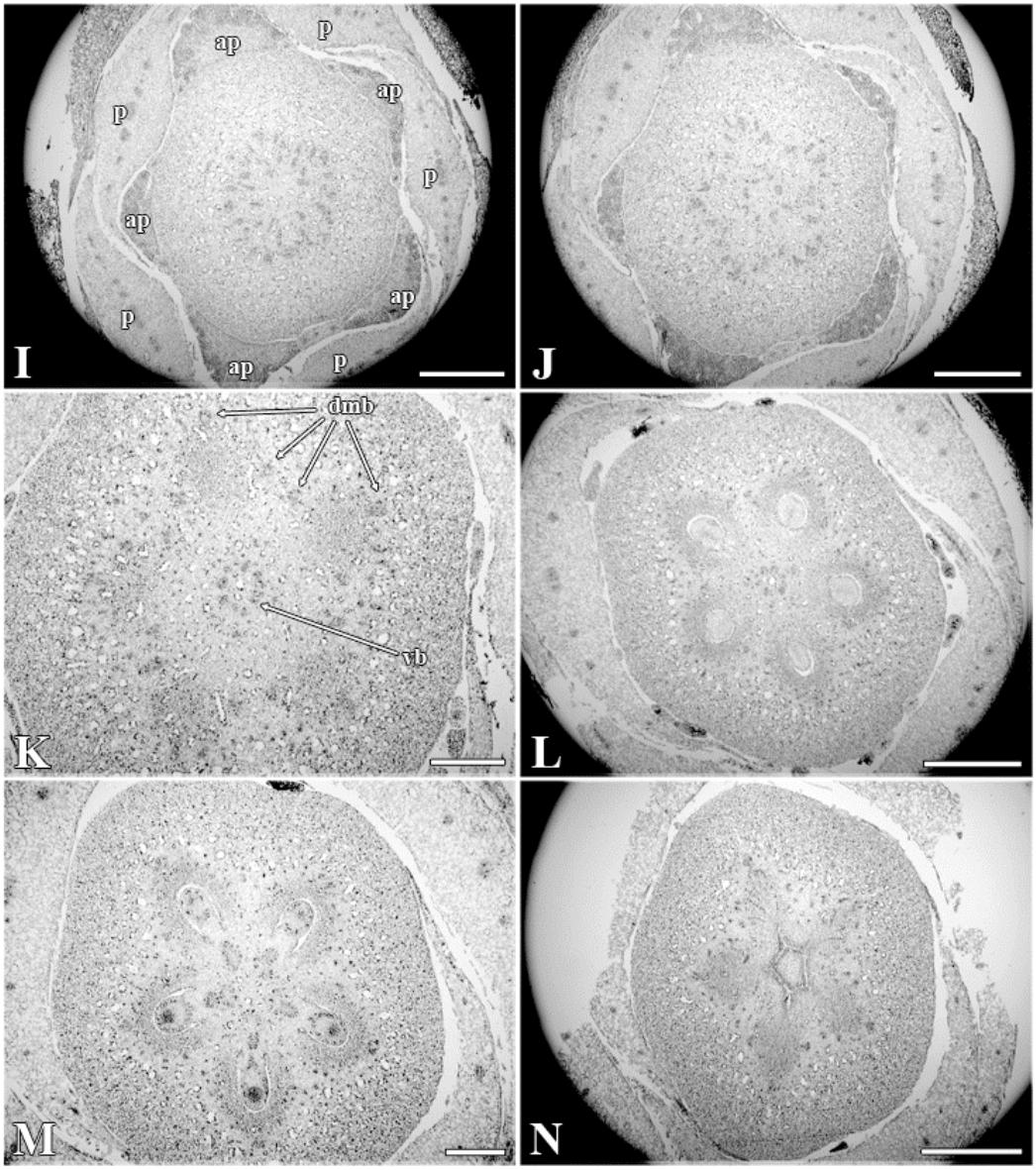

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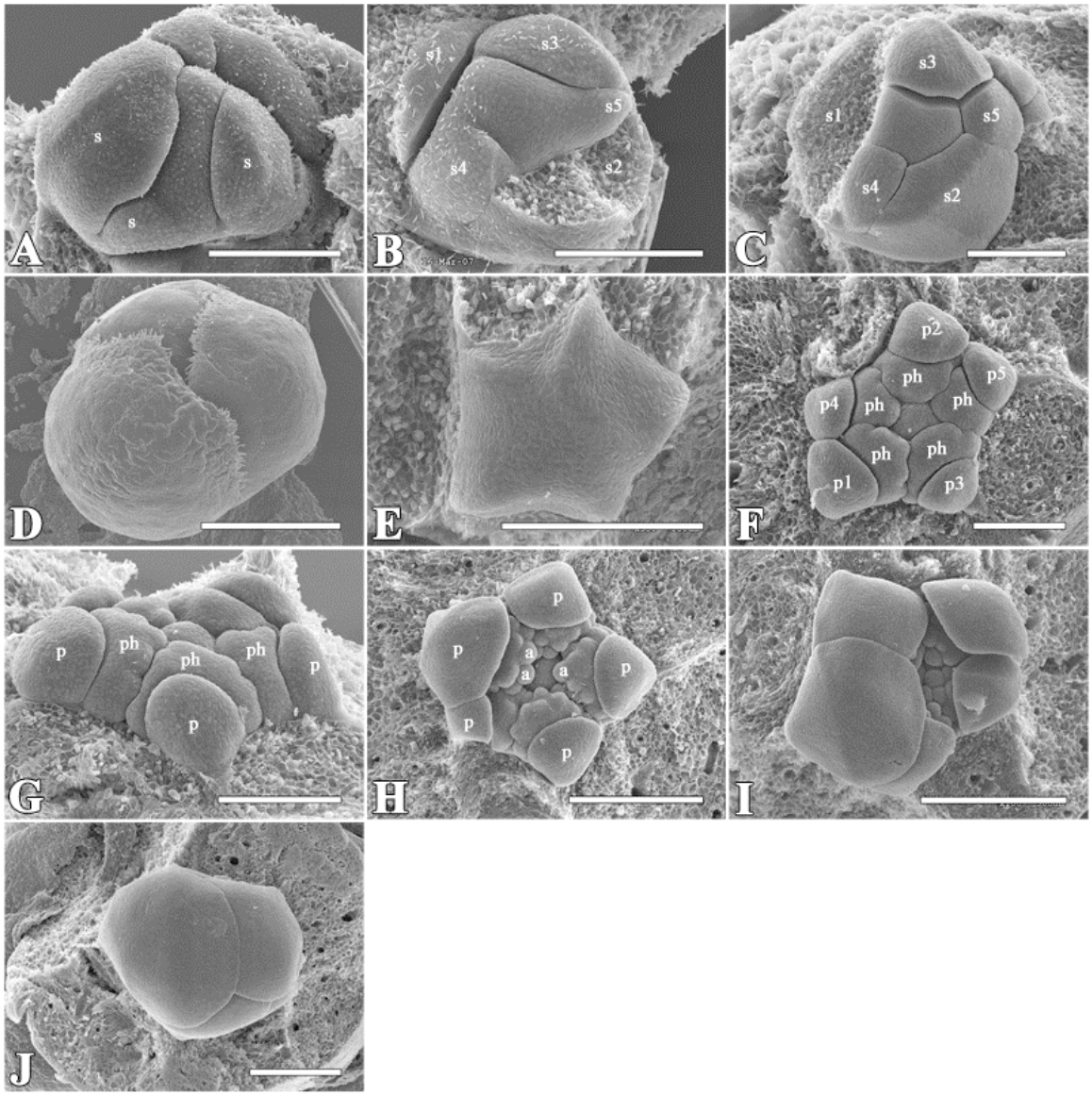
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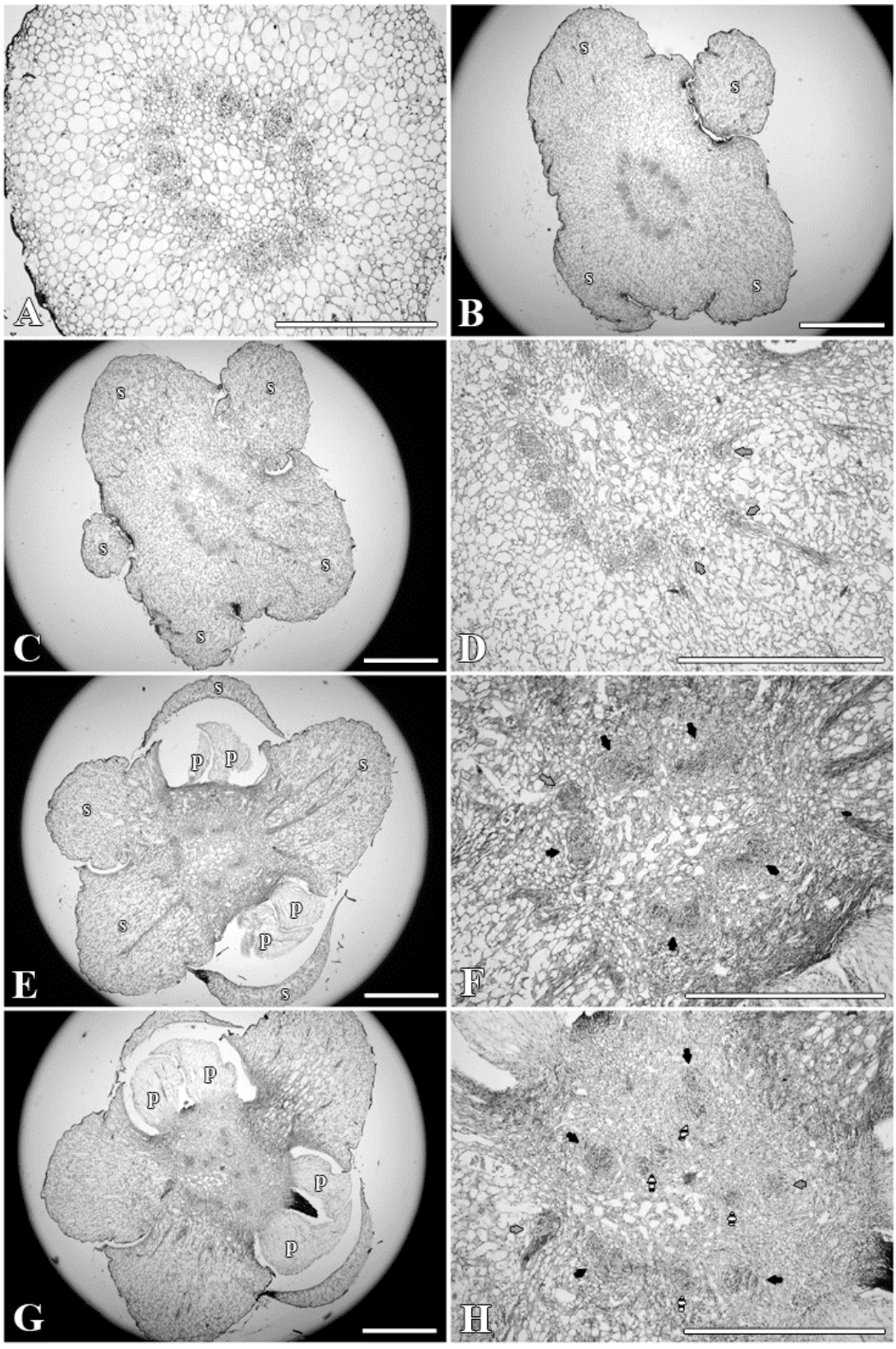

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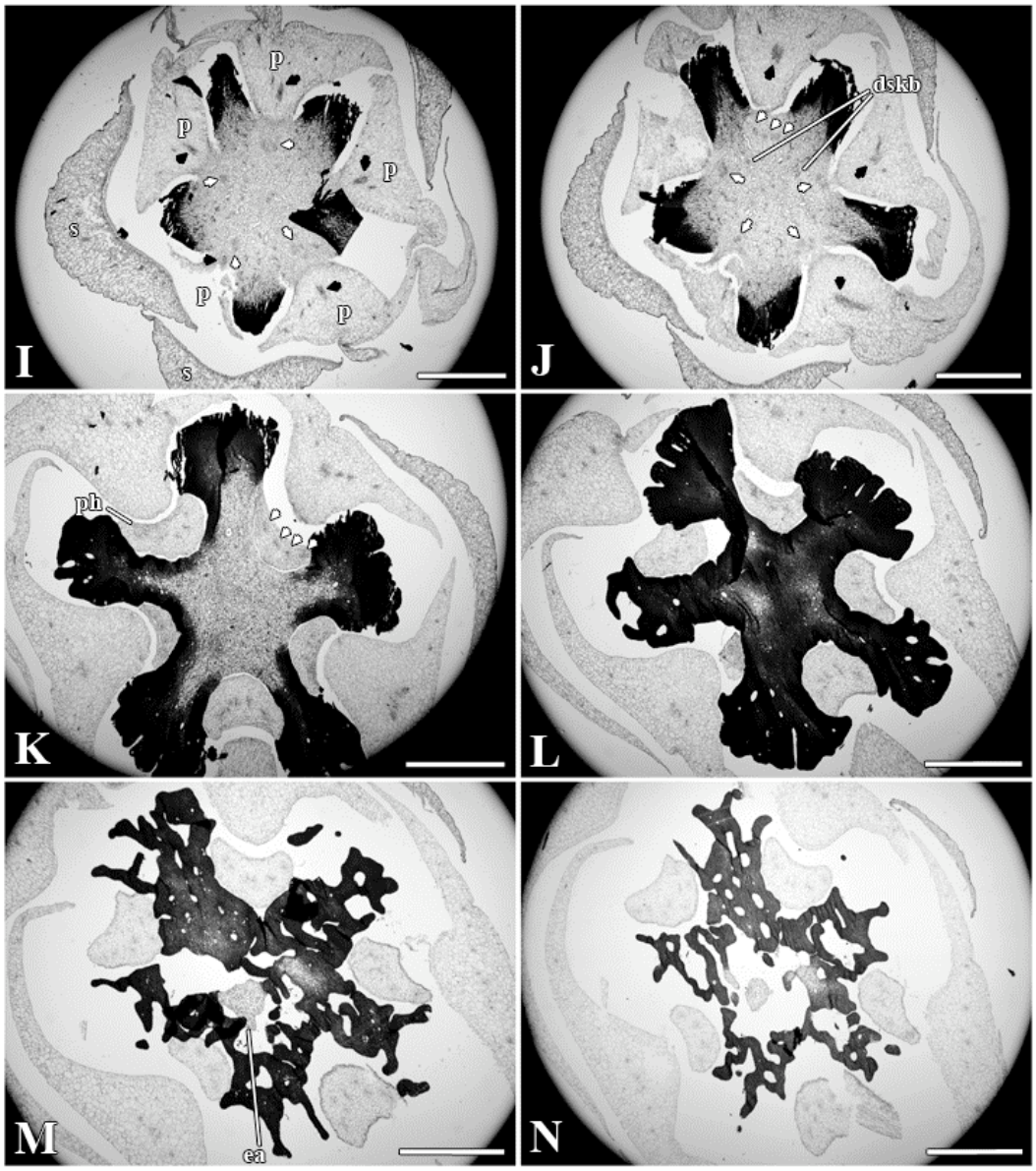

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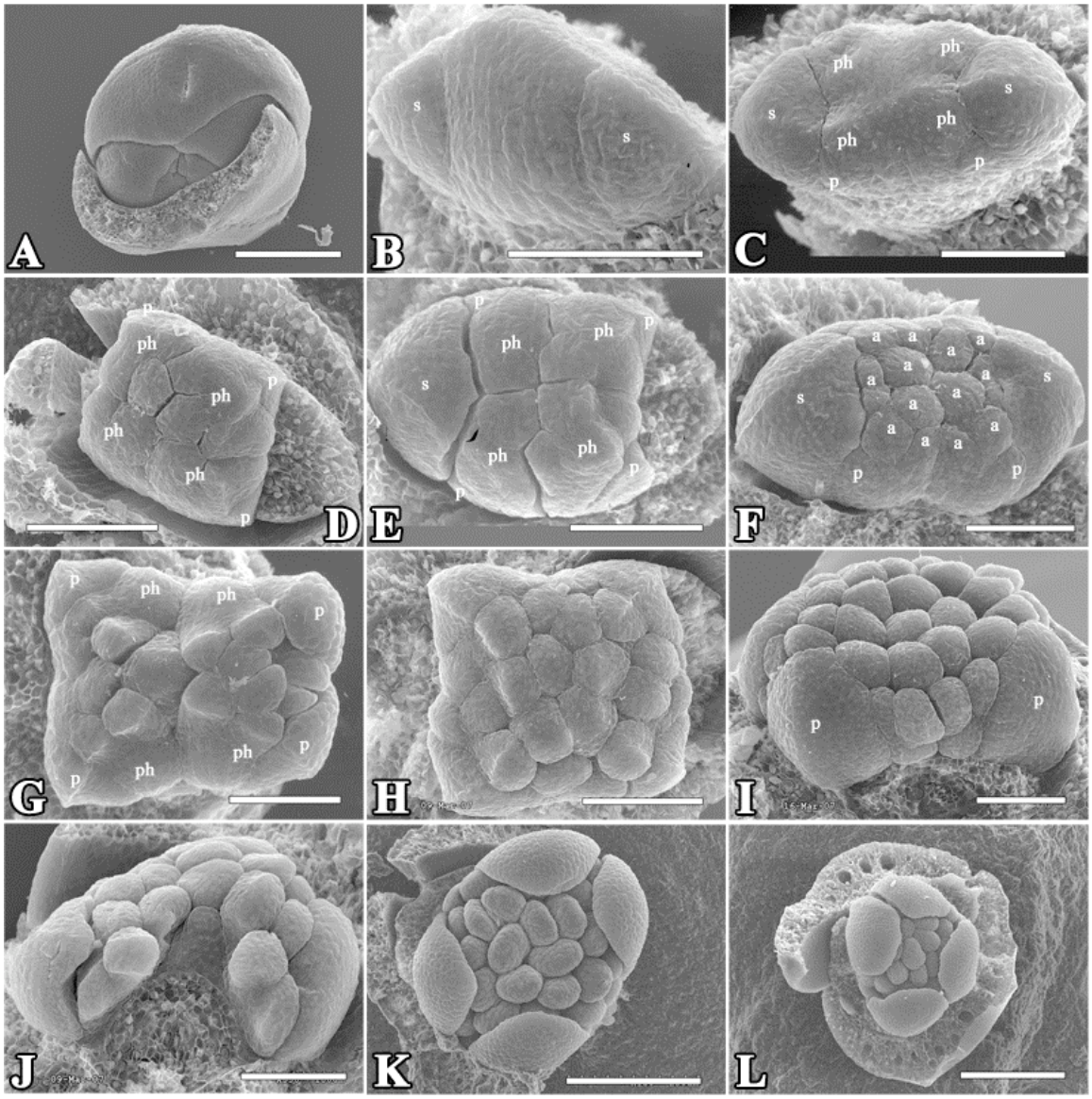
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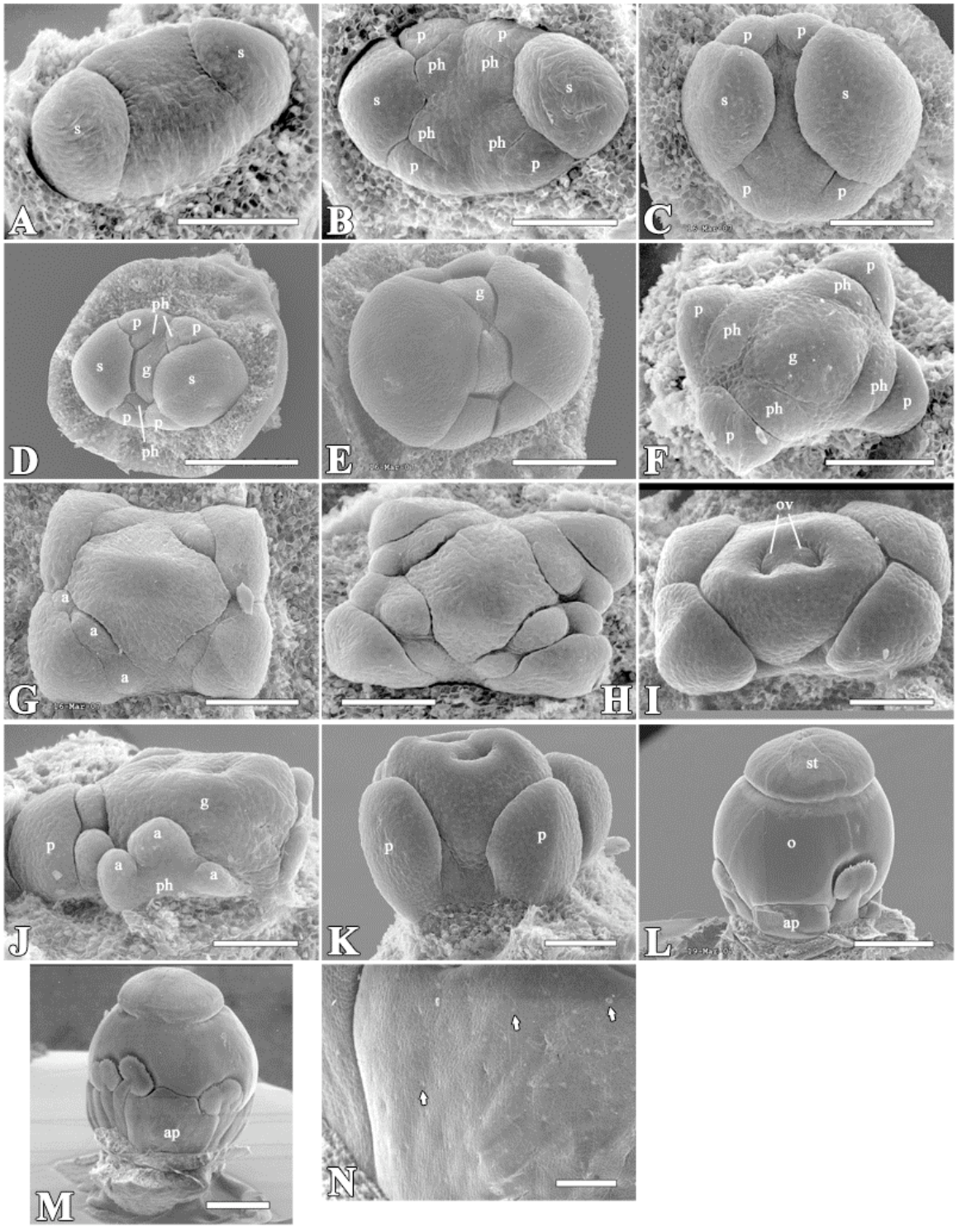
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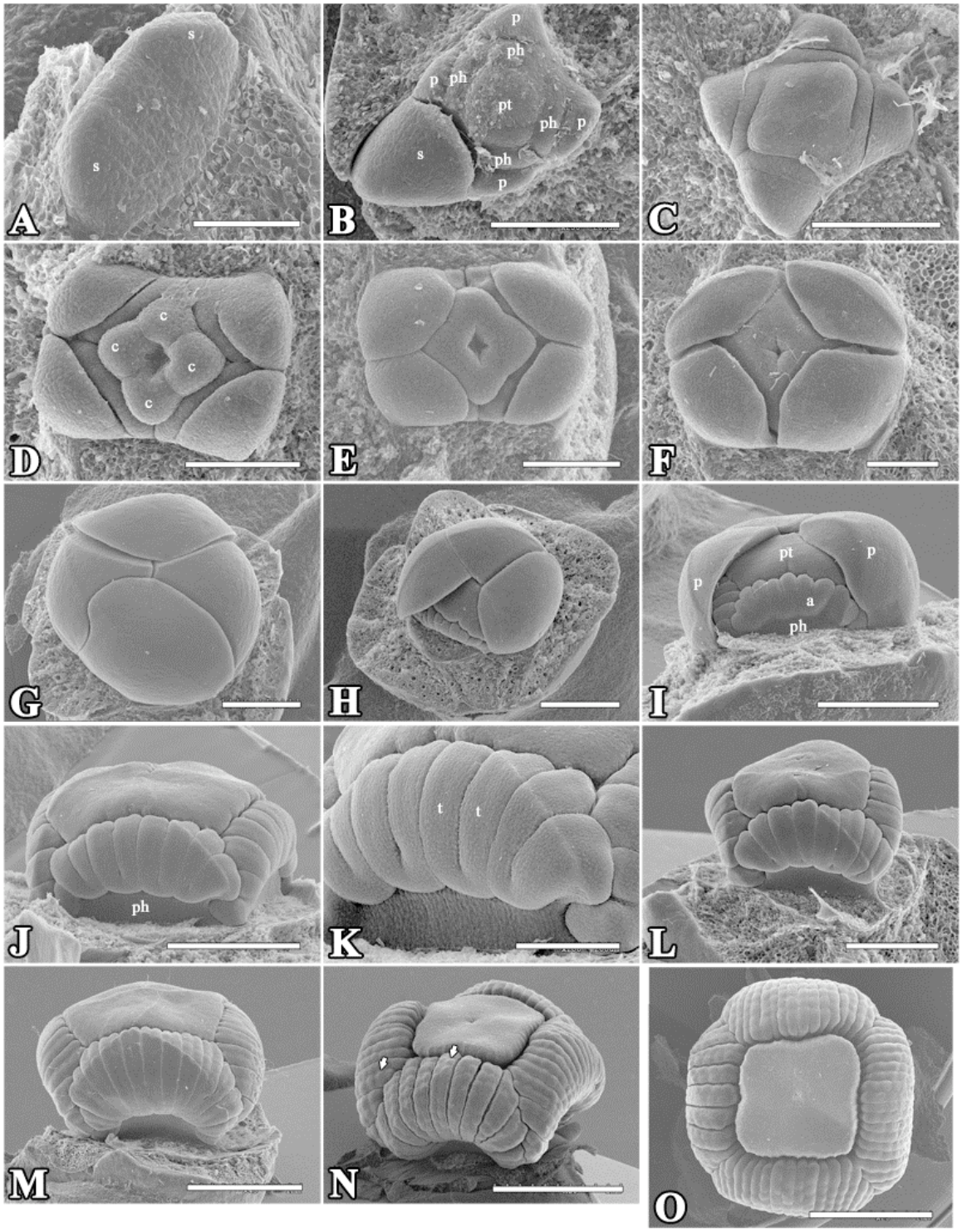

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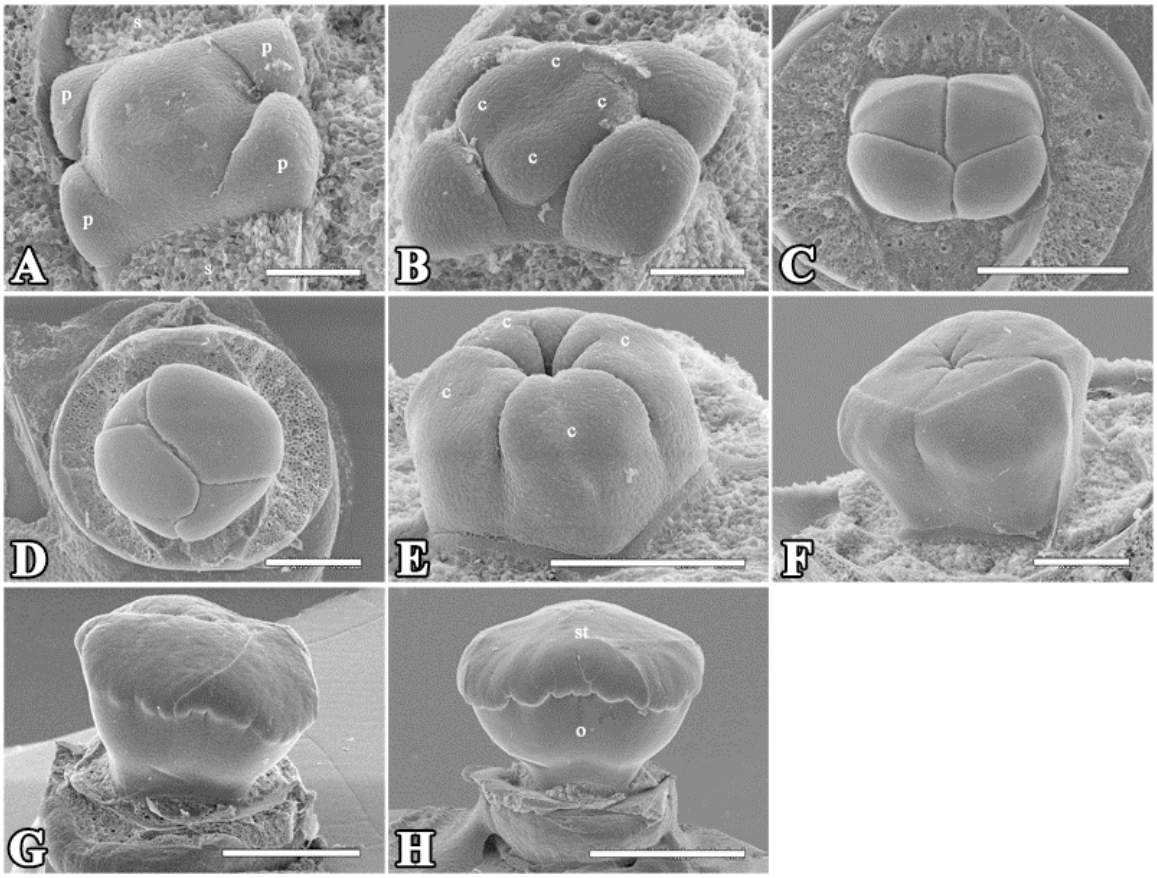

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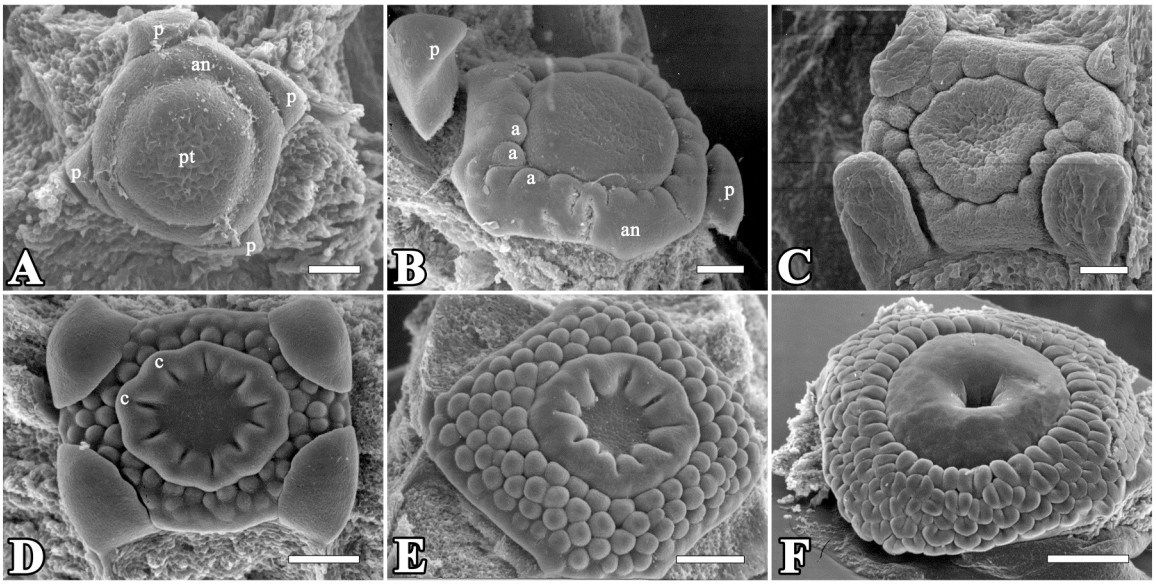

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