

**MOLECULAR SYSTEMATICS AND MORPHOLOGICAL CHARACTER
 EVOLUTION OF THE CONDAMINEAE (RUBIACEAE)¹**

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- *Premise of the study:* The Condamineae have in previous molecular studies been shown to be part of an early-divergent clade within the subfamily Ixoroideae, together with the tribes Calycophylleae, and Hippotidae, and genera of the former Cinchoneae and Rondeletieae. Generic relationships within this clade have, however, remained largely unresolved.
- *Methods:* In this study, the systematics of the Condamineae was further examined by phylogenetic reconstruction of six cp-DNA regions and one nrDNA region using parsimony and Bayesian Markov chain Monte Carlo inference. Morphological character evolution within the tribe was assessed by ancestral state reconstruction using likelihood optimization of characters onto Bayesian trees.
- *Key results:* Calycophylleae appears polyphyletic. “Hippotidae” is monophyletic but nested within the Condamineae. The phylogenetic hypotheses presented support a resurrection of the genera *Holtonia*, *Schizocalyx*, and *Semaphyllanthus*. Furthermore, *Bathysa* is found to be polyphyletic, *Tresanthera* is found nested within *Rustia*, and the taxonomically disputed genus *Dialypetalanthus* is here shown to be sister to a *Bothriospora*–*Wittmackanthus* clade. Morphological ancestral state reconstructions indicate that protogyny have evolved at least two times within the tribe and that indehiscent fruits, loculicidal fruit dehiscence, and intrapetiolar stipules have evolved independently several times. The occurrence of calycophylls (leaf-like calyx lobes), poricidal anthers, and winged seeds also appear homoplastic within the tribe.
- *Conclusions:* A diagnosis and delimitation of the tribe Condamineae is presented, with taxonomic proposals to synonymize *Tresanthera* and to transfer several species of *Bathysa* as well as *Phitopsis* to a resurrected *Schizocalyx*.

Key words: character evolution; Condamineae; *Dialypetalanthus*; Hippotidae; Ixoroideae; Rubiaceae; seed morphology; systematics.

Molecular phylogenetic studies have revealed many unexpected relationships in the systematics of Rubiaceae (reviewed by Bremer, 2009). In a study by Bremer et al. (1999), an early-divergent clade within the subfamily Ixoroideae was found that comprised genera previously classified in many different tribes, including Calycophylleae, Cinchoneae, Condamineae, Hippotidae, and Rondeletieae. This clade (in the present study, considered to constitute a single tribe and referred to as the Condamineae) included *Alseis*, *Calycophyllum*, *Capirona*, *Chimarrhis*, *Condaminea*, *Emmenopterys*, *Pentagonia*, *Pinckneya*, *Pogonopus*, *Rustia*, *Simira*, and *Warszewiczia*. Since then, many other genera have been demonstrated to be associated with the Condamineae, i.e., *Bathysa*, *Bothriospora*, *Dioicodendron*,

Dolichodelphys, *Ferdinandusa*, *Hippotis*, *Macbrideina*, *Macrocneum*, *Mastixiodendron*, *Parachimarrhis*, *Picardaea*, *Sommeria*, and *Wittmackanthus* (Rova, 1999; Rova et al., 2002), and *Mussaendopsis* (Razafimandimbison and Bremer, 2001). In addition, the enigmatic *Dialypetalanthus* was shown to be part of Ixoroideae in a molecular phylogenetic study by Fay et al. (2000) and as part of the Condamineae in a study by Bremer and Eriksson (2009). Previous molecular phylogenetic studies have indicated that the Condamineae include 30–35 genera, comprising ca. 300 species (Table 1; Govaerts et al., 2010), but have not been able to resolve the relationships within this tribe with strong support.

The Condamineae are morphologically very diverse (Fig. 1), and a number of morphological characters that are otherwise exceptionally rare within Rubiaceae are found within the tribe, including apopetalous, rotate corollas (*Dialypetalanthus*, *Mastixiodendron*, and *Mussaendopsis*), poricidal anthers (*Dialypetalanthus*, *Rustia*, and *Tresanthera*), polyandrous stamens (*Dialypetalanthus*), and pinnatifid leaf blades (several species of *Pentagonia* and *Simira*). *Dialypetalanthus* and *Mastixiodendron* have historically been difficult to fit into traditional schemes of Rubiaceae classification and have at times also been considered as belonging to, or being associated with other plant families, i.e., Dialypetalanthaceae or Myrtaceae, and Cornaceae, respectively (for historical reviews, see Darwin [1977], and Piesschaert et al. [1997]). Although of complex taxonomic history, the Condamineae can be considered as primarily including parts of the tribes Cinchoneae, Condamineae, and Rondeletieae sensu Hooker (1873), as well as the Hippotidae.

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An overview of the taxonomic history and tribal classifications of the associated genera is given in Table 1.

The Condamineae and the Rondeletiae have traditionally (Hooker, 1873; Schumann, 1891) been considered closely allied, both being characterized by many-seeded capsules, but distinguished by aestivation patterns of the corolla lobes (valvate in Condamineae vs. imbricate or contorted in Rondeletiae). Verdcourt (1958) found this distinction insufficient and reduced Condamineae to Rondeletiae, whereas Bremekamp (1966) upheld the distinction and, in addition, distinguished a tribe Simireae, claiming that the placentation and number of ovules in *Simira* indicated an isolated position in the family. Robbrecht (1988), in his comprehensive classification of Rubiaceae, largely followed the views of Hooker (1873) and Bremekamp (1966). Like Verdcourt (1958), Delprete (1996a, b) recognized only Rondeletiae, which he considered closely related to Calycophylleae and Cinchoneae.

The Cinchoneae have historically been distinguished from the Condamineae and the Rondeletiae by the presence of ascending, imbricate ovules and winged seeds (Hooker, 1873; Schumann, 1891). The tribe was investigated in a morphological cladistic study by Andersson and Persson (1991), and the authors transferred a number of genera from the Cinchoneae to a newly erected Calycophylleae (additional genera were included by Andersson [1995]). These genera (*Alseis*, *Calycophyllum*, *Capirona*, *Ferdinandusa*, *Macrocnemum*, and *Wittmackanthus*), and the tentatively included *Dolicholobium*, were shortly thereafter demonstrated as being associated with the Condamineae (cf. Bremer et al., 1999; Rova, 1999).

The “hippotides” (*Hippotis*, *Pentagonia*, *Sommerera*, and *Tammsia*; Rova and Andersson, 1995) have previously been placed in the Catesbaeae (Hooker, 1873) or the Mussaendeae (Schumann, 1891); both tribes were characterized by valvate corolla aestivation and indehiscent fruits with many seeds. These genera were transferred into two new tribes (Hippotidae and Tammsieae by García Kirkbride [1981]) of which the latter was reduced to synonymy by Rova and Andersson (1995). The Hippotidae sensu Rova and Andersson (1995) were soon thereafter also found to be associated with the Condamineae (Bremer et al., 1999; Rova et al., 2002). For more detailed historical reviews of Condamineae, see Delprete (1996a); Rondeletiae, see Delprete (1999); Cinchoneae, see Andersson and Persson (1991), Andersson (1995), and Andersson and Antonelli (2005); and Hippotidae, see Rova and Andersson (1995).

Molecular phylogenetic studies (Bremer et al., 1999; Rova, 1999; Rova et al., 2002) have revealed the Condamineae to be a diverse tribe with an unexpected phylogenetic position within the Ixoroideae, but have so far not provided much insight into the relationships within the tribe. In this sense, the Condamineae remain one of the least known groups within the family. The objectives of this study were to investigate the systematics and morphological character evolution of the Condamineae using phylogenetic hypotheses inferred from cpDNA and nrDNA.

MATERIALS AND METHODS

Taxon sampling—Included in this study were specimens from all the genera found to be associated with the Condamineae in the molecular phylogenetic studies discussed, as well as material from *Phitopsis multiflora*, *Tammsia anomala*, and *Tresanthera condamineoides*, previously not included in any molecular phylogenetic studies. An effort was made to sample material of the type of each genus; however, we were unsuccessful in obtaining DNA sequences from *Mastixiodendron pachyclados* (K. Schum.) Melch., *Semaphyllanthus obovata* (Ducke) L. Andersson, and *Simira tinctoria* Aubl. Sampling was increased for genera that did not appear monophyletic in preliminary analyses (*Bathysa*, *Elaeagia*, and *Rustia*). The ingroup comprised a sample of 71 species representing 32 genera, *Luculia gratissima* and 15 species from the subfamily Cinchonoideae and other representatives of the subfamily Ixoroideae were included as outgroups, however, the sampling of the fast evolving ITS and *trnH-psbA* markers was somewhat restricted due to difficulties in finding unambiguous alignments. An overview of the sampling and voucher specimens, is given in Appendix 1.

Scanning electron microscopy (SEM)—Seeds from herbarium material were mounted on aluminum stubs, gold-coated using an SEM coating unit E5100 (Polaron Equipment, Hertfordshire, UK), and subsequently photographed using a Stereoscan 260 (Cambridge Instruments, Cambridge, UK) scanning electron microscope. The images of some of the larger seeds (*Alseis*, *Calycophyllum*, *Capirona*, *Dialypetalanthus*, *Dolicholobium*, and *Ferdinandusa*) were prepared by combining several images obtained under the same conditions using Photoshop CS2 (Adobe Systems, San Jose, California, USA). Image backgrounds were removed using the same program. Voucher specimens are cited in the legend for Fig. 6.

DNA extraction, amplification, and sequencing—The chloroplast DNA (cpDNA) markers *matK*, *ndhF*, *rps16*, *trnH-psbA*, *trnS-G*, *trnT-F*, and the ITS region of the nuclear ribosomal DNA (nrDNA), previously used in phylogenetic inference in Rubiaceae (Andersson and Rova, 1999; Bremer et al., 1999; Persson, 2000; Razafimandimbison and Bremer, 2002; Rova et al., 2002; Andersson and Antonelli, 2005; Kainulainen et al., 2009), were included in the study. DNA was extracted, amplified, and sequenced as outlined in Kainulainen et al. (2009). Amplification of *matK* was done using the primers matK1198f and matK2053r (Andersson and Antonelli, 2005) and standard PCR settings. Sequences were assembled using the Staden software package v1.5.3 (Staden, 1996). Sequences new to this study (445) were deposited in GenBank (Appendix 1). Additional sequences (105) were obtained from GenBank (for references, see Appendix 1).

Data analyses—Sequences were aligned using Clustal W (default settings; Thompson et al., 1994), as implemented in the program BioEdit (Hall, 1999) and then manually adjusted. A number of inversions were excluded from the analyses. These correspond to base pairs 231–262 (*trnH-psbA*), 5413–5441 (*rps16*), 9330–9335, and 9714–9723 (*trnS-G*) of the *Coffea arabica* chloroplast genome (GenBank accession EF044213; Samson et al., 2007). In addition, two AT-rich regions in the *trnS-G* and *trnT-F* markers, respectively (corresponding to positions 9184–9203 and 47342–47370 of the *Coffea* chloroplast genome), were also excluded from analyses due to difficulties in finding unambiguous alignments. Aligned data sets (Appendix S1; see Supplemental Data online at <http://www.amjbot.org/cgi/content/full/ajb.1000090/DC1>) of (I) the nrDNA region (ITS), (II) the combined protein-coding cpDNA regions (*matK* and *ndhF*), and (III) the combined noncoding cpDNA regions (the *rps16* intron, *trnH-psbA*, *trnS-G*, *trnT-F*), were analyzed separately and in combination. Methods of phylogenetic reconstruction included maximum parsimony (MP) and Bayesian MCMC inference (BI; Yang and Rannala, 1997), using the programs PAUP* version 4.0b10 (Swofford, 2002) and MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) respectively. Gaps were treated as missing data.

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Fig. 1. Some of the morphological variation within the Condamineae. (A) *Emmenopterys henryi*, (B) *Dolicholobium oblongifolium*, (C) *Mussaendopsis beccariana*, (D) *Ferdinandusa elliptica*, (E) *Simira maxonii*, (F) *Wittmackanthus stanleyanus*, (G) *Pogonopus tubulosus*, (H) *Condaminea corymbosa*, (I) *Macbrideina peruviana*, (J) *Schizocalyx peruvianus* (*Bathysa peruviana*), (K) *Macrocnemum roseum*, (L) *Sommerera donnell-smithii*, (M) *Pentagonia wendlandii*. Image credits: A, PLANTCOL, Arboretum Kalmthout, Jan De Langhe; B and M, Johan Rova; C, copyright by Christian Puff, Faculty Centre of Biodiversity, Univ. Vienna; used with permission; D, E, I–K, Claes Persson; F, Claes Gustafsson; G, H, L, Robin Foster, The Field Museum).

TABLE 1. Year of publication and previously proposed tribal classifications of the genera of the Condamineae according to Hooker (1873), Schumann (1889, 1891), Robbrecht (1988), Andersson (1995), Delprete (1996a), and Bremer and Eriksson (2009). ! = excluded from Rubiaceae, CAL = Calycophylleae, CAT = Catesbaeeae, CHI = Chiococceae, CIN = Cinchoneae, CON = Condamineae, COP = Coptosapelteae, *inc* = *incertae sedis*, HIP = Hippotideae, MUS = Mussaendeae, RON = Rondeletiae, SIM = Simireae, TAM = Tammsieae.

| Genera | Year | Hooker | Schumann | Robbrecht | Andersson | Delprete | Bremer & Eriksson |
|---|------|------------------|------------------|------------|-------------------------|------------------|-------------------|
| <i>Alseis</i> Schott | 1827 | CIN | CIN | CIN | CAL | — | CON |
| <i>Bathysa</i> C. Presl | 1845 | RON | RON | RON | — | RON | CON |
| <i>Bothriospora</i> Hook. f. | 1870 | HAM | GAR | <i>inc</i> | — | — | — |
| <i>Calycophyllum</i> DC. | 1830 | CIN | CIN | CIN | CAL | — | CON |
| <i>Capirona</i> Spruce | 1859 | CIN | CIN | CIN | CAL | — | CON |
| <i>Chimarrhis</i> Jacq. | 1763 | CON | CON | CON | — | RON? | CON |
| <i>Condaminea</i> DC. | 1830 | CON | CON | CON | — | RON | CON |
| <i>Dialypetalanthus</i> Kuhlman | 1925 | — | — | ! | — | — | CON |
| <i>Diocodendron</i> Steyerl. | 1963 | — | — | CON | — | RON? | CON |
| <i>Dolichodelphys</i> K. Schum. & K. Krause | 1908 | — | GAR ⁵ | GAR | — | RON | CON |
| <i>Dolichobium</i> A. Gray | 1860 | CIN | CIN | CIN | CIN? ⁴ | — | CON |
| <i>Elaeagia</i> Wedd. | 1849 | RON | RON | RON | — | RON | — |
| <i>Emmenopterys</i> Oliv. | 1889 | — | CIN ⁶ | CIN | <i>inc</i> ⁴ | — | CON |
| <i>Ferdinandusa</i> Pohl | 1829 | CIN | CIN | CIN | CAL | — | CON |
| <i>Hipposis</i> Ruiz & Pav. | 1794 | MUS | MUS | HIP | — | — | CON |
| <i>Macbrideina</i> Standl. | 1929 | — | — | RON | — | RON | CON |
| <i>Macrocnemum</i> P. Browne | 1756 | CIN | CIN | CIN | CAL | — | — |
| <i>Mastixiodendron</i> Melch. | 1925 | — | — | CHI? | — | CHI? | — |
| <i>Mussaendopsis</i> Baill. | 1879 | — | CIN | CIN | COP ⁴ | — | CON |
| <i>Parachimarrhis</i> Ducke | 1922 | — | — | CON | — | RON | CON |
| <i>Pentagonia</i> Benth. | 1845 | CAT | MUS | HIP | — | — | CON |
| <i>Phitopsis</i> Hook. f. | 1871 | GAR | RON | <i>inc</i> | — | — | — |
| <i>Picardaea</i> Urb. | 1903 | — | — | CON | — | RON | CON |
| <i>Pinckneya</i> Michx. | 1803 | CON | CON | CON | — | RON | CON |
| <i>Pogonopus</i> Klotzsch | 1853 | CON | CON | CON | — | RON | CON |
| <i>Rustia</i> Klotzsch | 1846 | CON | CON | CON | — | RON | CON |
| <i>Semaphyllanthus</i> L. Andersson | 1995 | — | — | — | CAL | — | — |
| <i>Schizocalyx</i> Wedd. | 1854 | CIN | CIN | CIN | RON | RON ⁷ | — |
| <i>Simira</i> Aubl. | 1775 | CIN ¹ | RON ¹ | RON | — | RON | CON |
| <i>Sommeria</i> Schldtl. | 1835 | CAT | MUS | HIP | — | — | CON |
| <i>Tammisia</i> H. Karst. | 1861 | CAT | MUS | TAM | — | — | — |
| <i>Tresanthera</i> H. Karst. | 1859 | CON ² | CON | CON | — | RON | — |
| <i>Warszewiczia</i> Klotzsch | 1853 | RON | RON | RON | — | RON | CON |
| <i>Wittmackanthus</i> Kuntze | 1891 | RON ³ | RON ³ | CIN | CAL | — | CON |

¹As *Sickingia* Willd.; ²As *Rustia*; ³As *Pallasia* Klotzsch; ⁴Andersson and Persson (1991); ⁵Schumann and Krause (1908); ⁶Schumann (1897); ⁷Delprete (1997); dash (—) indicates genera not classified by author; ? indicates a tentative classification.

The MP analyses were performed using heuristic searches with the tree-bisection-reconnection (TBR) branch swapping algorithm, Multrees on, 1000 random sequence addition replicates, and a maximum of 10 trees saved per replicate. Clade support was estimated using 1000 bootstrap replicates (Felsenstein, 1985), with three random addition replicates per replicate. For the Bayesian inference analyses, substitution models suggested as best-fit to the data under the corrected Akaike information criterion (AICc; Akaike, 1974), as implemented in the program MrAIC v1.4.2 (Nylander, 2004), a script dependent on the program PHYML v2.4.1 (Guindon and Gascuel, 2003), were used for each data set (I, GTR+G; II, GTR+I+G; III, GTR+I+G). Model parameter estimates (except topology) were unlinked among data sets when analyzed in combination. The analyses comprised two runs of four chains each, which were monitored for 20×10^6 generations, with every 1000th generation being sampled, and a chain heating parameter of 0.15. Trees sampled before stable posterior probability (PP) values had been reached, were excluded from consensus as a burn-in phase (initial 50% of the sampled trees). Standard deviations of split frequencies between separate runs were calculated using the program splitsmb v.0.1.1 (Lakner and Ronquist, 2008). Nodes receiving a bootstrap support (BS) of <70% in the MP analyses, or a PP of <0.95 in the BI analyses, were not considered as well supported.

All BI analyses performed well as judged from parameter move acceptance rates (ITS, 10–26%; plastid coding, 10–63% except state frequencies [4%]; plastid noncoding, 6–57%, except state frequencies [2%]; and similarly for the combined data sets), and chain swap percentages (ITS, 7–20%; plastid coding, 20–25%, except <1% for one chain in both analyses; plastid noncoding, 12–21%; plastid combined, 33–46%; all combined, 17–28%). Initial analyses using the default chain temperature value (0.2) did not mix as well. The mean standard deviations of split frequencies between the two separate analyses for each

data set were low (ITS, 0.005; plastid coding, 0.005; plastid noncoding, 0.003; plastid combined, 0.005; all combined, 0.003). The potential scale reduction factor [PSRF] was 1.0 for all parameters in all analyses.

Morphological ancestral state reconstructions—Morphological information was mainly obtained from literature (primarily from protologues; Schumann [1889, 1891], Andersson and Persson [1991], Andersson [1994, 1995], Andersson and Rova [2004], Rova and Andersson [1995], Delprete [1996a, 1999], Pennington et al. [2004], and Taylor et al. 2004]), complemented with observations of herbarium material (studied at GB, MO, S, and UPS). Ancestral character states were reconstructed for the following morphological characters: stipule type, flower breeding systems, corolla lobe aestivation, calycophylls, fruit type, and seed wings. Stipules were coded as (0) interpetiolar, (1) intrapetiolar, or (2) calyprate; breeding systems as (0) protandrous, (1) protogynous, (2) monoecious, (3) dioecous, or (4) heterostylous; aestivation as (0) imbricate, (1) left-contorted, (2) valvate, or (3) open; calycophylls as (0) absent, or (1) present; fruit type as (0) septicidal, (1) loculicidal, or (3) indehiscent; seed wings as (0) absent, (1) bipolar, (3) lateral, or (4) orbicular. The morphological data matrix is included in Appendix S2.

Using the scripts catmb.pl (written by Johan Nylander, Stockholm University) and seltree-mini.pl (written by Torsten Eriksson), we randomly selected 1000 trees from the postburn-in trees generated in the MrBayes analyses of the combined data. Ancestral states were reconstructed across these trees using maximum likelihood (Mk1 model—equal probability of change between states), as implemented in the program Mesquite v.2.72 (Maddison and Maddison, 2010), then plotted on the tree with the highest marginal likelihood (selected using the script mbscan v.1.0.0, written by Torsten Eriksson).

RESULTS

A summary of the tree data and statistics from the analyses is given in Table 2. There were no well-supported nodes that conflicted from the Bayesian inference analyses and the maximum parsimony analyses. In the phylogenetic hypotheses from the nrDNA data, the Condamineae were resolved as monophyletic in both BI and MP analyses; however, this node was well supported only in the former (PP, 1.00). Within the Condamineae, a basal trichotomy was inferred, consisting of *Dioicodendron*, an *Emmenopterys-Pinckneya* clade (PP, 1.00), and a crown group (PP, 1.00; BS, 70%) of the remaining genera of the tribe. The crown group was poorly resolved and comprised six clades: (I) *Ferdinandusa*; (II) a “Malesian-Pacific clade” consisting of *Dolicholobium*, *Mastixiodendron*, *Mussaendopsis* (PP, 1.00); (III) a “septicidal clade” consisting of *Alseis*, *Bathysa*, *Bothriospora*, *Calycophyllum*, *Capirona*, *Chimarrhis*, *Dialypetalanthus*, *Dolichodelphys*, *Parachimarrhis*, *Simira*, *Warszewiczia*, and *Wittmackanthus* (PP, 0.99); (IV) a *Picardaea* and *Pogonopus* clade (PP, 1.00; BS, 91%); (V) a clade consisting of *Bathysa*, *Condaminea*, *Elaeagia*, *Macbrideina*, and *Phitopsis* (PP, 1.00); and (VI) a clade consisting of the hippotides (*Hippotis*, *Pentagonia*, and *Sommeria*), and *Macrocnemum*, *Rustia*, and *Tresanthera* (PP, 1.00; BS, 93%). The 95% majority rule consensus tree from the Bayesian analysis of the nrDNA data are shown in Fig. 2, with bootstrap support values from the MP analysis. Besides the well-supported clades shown in the figure, a number of additional clades were well supported in the MP analysis. These were a *Mussaenda-Posoqueria* clade (BS, 77%), a *Bathysa australis-B. stipulata* clade (BS, 73%), and a *Calycophyllum multiflorum-C. spruceanum* clade (BS, 87%).

The separate phylogenetic hypotheses from the BI analyses of the protein-coding genes and of the noncoding cpDNA data sets (trees not shown) were congruent in all but one node (*Simira corumbensis* formed a sister group to remaining *Simira* in the former and a sister group to *Pogonopus* in the latter). However, combining the data resulted in increased support for the former position; consequently, we did not consider combining the plastid data sets as problematic. In analyses of the combined cpDNA data, the Condamineae appeared monophyletic and strongly supported (PP, 1.00; BS, 100%). Within the tribe, *Emmenopterys* and *Pinckneya* formed a basal trichotomy with a Condamineae crown group (PP, 1.00). *Dioicodendron* was unresolved within the crown group, as were a number of other genera (*Condaminea*, *Macbrideina*, and *Picardaea*). The Malesian-Pacific clade (PP, 1.00; BS, 100%), the septicidal clade (PP, 1.00), and the hippotide-*Macrocnemum-Rustia-Tresanthera* clade (PP, 1.00; BS, 76%), were all supported. Within the

latter clade, the hippotides appeared monophyletic (PP, 1.00). The 95% majority rule consensus tree from the Bayesian analysis of the cpDNA data are shown in Fig. 3, with bootstrap support values from the MP analysis. In the MP analysis, the Condamineae formed a sister group to an Ixoroideae crown group (*Alberta*, *Coffea*, *Crossopteryx*, *Mussaenda*, *Sabicea*, *Scyphiphora*, and *Steenisia*; BS, 71%).

Although generally congruent with analyses of the nrDNA data, the analyses of the cpDNA data supported a number of clades that were incongruent with the topology inferred from the nrDNA data. These incongruences were (I) *Ferdinandusa* as sister group to a *Parachimarrhis-Simira* clade (PP, 1.0), (II) *Pogonopus* as sister to *Elaeagia myriantha* (PP, 0.97), (III) *Simira corumbensis* as sister to the rest of the *Simira* species (PP, 1.0), (IV) *S. glaziovii* as sister to a *S. cordifolia-S. maxonii* clade (PP, 0.98; BS, 79%), and (V) *Rustia rubra* as sister to *R. viridiflora* (PP, 1.00; BS, 80%). In the MP and BI analyses of the combined cpDNA and nrDNA data (Fig. 4), the support values for the *Parachimarrhis-Simira* clade were increased compared to the corresponding values from nrDNA data alone, and the support values for the *Picardaea-Pogonopus* clade were unchanged. Consequently, these incongruences (I-II) were not considered as strong conflicts. In contrast, the support values within the *Simira* and *Rustia* clades were reduced relative to corresponding values in the separate analyses, indicating a conflict in the phylogenetic signal for incongruences III–V. Therefore *Rustia rubra*, *Simira corumbensis*, and *S. glaziovii* were excluded in the final analyses of the combined data.

Combining the data resulted in somewhat increased resolution (Fig. 4) and, in general, higher support values. As in the nrDNA tree, *Dioicodendron*, *Emmenopterys*, and *Pinckneya* were inferred as early-diverging clades within the tribe. *Dioicodendron* was in the BI analyses sister to the remaining tribe. However, bootstrap support for this node was <70%. Within the Condamineae crown group (PP, 1.00; BS, 91%), four clades were resolved; *Ferdinandusa*, the Malesian-Pacific clade (PP, 1.00; BS, 97%), the septicidal clade (PP, 1.00; BS, 82%), and a “loculicidal clade” (PP, 1.00), comprising the genera *Condaminea*, *Elaeagia*, *Hippotis*, *Macbrideina*, *Macrocnemum*, *Pentagonia*, *Phitopsis*, *Picardaea*, *Pogonopus*, *Rustia*, *Sommeria*, *Tammsia*, and *Tresanthera*. Besides the supported clades indicated in Fig. 4, two additional clades were well supported in the MP analysis: a *Alseis-Bothriospora-Dialypetalanthus-Wittmackanthus* clade (77%) and a *Rustia alba-R. bilsana-R. formosa-R. thibaudioides-R. viridiflora-Tresanthera* clade (77%). The Calycophylleae sensu Andersson (1995) were found to be polyphyletic, whereas the Hippotideae sensu Rova and Andersson (1995) were resolved as monophyletic (PP, 1.00), nested within the loculicidal clade.

TABLE 2. Statistics and parameter estimates for the different DNA regions and data sets. Values in parenthesis are specific to the Condamineae, i.e., excluding outgroup taxa. PIC = Parsimony informative characters.

| Data set | ITS | matK | ndhF | rps16 | trnH-psbA | trnS-G | trnT-L-F | cpDNA | Combined |
|--------------------------|---------------|---------------|--------------|--------------|-------------|--------------|--------------|--------------|---------------|
| Number of taxa | 71 (65) | 84 (70) | 57 (42) | 88 (72) | 75 (72) | 83 (68) | 89 (73) | 91 (75) | 89 (73) |
| Excluded characters | 0 (0) | 0 (0) | 0 (0) | 34 (33) | 38 (38) | 178 (168) | 67 (49) | 317 (288) | 317 (288) |
| Included characters | 774 (768) | 780 (762) | 2140 (2116) | 948 (853) | 420 (400) | 1482 (1357) | 2198 (1974) | 7968 (7462) | 8742 (8230) |
| Variable characters | 384 (348) | 307 (189) | 642 (328) | 285 (153) | 121 (102) | 448 (224) | 698 (348) | 2501 (1344) | 2885 (1692) |
| PIC | 291 (265) | 156 (93) | 320 (122) | 142 (77) | 62 (52) | 212 (103) | 335 (169) | 1227 (616) | 1518 (881) |
| % informative characters | 37.60 (34.51) | 20.00 (12.20) | 14.95 (5.77) | 14.98 (9.03) | 14.76 (13) | 14.30 (7.59) | 15.24 (8.56) | 15.40 (8.26) | 17.36 (10.70) |
| Consistency index (CI) | 0.41 (0.44) | 0.64 (0.73) | 0.68 (0.81) | 0.70 (0.79) | 0.71 (0.68) | 0.68 (0.69) | 0.77 (0.85) | 0.69 (0.75) | 0.61 (0.62) |
| Retention index (RI) | 0.65 (0.69) | 0.68 (0.77) | 0.69 (0.69) | 0.75 (0.83) | 0.79 (0.76) | 0.68 (0.71) | 0.77 (0.84) | 0.70 (0.74) | 0.67 (0.70) |

Note: PIC = parsimony informative characters

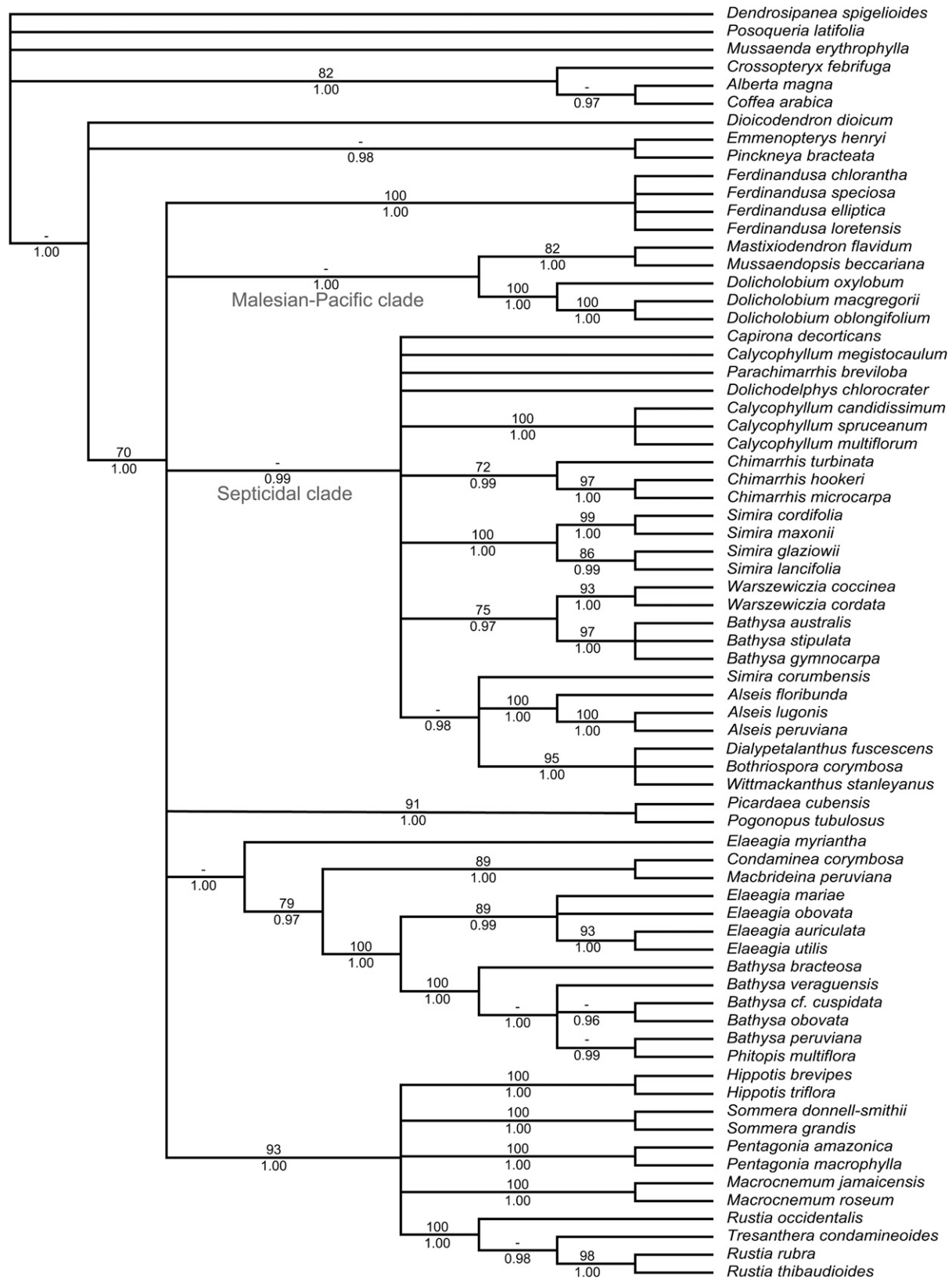


Fig. 2. The 95% majority rule consensus tree of all trees sampled after the burn-in in the Bayesian analysis of the nrDNA data (ITS region). Posterior probabilities are indicated below branches and bootstrap support values above. A hyphen (-) indicates a bootstrap support value <70%. Note that a number of additional nodes were supported in the maximum parsimony analysis (see Results).

Morphological character state reconstructions (Fig. 5), supported interpetiolar stipules, protandrous flowers without calycophylls, and capsules with septicidal dehiscence as ancestral states for the Condamineae, whereas the states of corolla lobe aestivation and seed wing were equivocal. Protogyny appears to have evolved independently at least two times within Condamineae, whereas intrapetiolar stipules, loculicidal capsules, and indehiscent fruits appear to have evolved independently at least three times.

DISCUSSION

In this study, we investigated the systematics of the Condamineae using phylogenetic reconstruction of molecular data. Separate analyses of cpDNA and nrDNA each resolve the tribe as monophyletic. The tribe is morphologically very diverse and not easily characterized (see Synopsis), nor are any potential morphological synapomorphies known. Still, because molecular data strongly shows that the Condamineae are monophyletic, we do not consider any alternative classification (subdivision) as being more appropriate. Below we characterize the resolved clades of the Condamineae and discuss the inferred systematic relationships. Unless otherwise indicated, the phylogenetic hypothesis referred to is that from the combined data set (Fig. 4). The informal names “the hippotides” (Rova and Andersson, 1995), “the Malesian-Pacific clade”, “the loculicidal clade”, and “the septicidal clade” are used for the convenience of the discussion.

Systematic relationships—*Dioicodendron*—The position of *Dioicodendron* is not unambiguously resolved. The genus is part of a basal polytomy together with *Emmenopterys*, *Pinckneya*, and a Condamineae crown group in the nrDNA phylogenetic hypotheses (Fig. 2), whereas it is unresolved within the crown group or within the entire tribe in the BI and MP analyses of cpDNA data, respectively (Fig. 3). In the BI analysis of the combined data (Fig. 4), *Dioicodendron* appears as sister to the remaining Condamineae. *Dioicodendron* occurs at medium to high elevations in the Andes of northwestern South America (Delprete, 1999). It is the only dioecious genus in the Condamineae, with small, greenish white, and relatively unspecialized flowers (in concordance with a general morphology of dioecious tropical trees as discussed by Bawa [1975]). *Dioicodendron* can be further characterized by valvate corolla lobe aestivation; spreading, \pm foliose, persistent stipules; and septicidal capsules with horizontally inserted seeds. The seeds of *Dioicodendron* are somewhat similar to seeds of *Picardaea* and *Pogonopus*, being 3–5-angular with a granulate and irregularly reticulate testa (Fig. 6Z) (Delprete, 1999).

Emmenopterys—In the phylogenetic hypotheses from the nrDNA data, *Emmenopterys* forms a sister group to *Pinckneya*. Support for this clade is not found in analyses of cpDNA data, nor in BI analyses of the combined data. Both genera are deciduous trees, with interpetiolar caducous stipules, imbricate corolla aestivation, calycophylls, fruits with caducous calyces, and winged seeds. However, unlike *Pinckneya*, the flowers of *Emmenopterys* have funnelliform corollas, and included stamens and stigmas (Fig. 1A). Furthermore, the capsules of *Emmenopterys* are oblong-cylindrical to fusiform with the disk portion shortly prolonged into a rounded beak (C. M. Taylor, Missouri Botanical Garden, personal communication). The dehiscence is

septicidal, and the seeds are vertically inserted and imbricate and have bipolar wings (Oliver, 1889). *Emmenopterys* is restricted to southern China–northern Vietnam.

Pinckneya—As mentioned, *Pinckneya* is morphologically similar to *Emmenopterys* in a number of characters. However, *Pinckneya* differs in having flowers with tubular corollas and exerted stamens and stigmas. Furthermore, the capsules of *Pinckneya* are lenticellate, semiglobose, and bilobed with a somewhat convex apex. The dehiscence is loculicidal, and the seeds are horizontally inserted in two rows and have wide orbicular wings (Delprete, 1996c; cf. fig. 8G in Delprete, 1999). *Pinckneya* is endemic to southeastern USA. The *Emmenopterys*–*Pinckneya* clade could consequently provide another example of a well-established southeastern Asia–southeastern United States disjunct distribution pattern found in many other extant plant groups. However, the phylogenetic relationship between the two genera requires further study.

Ferdinandusa—The neotropical genus *Ferdinandusa* occurs in an unresolved position in the phylogenetic hypotheses from nrDNA data, whereas it is inferred as sister group to a *Parachimarrhis*–*Simira* clade by cpDNA data. This latter clade is not retrieved in the analyses of the combined data. No potential synapomorphies are known supporting this relationship (with the possible exception of relatively large seeds). The genus is chiefly South American, with most species concentrated in Brazil (Steyermark, 1972). *Ferdinandusa* has left-contorted corolla lobe aestivation, septicidal fruit dehiscence, and ascending imbricate seeds with a bipolar wing (Fig. 6B), characters states shared with several genera of the Malesian-Pacific clade as well as the septicidal clade (i.e., *Capirona*, *Dolicholobium*, *Mussaendopsis*, and *Semaphyllanthus*).

Malesian-Pacific clade—In the inferred phylogenetic hypotheses of nrDNA as well as cpDNA, *Dolicholobium*, *Mastixiodendron*, and *Mussaendopsis* form a well-supported clade; however, neither data set, nor the combined data, resolves the position of this clade within the tribe with strong support. All three genera are distributed in the Old World (*Dolicholobium*, Malesia-southwestern Pacific; *Mastixiodendron*, Moluccas-Fiji; *Mussaendopsis*, Malesia-Sulawesi). Within the clade, *Dolicholobium* forms a sister group to the *Mastixiodendron*–*Mussaendopsis* clade. A possible synapomorphy for the Malesian-Pacific clade are paired-axillary inflorescences confined to the upper nodes of the shoot. With the exception of *Mussaendopsis celebica* Bremek., *Mastixiodendron* and *Mussaendopsis* both have \pm apetalous, rotate corollas (Merrill and Perry, 1945; Darwin, 1977; Puff and Igersheim, 1994). The aestivation in *Mussaendopsis* is left-contorted (Oliver, 1895; cf. fig. 5D in Puff and Igersheim, 1994), as is the aestivation of *Dolicholobium* (Bremer, 1987). In *Mastixiodendron*, the aestivation is valvate (Darwin, 1977). *Mastixiodendron* also differs in having indehiscent drupaceous fruits (autapomorphic within the clade [Fig. 5]), with solitary and pendulous ovules (Darwin, 1977), whereas the fruits of *Dolicholobium* and *Mussaendopsis* are septicidal capsules with numerous, ascending ovules (Jansen and Ridsdale, 1983; Puff and Igersheim, 1994).

Septicidal clade—This clade is resolved in the phylogenetic hypotheses from nrDNA and the combined data. It contains several genera previously classified as Cinchoneae/Calycophylleae (*Alseis*, *Calycophyllum*, *Capirona*, and *Wittmackanthus*),

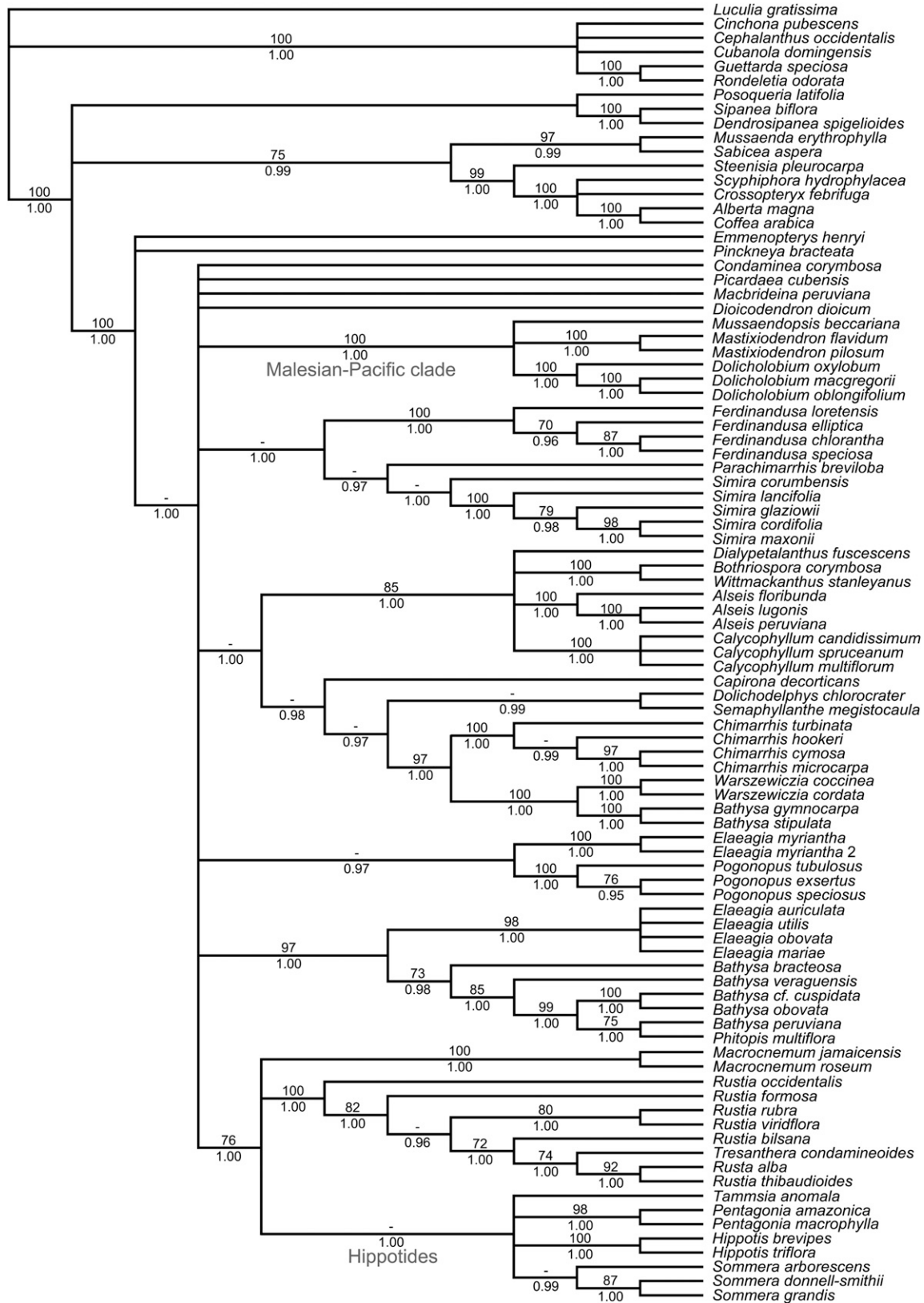


Fig. 3. The 95% majority rule consensus tree of all trees sampled after the burn-in in the Bayesian analysis of the cpDNA data (*matK*, *ndhF*, *rps16* intron, *trnH-psbA*, *trnSG*, and *trnT-F*). Posterior probabilities are indicated below branches and bootstrap support values above. A hyphen (-) indicates a bootstrap support value <70%. Note that a number of additional nodes were supported in the maximum parsimony analysis (see Results).

Condamineae (*Chimarrhis* and *Parachimarrhis*), or Rondeletieae (*Bathysa*, *Simira*, and *Warszewiczia*), and a number of genera of differing taxonomic classification (*Bothriospora*, *Dolichodelphys*, and *Dialypetalanthus*). The septicidal clade can be subdivided into three main clades: (I) a *Parachimarrhis-Simira* clade; (II) a clade consisting of *Alseis*, *Bothriospora*, *Calycophyllum*, *Dialypetalanthus*, and *Wittmackanthus*; and (III) a clade consisting of *Bathysa*, *Chimarrhis*, *Dolichodelphys*, and *Warszewiczia*. *Capirona* is unresolved within the septicidal clade.

As suggested by the informal name, most genera of the clade have septicidal capsules. However, *Parachimarrhis* and *Simira* have loculicidal capsules, and *Bothriospora* has indehiscent fruits (berries), both fruit types apparently derived in the clade (Fig. 5). Aestivation is in general imbricate, although some genera have left-contorted (*Capirona* and *Semaphyllanthe*), or open (some species of *Alseis* and *Simira*) corolla lobes in bud.

Simira and *Parachimarrhis*—Bremekamp (1966) considered the placentation and ovule number of *Simira* as indicating an isolated position within Rubiaceae and classified the genus in the monogeneric Simireae. In the phylogenetic hypothesis here presented, *Simira* forms a sister group to the monotypic genus *Parachimarrhis*. *Simira* is widely distributed in the neotropics, occurring from Mexico to the Atlantic forest of southern Brazil. *Parachimarrhis* occurs in lowland Amazonian rain forest (Delprete, 1999). Delprete (1999), suggested a close relationship of these two genera primarily on their bilobular fruits and crescent-shaped seeds with a lateral wing (Fig. 6K), inserted horizontally in two rows. Although nested within the septicidal clade, both genera have loculicidal capsules. *Parachimarrhis* differs from *Simira* in its smaller capsules and seeds and its yellowish wood (the wood of *Simira* typically turns reddish when exposed to air; Delprete, 1999).

Alseis, *Calycophyllum*, and *Semaphyllanthe*—Andersson (1995) transferred a number of species of *Calycophyllum* [*C. intonsum* Steyer., *C. megistocaulum* (K. Krause) C. M. Taylor, *C. merumense* Steyer., *C. obovatum* (Ducke) Ducke, *C. spectabile* Steyer., *C. venezuelense* Steyer.] to a new genus, *Semaphyllanthe*. Distinguishing characters included free stipules (vs. calyptrate), plane bracts (vs. enlarged bracts enclosing the floral buds), contorted (vs. imbricate) aestivation, and filaments attached in the lower part of the corolla tube (vs. in the throat). This distinction was not recognized by Delprete (1996b), who treated *Calycophyllum* in the traditional sense. In this study, *C. megistocaulum* is not found associated with the type *C. candidissimum*, hence supporting a generic distinction. *Semaphyllanthe* will consequently be discussed as a separate genus. However, it should be noted that *S. megistocaula* is the only included species and that this species was considered aberrant by Andersson (1995). Clearly, the generic delimitations within this group needs to be further studied. *Calycophyllum* sensu Andersson was restricted to *C. candidissimum*, *C. multiflorum*, and *C. spruceanum*. These are all included in this study, where the (mainly) Central American *C. candidissimum* is found as sister group to the South American species *C. multiflorum* and *C. spruceanum*. In the MP analyses of the combined data, *Calycophyllum* (s.s.) forms a sister group (BS, 77%) to a clade consisting of *Alseis*, *Bothriospora*, *Dialypetalanthus*, and *Wittmackanthus*. No morphological synapomorphies for this clade are known.

Alseis is a widely distributed genus occurring from southern Mexico to Peru and in eastern Brazil. *Alseis* is unusual within Rubiaceae in its deciduous habit and protogynous flowers

(Taylor, 2002) and can be further characterized by subspicate inflorescences and pendent placentas (Andersson, 1995).

Dialypetalanthus, *Bothriospora*, and *Wittmackanthus*—The monotypic *Dialypetalanthus*, from the southern Amazon basin, is morphologically aberrant in Rubiaceae on account of its flowers with free petals, numerous stamens, and porate anthers. These character states have complicated the correct classification of the genus. Furthermore, *Dialypetalanthus* is also unusual within the Condamineae in having deeply lobed, persistent stipules that form a low sheath around the stem (Hutchinson, 1973; Piesschaert et al., 1997 (interpreted by some authors as intrapetiolar). When Kuhlmann (1925) described *Dialypetalanthus*, he considered it as part of Rubiaceae and associated with tribe Cinchoneae. However, subsequent classifications have recognized *Dialypetalanthus* as constituting a monotypic family (Dialypetalanthaceae), part of Myrtales (Rizzini and Occhioni, 1949), Rubiales (Hutchinson, 1973), Rosales (Cronquist, 1981), or Gentianales (Piesschaert et al., 1997). Fay et al. (2000), in a molecular phylogenetic study, found the genus nested within Rubiaceae in subfamily Ixoroideae, and Bremer and Eriksson (2009) found the genus to be associated with the Condamineae. In this study, *Dialypetalanthus* forms a strongly supported sister group to *Bothriospora* and *Wittmackanthus*, indicating that the unique floral morphology of *Dialypetalanthus* is derived. Although differing in floral characters, the fruits of *Dialypetalanthus* have numerous ascending and imbricate seeds (Piesschaert et al., 1997) and a fruit and seed structure similar to that of *Capirona* and *Calycophyllum* (Fig. 6G) (Pennington et al., 2004).

The sister group to *Dialypetalanthus* is a clade of *Bothriospora* and *Wittmackanthus*, two monotypic genera from northern South America (the latter is also found in eastern Panama). The three genera appear somewhat morphologically disparate, and no synapomorphies are known. *Bothriospora* is unique within the Condamineae in having 4–5-locular, yellow berries. The seeds of *Bothriospora* are horizontally inserted, imbricate, oblong, exalate, and tuberculate (Hooker, 1870; Schumann, 1889). *Wittmackanthus* is similar to *Calycophyllum* (Steyermark and Kirkbride, 1975), from which it differs in having unequal and included stamens and subspicate inflorescences similar to those of *Alseis* (Andersson, 1995). However, unlike *Alseis*, the lowermost flowers of the inflorescence often have pink-lilac calyophylls (Fig. 1F) (Claßen-Bockhoff, 1996).

Capirona—*Capirona*, a widely distributed genus in northern South America, was in the study by Andersson (1995) closely associated with *Calycophyllum* (s.l.). In this study, the phylogenetic position of the genus is unresolved within the septicidal clade. *Capirona*, like *Semaphyllanthe* has contorted aestivation and filaments attached near the corolla base. However, unlike the latter *Capirona* has intrapetiolar stipules (autapomorphy, Fig. 5) and large and foliaceous bracts (Schumann, 1889). *Calycophyllum*, *Capirona*, and *Semaphyllanthe* (as well as *Wittmackanthus*), all have smooth, papery, reddish-brown exfoliating bark (Gentry, 1993; Pennington et al., 2004).

Bathysa, *Chimarrhis*, *Dolichodelphys*, and *Warszewiczia*—*Bathysa* appears polyphyletic in the phylogenetic hypotheses of both cpDNA and nrDNA data. *Bathysa australis*, *B. gymnocarpa*, and the type, *B. stipulata* are from the Brazilian Atlantic rainforest and form a sister group to *Warszewiczia*, whereas species of western South America (*B. bracteosa*, *B. obovata*,

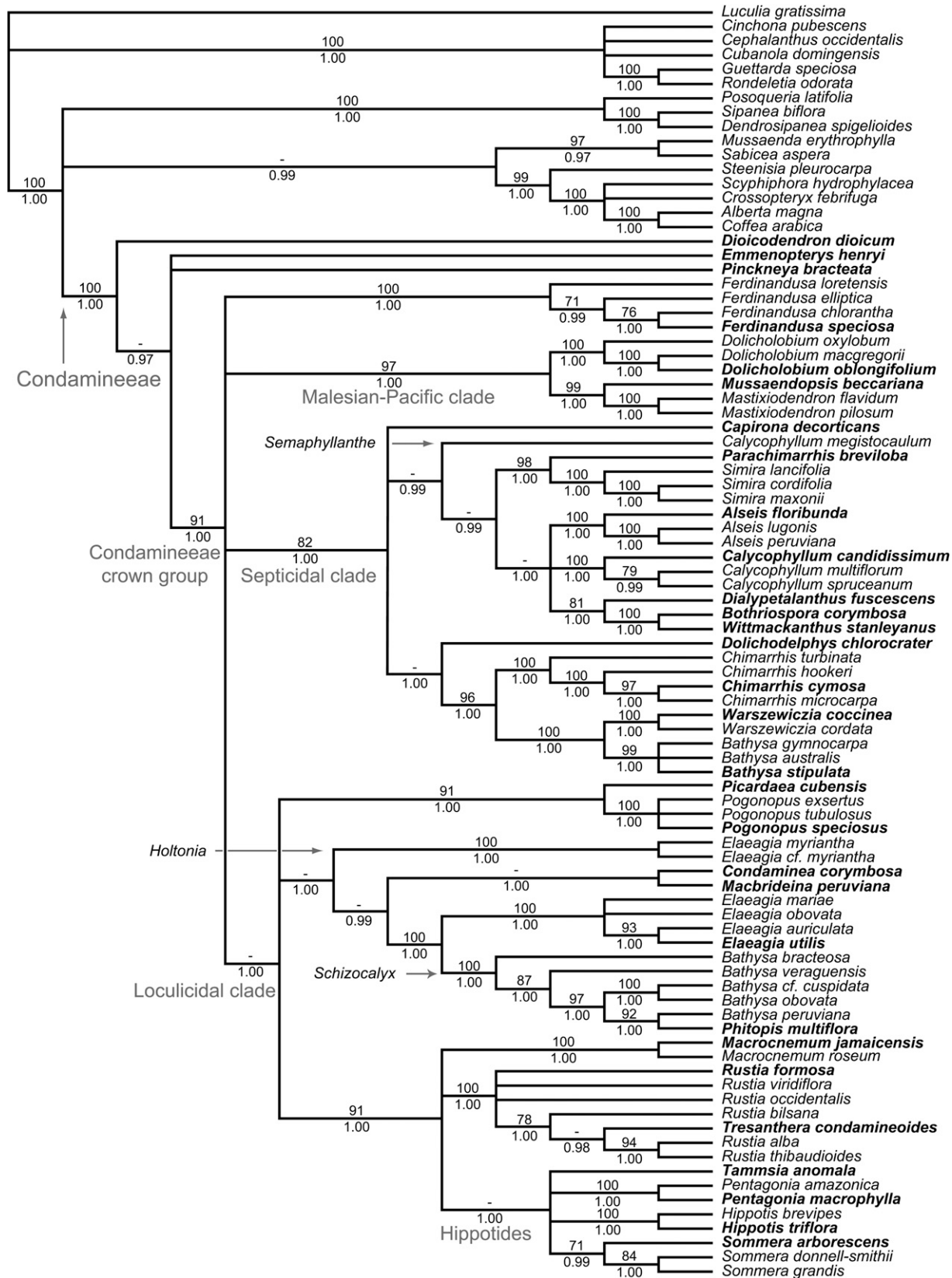


Fig. 4. The 95% majority rule consensus tree of all trees sampled after the burn-in in the Bayesian analysis of the combined data. Posterior probabilities are indicated below branches and bootstrap support values above. A hyphen (-) indicates a bootstrap support value <70%. Species names in bold represent types of genera. Note that a number of additional nodes were supported in the maximum parsimony analysis (see Results).

and *B. peruviana*) and Panama (*B. veraguensis*) form a sister group to *Elaeagia*. Curiously, the Brazilian species *B. cuspidata* is part of the latter clade (from here on termed the *Schizocalyx* clade). Also nested within *Schizocalyx* is *Phitopsis*.

Morphologically, *Bathysa* and *Schizocalyx* are distinct. In *Bathysa*, the stipules are persistent (except in *B. gymnocarpa*; Germano-Filho, 1999) and free. In contrast, the stipules of *Schizocalyx* are early-caducous, coherent-connate, and calyptrate in bud (Hooker, 1873; Rova and Andersson, 1995; Germano-Filho, 1999). Interestingly, the stipules of *Elaeagia*, the sister group of *Schizocalyx*, are also united and calyptrate in bud, being oriented perpendicularly to the subtending leaves (Taylor, 2001; Taylor et al., 2004). However, unlike the stipules of *Schizocalyx*, the stipules of *Elaeagia* split into intrapetiolar sections as new leaves emerge, and these sections are more or less persistent (in some species only the lower portion persists; Taylor, 2001). The corolla of *Bathysa* is hypocrateriform, cream-colored or greenish yellow, and predominantly tetramerous (although *B. australis* can be pentamerous; Germano-Filho, 1999), whereas the corolla in *Schizocalyx* is funnelliform, white, and pentamerous. The corolla lobes of *Bathysa* are imbricate in bud, whereas examined specimens of *B. bracteosa* and *B. peruviana* have left-contorted aestivation (in the original description of *B. bracteosa* by Weddell [1854] as *Schizocalyx bracteosus*), the aestivation is described as contorted-imbricate, whereas Hooker [1873] described the aestivation as strictly contorted).

Flowers of *Schizocalyx*, as of most Condamineae, are protandrous. However, we observed specimens of *B. australis*, *B. gymnocarpa*, *B. mendoncae* K. Schum., and *B. stipulata* with floral buds with stamens still included, but exerted and expanded styles, indicating protogyny. Schumann (1891), described *B. stipulata* as being gynodioecious and included an illustration showing a flower bud with protruding stigma (fig. 9F in Schumann, 1891), a description likely based on a misinterpretation of protogynous flowers in a female stage. A similar illustration of a flower bud with protruding style (although with unexpanded stigma lobes) is also seen with the description of *Bathysa pittieri* (Standl.) Steyerl, by Steyermark (1974, fig. 65d). Protogyny may consequently be an additional character state in diagnosing *Bathysa*.

In *Bathysa*, the capsules split open septicidally across the whole fruit (cf. fig. 9G in Schumann, 1891; figs. 3E and 4E in Germano-Filho, 1999), but the examined specimens of *B. obovata*, *B. peruviana*, and *B. veraguensis* displayed loculicidal dehiscence. Furthermore, in these species as well as in *B. cuspidata*, only the apex of the capsule split open; the lower part as well as the persistent calyx remain intact, with the calyx forming a tube through which the capsule opening can be seen (cf. Gentry, 1993; fig. 1D in Delprete, 1997; fig. 9A in Germano-Filho, 1999). Loculicidal capsules that split at the apex are also found in *Elaeagia* (Taylor, 2001).

On the basis of these observations, *Schizocalyx* is clearly morphologically distinct from *Bathysa*. The latter has free and usually persistent stipules, protogynous flowers with hypocrateriform, usually tetramerous corollas, and imbricate aestivation, and capsules dehiscing septicidally across hypanthium. In contrast, *Schizocalyx* has coherent-connate, calyptrate, early-caducous stipules, protandrous flowers with funnelliform pentamerous corollas and left-contorted aestivation, and capsules loculicidal at the apex. Inferred molecular phylogenetic hypotheses as well as morphology indicate that *Bathysa* is not monophyletic as presently circumscribed, and we consequently propose a resurrection of *Schizocalyx* (see Synopsis).

As mentioned, *Bathysa* forms a sister group to *Warszewiczia*. These two genera are in turn nested within a clade which also includes *Chimarrhis* and *Dolichodelphys*. *Warszewiczia* and *Chimarrhis* are both widespread in southern Central America and northern South America (the latter also in the Caribbean), whereas *Dolichodelphys* is a rare genus restricted to northwestern South America (Venezuela to Peru; Delprete, 1999). *Chimarrhis* and *Warszewiczia* both have protogynous flowers (Taylor et al., 2004), as do *Bathysa* (see above); consequently, protogyny appears to be a synapomorphy for the clade consisting of *Bathysa*, *Chimarrhis*, and *Warszewiczia* (Fig. 5).

Loculicidal clade—This clade is resolved in the phylogenetic hypotheses of the nrDNA and the combined data. The clade contains most of the genera of the traditional Condamineae sensu Hooker (1873), i.e., *Condaminea*, *Picardaea*, *Pogonopus*, *Rustia*, and *Tresanthera*, but also the genera *Elaeagia*, *Macbrideina*, *Macrocnemum*, and *Schizocalyx* (including *Phitopsis*), as well as the Hippotidae sensu Rova and Andersson (1995; *Hippotis*, *Pentagonia*, *Sommerera*, and *Tammsia*). The loculicidal clade can be subdivided into (I) a *Picardaea-Pogonopus* clade; (II) a clade consisting of *Condaminea*, *Elaeagia*, *Macbrideina*, *Phitopsis*, and *Schizocalyx*; and (III) a hippotide-*Macrocnemum-Rustia* clade. All the genera have loculicidal capsules (except the hippotides, which all have indehiscent fruits), typically with horizontally inserted, compressed, polymorphic, and exalate seeds (Fig. 6Q–Y).

Picardaea and Pogonopus—Support for this clade comes from nrDNA. Both genera share short-triangular, persistent stipules, (reduplicate) valvate aestivation, caducous calyx in fruit, and irregularly angled, horizontally compressed seeds with reticulate and granulate testa (Fig. 6S, T) (Aiello, 1979; Delprete, 1999). In the phylogenetic analyses of the cpDNA data, *P. tubulosus* of the southern hemisphere (Peru to northern Argentina) is sister to *P. exsertus* (southern Mexico to northern Colombia) and *P. speciosus* (northern Colombia and Venezuela) of the northern hemisphere. *Picardaea* is a Caribbean endemic found only in Cuba and Hispaniola.

Condaminea and Macbrideina—In the phylogenetic hypotheses of the nrDNA and combined data, *Condaminea* forms a sister group to the monotypic genus *Macbrideina*. *Condaminea* occurs from Costa Rica to Bolivia, and *Macbrideina* occurs from Colombia to northwestern Bolivia. Gentry (1993) considered the two genera closely related on account of similar capsules. Both genera share large, caducous, intrapetiolar stipules. Whereas the stipules of *Condaminea* are deeply divided, lanceolate, and connate (interpreted as two pairs of usually connate inter- and intrapetiolar stipules by Delprete [1999]), the stipules of *Macbrideina* are oval-oblong, entire, and free. Both genera have rather large flowers with thick, funnel-salver-shaped corollas, although with differing aestivation patterns (valvate in *Condaminea*, vs. imbricate in *Macbrideina*; Fig. 1H, I).

Elaeagia and Holtonia—In the phylogenetic hypotheses presented, *Elaeagia* forms a strongly supported sister group to the *Schizocalyx* clade. As discussed, this relationship is morphologically supported by left-contorted aestivation, calyptrate stipules in bud, and capsules with (loculicidal) dehiscence restricted to the apices. However, *Elaeagia* does not appear to be monophyletic because *Elaeagia myriantha* forms a sister group to the *Condaminea-Elaeagia-Macbrideina-Schizocalyx* clade.

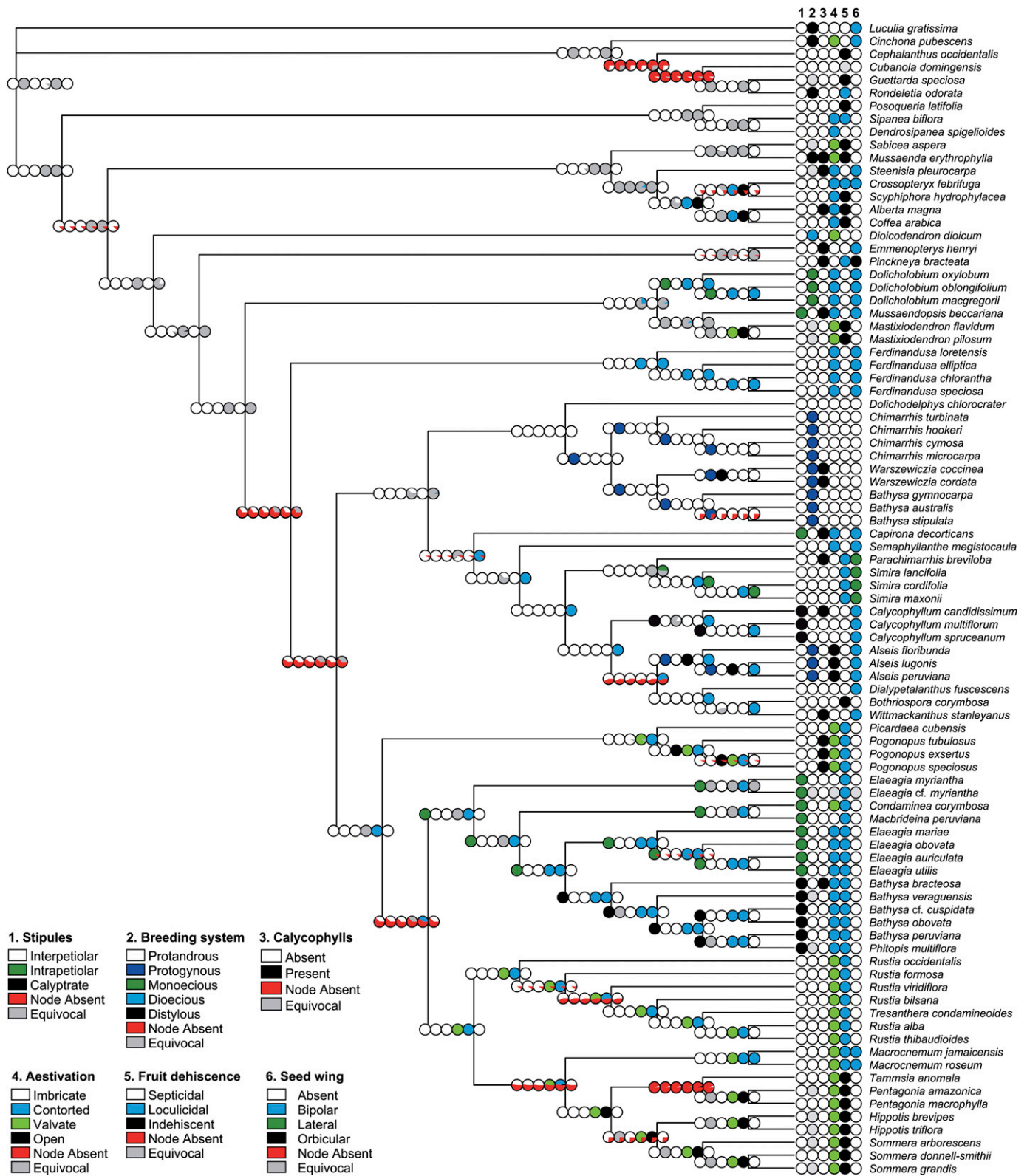


Fig. 5. Ancestral state reconstructions for the type of (1) stipules, (2) flower breeding systems, (3) calycophylls, (4) corolla lobe aestivation patterns, (5) fruit dehiscence patterns, and (6) seed wings of the Condamineae. Character states were estimated using likelihood across a randomly selected subset of 1000 postburn-in trees from the Bayesian inference analyses of the combined data and plotted on the tree with the highest marginal likelihood. Pie charts indicate the estimated likelihood of a character state being present at a given node. Unknown character states are indicated in pale gray.

Elaeagia myriantha was first described by Standley (1930) as *Sickinga myriantha* and shortly thereafter reconsidered as *Holtonia myriantha* by the same author (Standley, 1932). *Holtonia myriantha* was later transferred to *Elaeagia* by Taylor and Hammel (1993). *Elaeagia myriantha* differs from typical *Elaeagia* in having imbricate aestivation (Steyermark, 1972 [as *Simira myriantha*]; Burger and Taylor, 1993) and, furthermore, appears distinct by corollas with erect (vs. recurved) lobes, truncate-divided stipules that persists intact (Taylor and Hammel, 1993), and more complete capsular dehiscence (Dwyer, 1980, as *Deppea panamensis*). The phylogenetic hypotheses from nrDNA, and cpDNA both support the resurrection of the name *Holtonia*, and consequently, it will be discussed as a separate genus. *Elaeagia* is widely distributed in the neotropics, from Mexico and Cuba in the north to Bolivia in the south. *Holtonia* occurs from southern Costa Rica to northern Peru (Taylor and Hammel, 1993).

Phitopsis—As mentioned, *Phitopsis* is nested within the *Schizocalyx* clade. Delprete (1999) considered *Bathysa* and *Phitopsis* as closely related and possibly congeneric. Like *Schizocalyx*, *Phitopsis* also has loculicidal capsules that open only at the apex (Schumann, 1888; cf. figure 11B in Schumann, 1891) and funnelform corollas with left-contorted aestivation (Hooker, 1871; Standley, 1936; Bremer, 1987). According to Hooker (1871), the stipules of *Phitopsis* are free and divided in two on each side (considered as a primitive character state by Robbrecht [1988]). This description is likely based on a misinterpretation of axillary buds. In contrast, Schumann (1889) described the stipules as coherent-connate and early-caducous. The calyx of *Phitopsis* is closed before anthesis and subsequently splits unequally into two or three lobes during anthesis (Hooker, 1871; Standley, 1931). Notably, this pattern of calyx development is commonly found in *Schizocalyx peruvianus* (*Bathysa peruviana*, Fig. 1J) as well (Standley, 1936), the species resolved as sister group to *Phitopsis* in the phylogenetic hypothesis. *Phitopsis* occurs on the Amazonian slopes of the Andes (Delprete, 1999). Based on our present results, we propose that *Phitopsis* should be synonymized with *Schizocalyx* (see Synopsis).

Macrocneum—According to this study, *Macrocneum* is part of a well-supported clade with *Rustia* and the hippotides. The relationships among these three clades are unresolved. *Macrocneum* occurs in southern Central America, northwestern South America, and Jamaica (Andersson, 1994). The genus is aberrant within the loculicidal clade in having seeds with a relatively long, bipolar wing (Fig. 6H) and has historically been classified in Cinchoneae (see Table 1). This character appears autapomorphic in the ancestral state reconstruction (Fig. 5). The capsules of *Macrocneum* are similar to those of *Picardaea* in that dehiscence is loculicidal with the base and apex of the capsule remaining intact (Andersson, 1994; Delprete, 1999).

Rustia and *Tresanthera*—In the phylogenetic hypotheses, *Tresanthera condamineoides* is nested within *Rustia*, although the relationships within this clade are incongruent between the data sets. *Tresanthera* occurs in the coastal forest of Venezuela to the island of Tobago, whereas *Rustia* has a disjunct distribution, with one center of diversity in the northwestern South America to southern Central America, and another in the Atlantic forest of southern Brazil (Delprete, 1999). Both genera have leaves with pellucid glands and anthers opening by pores (in

Rustia by two apical pores, and in *Tresanthera* by a single pore below the apex; Delprete, 1999). *Tresanthera* was included in *Rustia* by Hooker (1873; as *R. pauciflora*), but Karsten (1887) argued for the recognition of *Tresanthera* and considered it distinct from the species of *Rustia* described at that time in a number of additional characters (i.e., corolla and filament pubescence, shape of filaments, anthers, styles and seeds, and capsule woodiness). In spite of this, an inclusion of *Tresanthera* in *Rustia* is supported by this study, and consequently we propose the reduction of the former to synonymy (see Synopsis).

The hippotides—Tribe Hippotideae sensu Rova and Andersson (1995), composed of *Hippotis*, *Pentagonia*, *Sommeria*, and *Tammsia*, are in this study found to be monophyletic and nested within the Condamineae. As mentioned, the hippotide clade forms a trichotomy with the *Macrocneum* and *Rustia* clades. Relationships within this clade, as well as the relationships within the hippotides (which receives only weak support in the phylogenetic analyses), needs further study. The hippotides occur from Mexico to Bolivia. This group can be readily recognized by its axillary inflorescences, indehiscent fruits, and subepidermal fiber bundles in the leaf blade that cause a characteristic striation or moiré pattern between the leaf veins (Rova and Andersson, 1995; Andersson and Rova, 2004). According to Rova and Andersson (1995), the hippotides commonly also have a mixed parietal and central placentation (i.e., the ovary is bilocular at base, but becomes unilocular toward the apex, although not always; cf. Cornejo, 2006).

Morphological character evolution—Tribal classification of Rubiaceae was traditionally (Hooker, 1873; Schumann, 1891) based on a few key diagnostic characters including ovule number, fruit type (indehiscent vs. dehiscent fruits), dehiscence pattern, corolla lobe aestivation, and presence/absence of seed wing. Because none of these characters are uniform within the Condamineae and all but ovule numbers appear to be homoplastic in relation to the phylogenetic hypotheses presented in this study, the complex taxonomic history of the tribe (see Table 1) is not surprising. Consequently, morphological characterization of the tribe (see Synopsis) is difficult, and unique tribal morphological synapomorphies are unknown. Although appearing more or less homoplastic at tribal level (Fig. 5), the characters mentioned do have some phylogenetic utility in characterizing clades within the tribe and are further discussed next.

Anther dehiscence—A transition to poricidal anthers appears to have occurred independently at least twice within the Condamineae: in ancestral *Dialypetalanthus* and in the *Rustia-Tresanthera* lineage. Indeed, the dehiscence patterns differ even within the latter clade. In *Rustia*, the anthers open by two distinct apical pores, whereas in *Tresanthera* the thecae have a common pore just below the apex (Delprete, 1999). In *Dialypetalanthus*, an apical appendage of the thecae splits open leaving a ±rounded pore at the apex, although sometimes the pollen sac may split further across its entire length (Hutchinson, 1973; Piesschaert et al., 1997).

Breeding system—Within the Condamineae, both dichogamous and hercogamous breeding systems occur. Hercogamous flowers are found in *Dioicodendron* and *Dolicholobium*, of which the former is dioecious and the latter monocious (Burkill, 1900; Schumann and Lauterbach, 1901; Steyermark, 1963). Most of the Condamineae genera have protandrous flowers,

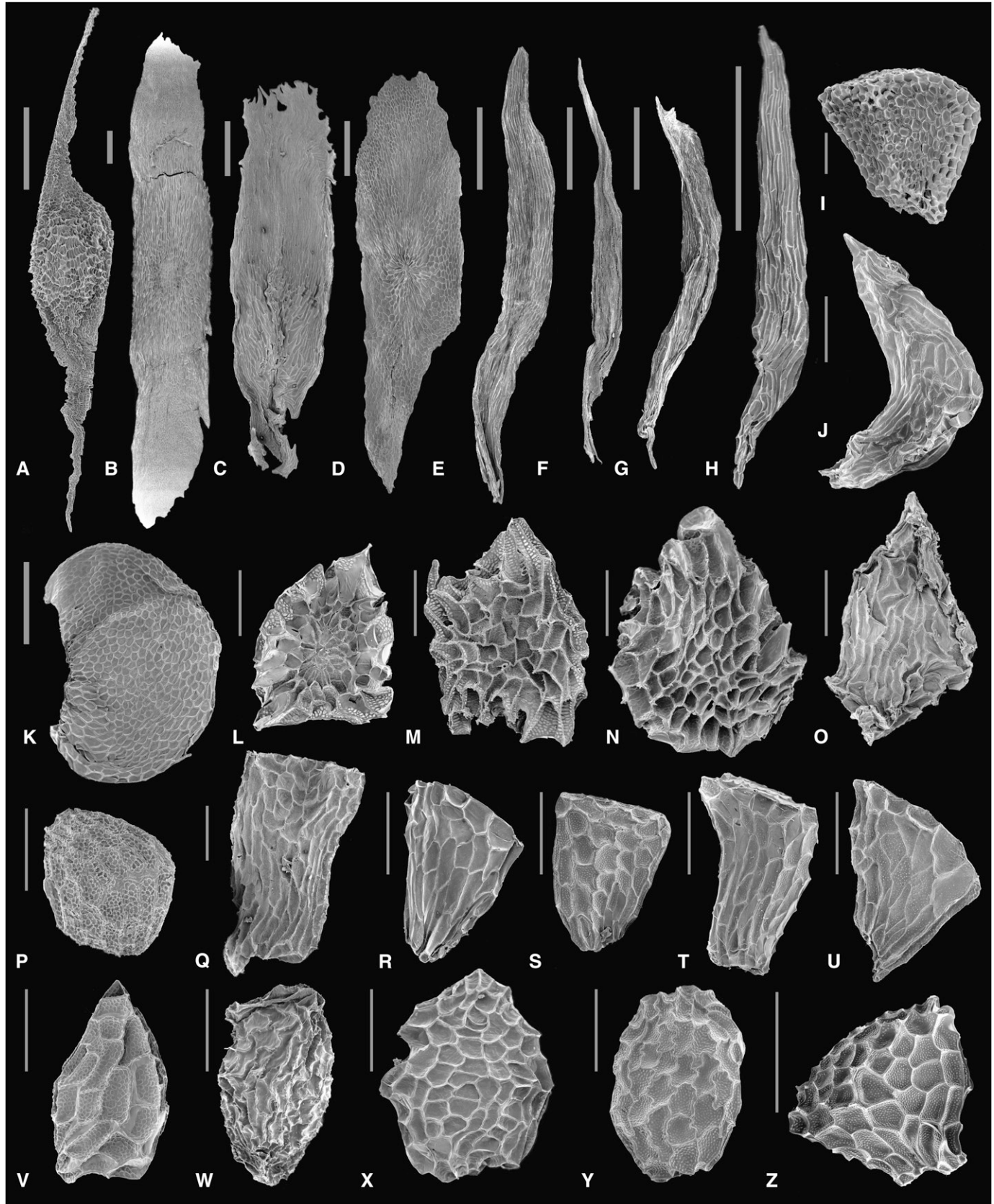


Fig. 6. Seeds of the Condamineae (SEM). (A) *Dolicholobium oblongifolium* (Smith 5109, S); (B) *Ferdinandusa guainiae* (Vásquez & Jaramillo 10151, MO); (C) *Capirona decorticans* (Mori & Bolten 8413, S); (D) *Semaphyllanthus megistocaulis* (*Calycophyllum megistocaulis*; Prance et al., 5483, S);

but several genera within the septicidal clade are protogynous, i.e., *Alseis*, *Chimarrhis*, and *Warszewiczia* (Taylor et al., 2004). In addition, we found indications of protogyny in several species of *Bathysa* (see above). Consequently, protogyny appears to have originated at least twice within the tribe, in the ancestral lineage of *Alseis*, and in the ancestral lineage of the *Bathysa-Chimarrhis-Warszewiczia* clade (Fig. 5).

Calycophylls—Expanded, showy calyx lobes, i.e., calycophylls, occur in many species within the group, primarily within the septicidal clade. Calycophylls are found in both *Emmenopterys* and *Pinckneya*, as well as in *Mussaendopsis* of the Malesian-Pacific clade. In the septicidal clade, calycophylls occur in *Capirona*, *Parachimarrhis*, and *Wittmackanthus*, as well as in some species of *Calycophyllum*, *Chimarrhis*, *Semaphyllanthe*, *Simira*, and *Warszewiczia*. In the loculicidal clade, calycophylls only occur in *Pogonopus* and *Schizocalyx bracteosus* (*Bathysa bracteosa*). The occurrence of calycophylls is hence homoplastic and has been suggested to be a result of a “latent potential of the calyx to enlarge all or some of its elements” (Claßen-Bockhoff, 1996, p. 365).

Corolla lobe aestivation—Several different corolla lobe aestivation patterns are frequent within the Condamineae. The character evolution appears complex, and the ancestral state reconstruction is equivocal (Fig. 5). The genera of the loculicidal clade generally have valvate aestivation, although the *Elaeagia-Schizocalyx* clade is characterized by left-contorted aestivation. It is notable that, *Holtonia myriantha* (*Elaeagia myriantha*), which falls outside *Elaeagia* in the phylogenetic analyses, has buds with imbricate aestivation. In the septicidal clade, aestivation is generally imbricate. Exceptions are found in *Capirona* and *Semaphyllanthe*, both of which have left-contorted aestivation. Left-contorted aestivation also occurs in *Dolicholobium* and *Mussaendopsis* (of the Malesian-Pacific clade), and *Ferdinandusa* (unresolved in the phylogenetic tree). It should also be noted that within the septicidal clade, some species of both *Alseis* and *Simira* have open aestivation (Taylor, 2002; Taylor et al., 2004) and that the buds of *Chimarrhis*, although appearing valvate, are in fact narrowly imbricate (Delprete, 1996b). *Dioicodendron* and *Mastixiodendron* have valvate aestivation, whereas the corollas of *Emmenopterys* and *Pinckneya* are imbricate.

Fruit types—Most genera of the tribe have capsular fruits, and the pattern of capsular dehiscence appears to be useful in characterizing the major clades of the tribe. Septicidal capsule appears to be the ancestral character state (Fig. 5) and is found in *Ferdinandusa*, most genera of the Malesian-Pacific clade and the “septicidal clade” (the relationships between these clades are unfortunately not resolved), as well as in *Dioicodendron* and *Emmenopterys*. Loculicidal capsules are characteristic of *Pinckneya*, the *Parachimarrhis-Simira* clade, and the “loculi-

cidal clade” (autapomorphic in the former, and synapomorphic for the latter two clades; Fig. 5). Within the loculicidal clade, a clade consisting of *Elaeagia* and *Schizocalyx* can be further characterized by incomplete fruit dehiscence (i.e., only the apex of the capsules split open). Indehiscent fruits appear to have evolved independently at least three times, i.e., in ancestors of *Bothriospora* (berries), *Mastixiodendron* (drupes), and the hippotides (baccate).

Conspicuous lenticels are present on the fruits of *Dolicholobium*, *Pentagonia*, *Pinckneya*, *Pogonopus*, *Rustia* (some species), *Simira*, and *Tresanthera*. Consequently, this character appears homoplastic.

Seed wings—The occurrence of a seed wing has been considered an important character in Rubiaceae systematics and has been used in the diagnosis of Cinchoneae. However, the distinction of what constitutes a wing is not always obvious. Several genera have polymorphic seeds frequently with a (membranaceous) marginate testa surrounding the central seed body, a feature that some authors considered to be a wing. In particular, such seeds are found in *Bathysa*, *Chimarrhis*, and *Macbrideina*, but also in *Condaminea*, *Dioicodendron*, *Elaeagia*, and *Rustia* (cf. Burger and Taylor, 1993; Delprete, 1996a, 1999). Three forms of consistent testal extensions are here considered as wings: an extended bipolar (Fig. 6A–H), lateral (Fig. 6K), or orbicular wing (cf. fig. 8G in Delprete, 1999). Capsular-fruited genera of the Malesian-Pacific and the septicidal clades generally have seeds with a bipolar, \pm concentric wing (Fig. 6A–H), as do seeds of *Emmenopterys*, *Ferdinandusa*, and *Macrocnemum*. With the exception of the latter, the genera of the loculicidal clade do not have clearly winged seeds. As noted by Delprete (1996a), the ovule insertion angle and the occurrence and type of seed wing appear correlated. Genera with ascending-vertical (imbricate) seeds tend to have a long, bipolar, \pm concentric wing, whereas genera with horizontally inserted (compressed) seeds do not. Capsules (loculicidal) with seeds inserted horizontally in two rows occur in the genera *Pinckneya*, *Parachimarrhis*, and *Simira*, all of which have (relatively large) seeds with a surrounding orbicular wing (the former), or lateral wing (the two latter). The ancestral state reconstruction of the character is equivocal (Fig. 5).

Stipules—Stipules are often very useful in distinguishing Rubiaceae genera (Gentry, 1993). The stipules of taxa in the Condamineae are predominantly interpetiolar and caducous, and the occurrence of persistent stipules appears homoplastic. Stipules are in general persistent in *Bathysa*, *Capirona*, *Chimarrhis* (subgen. *Pseudochimarrhis*), *Dialypetalanthus*, *Dioicodendron*, *Dolichodelphys*, *Dolicholobium*, *Elaeagia* (some species), *Holtonia*, *Picardaea*, *Pogonopus*, and *Wittmackanthus*. Intrapetiolar stipules, otherwise rare in Rubiaceae (Robbrecht, 1988), appear to have evolved independently in several lineages, being present in *Capirona*, *Condaminea*, *Elaeagia*, *Holtonia*,

←

(E) *Calycophyllum spruceanum* (Krukoff 4768, S); (F) *Alseis floribunda* (Regnell 1524, UPS); (G) *Dialypetalanthus fuscescens* (Ferreira 9018, NY); (H) *Macrocnemum roseum* (Camp E-838, S); (I) *Sommeria donnell-smithii* (Rova & Sundbaum 2407, S); (J) *Rustia formosa* (Heringer et al., 5082, NY); (K) *Parachimarrhis breviloba* (Rimachi 10501, MO); (L) *Chimarrhis microcarpa* (Prance et al., 4452, S); (M) *Chimarrhis ekmanii* (Ekman 5246, S); (N) *Bathysa australis* (Jönsson 210a, S); (O) *Warszewiczia coccinea* (Rova et al., 2170, S); (P) *Warszewiczia schwackei* (Prance & Silva 58505, S); (R) *Macbrideina peruviana* (Gutiérrez 63, NY); (Q) *Condaminea corymbosa* (Harling & Ståhl 26936, S); (S) *Picardaea cubensis* (Ekman H6754, S); (T) *Pogonopus exsertus* (Dwyer 15265, MO); (U) *Schizocalyx obovatus* (*Bathysa obovata*; Persson & Gustafsson 240, S); (V) *Schizocalyx peruvianus* (*Bathysa peruviana*; Asplund 13298, S); (W) *Elaeagia cubensis* (Ekman 5457, S); (X) *Elaeagia utilis* (Core 649, S); (Y) *Holtonia myriantha* (*Elaeagia myriantha*; Delprete & Verduga 6406, UPS); (Z) *Dioicodendron dioicum* (Zarucchi & Echeverry 4780, MO). Thick scale bars = 1.00 mm, thin scale bars = 0.25 mm.

Macbrideina, and *Mussaendopsis*. Consequently, intrapetiolar stipules may be a synapomorphy for the *Condaminea-Elaeagia-Holtonia-Macbrideina-Schizocalyx* clade, from which the calyptrate stipule type of *Schizocalyx* would then be derived (the stipular development in these clades needs further study). The occurrences of intrapetiolar stipules in *Capirona* and *Mussaendopsis* appear autapomorphic (Fig. 5). Puff and Igersheim (1994) found that the intrapetiolar stipules of *Mussaendopsis* originate from an interpetiolar position.

Synopsis—Condamineae Hook. f., *Genera Plantarum* 2: 12 (1873). Type *Condaminea* DC.

Small trees to tall canopy trees, shrubs, or rarely monocal treelets. Stipules calyptrate, inter- or intrapetiolar, entire or bifid, early-caducous-persistent. Inflorescences terminal and/or axillary, mostly many-flowered. Flowers hermaphrodite, mostly protandrous, in some genera protogynous, rarely dioecious (*Dioicodendron*), or monoecious (*Dolicholobium*). Calyx mostly persistent, in several taxa with one lobe expanded and showy. Corolla aestivation left-contorted, imbricate, valvate or rarely open. Ovary 2-locular (5-locular in *Bothriospora*, or 1–2 locular), typically with numerous ovules. Fruits mostly loculicidal or septical capsules, in some genera indehiscent. Seeds diverse, with reticulate testa, usually small to minute, compressed, and polymorphic, with or without a membranaceous border, alternatively with a distinct bipolar-, lateral-, or orbicular wing.

Genera included: *Alseis*, *Bathysa*, *Bothriospora*, *Calycophyllum*, *Capirona*, *Chimarrhis*, *Condaminea*, *Dialypetalanthus*, *Dioicodendron*, *Dolichodelphys*, *Dolicholobium*, *Elaeagia*, *Emmenopterys*, *Ferdinandusa*, *Hippotis*, *Holtonia*, *Macbrideina*, *Macrocnemum*, *Mastixiodendron*, *Mussaendopsis*, *Parachimarrhis*, *Pentagonia*, *Picardaea*, *Pinckneya*, *Pogonopus*, *Rustia*, *Schizocalyx*, *Semaphyllanthus*, *Simira*, *Sommeria*, *Tammsia*, *Warszewiczia*, and *Wittmackanthus*.

As shown earlier, the genus *Bathysa* is not monophyletic. The type *B. stipulata*, as well as *B. australis* and *B. gymnocarpa*, form a sister group to *Warszewiczia*, whereas another group of species (*B. bracteosa*, *B. cuspidata*, *B. obovata*, and *B. peruviana*) including the genus *Phitopsis*, forms a sister group to *Elaeagia*. This second group is characterized by connate and early caducous stipules (vs. free and mostly persistent stipules), relatively large, funnel-shaped corollas with left-contorted aestivation (vs. small, hypocateriform corollas with imbricate aestivation), loculicidal capsules with only apical dehiscence (vs. septical capsules splitting entirely), and protandrous flowers (vs. protogynous flowers). One of the species of this group, *B. bracteosa*, was originally described as *Schizocalyx bracteosus* by Weddell (1854). To render *Bathysa* monophyletic, we propose to resurrect *Schizocalyx*, with a subsequent transfer of the species *B. cuspidata*, *B. obovata*, *B. peruviana*, and *B. veraguensis*, that conform to the description above. Of the *Bathysa* species not included in this study, *B. mendoncaei*, *B. nicholsonii* K. Schum., and *B. sylvestrae* Germano-Filho & M. Gomes, as well as *B. pittieri* (cf. description by Steyermark, 1974), conform to *Bathysa*. Regarding *B. bathysoides* (Steyermark) Delprete and *B. perijaensis* (Steyermark) Delprete (both originally described as *Chimarrhis*), as well as *B. multiflora* L. O. Williams and *B. panamensis* Dwyer, we are unable to suggest a classification; we have not studied materials for these taxa, and available descriptions are inadequate. The position of *Phitopsis* as sister to *B. peruviana* suggests that this taxon (two species) is also best accommodated in *Schizocalyx*. The name *Schizocalyx* is older than *Phitopsis* and consequently has priority.

Schizocalyx Wedd., *Ann. Sci. Nat., Bot.*, IV, 1: 73 (1854). Type: *Schizocalyx bracteosus* Wedd.

Phitopsis Hook. f., *Hooker's Icon. Pl.* 11: t. 1093 (1871). Type: *Phitopsis multiflora* Hook. f.

Trees. Leaves petiolate, ±pubescent. Stipules calyptrate in bud, early caducous. Inflorescence terminal, many-flowered, ±pubescent. Flowers protandrous. Calyx persistent, truncate–deeply lobed, sometimes unequally lobed. Calycophylls present in *S. bracteosus*. Corolla white, funnellform, lobes left-contorted in bud. Ovary 2-locular, with numerous horizontally inserted ovules. Fruits capsular, splitting loculicidally only in apical portion. Seeds minute, polymorphic, reticulate, not winged.

Schizocalyx cuspidatus (A. St.-Hil.) Kainul. & B. Bremer, comb. nov. Basionym: *Exostema cuspidatum* A. St.-Hil., *Pl. Usuel. Bras.*: t. 3 (1824). Type: S. Brazil. Saint-Hilaire B1-970 (P!, image seen).

Schizocalyx multiflorus (Hook. f.) Kainul. & B. Bremer, comb. nov. Basionym: *Phitopsis multiflora* Hook. f., *Hooker's Icon. Pl.* 11: t. 1093 (1871). Type: Peru, San Martín. *Mathews* 1639 (K!, image seen).

Schizocalyx obovatus (K. Schum. ex Standl.) Kainul. & B. Bremer, comb. nov. Basionym: *Bathysa obovata* K. Schum. ex Standl., *Publ. Field Mus. Nat. Hist., Bot. Ser.* 7: 280 (1931). Type: Peru. *Ruiz & Pavón s.n.* (B!, image seen).

Schizocalyx peruvianus (K. Krause) Kainul. & B. Bremer, comb. nov. Basionym: *Bathysa peruviana* K. Krause, *Verh. Bot. Vereins Prov. Brandenburg* 50: 96 (1908 publ. 1909). Type: Peru. *Ule* 6768 (B!, image seen).

Schizocalyx sterculioides (Standl.) Kainul. & B. Bremer, comb. nov. Basionym: *Phitopsis sterculioides* Standl., *Publ. Field Mus. Nat. Hist., Bot. Ser.* 8: 341 (1931). Type: Peru, Junín. *Killip & Smith* 25747 (F!, image seen).

Schizocalyx veraguensis (Dwyer) Kainul. & B. Bremer, comb. nov. Basionym: *Bathysa veraguensis* Dwyer, *Ann. Missouri Bot. Gard.* 67: 40 (1980). Type: Panama, Veraguas, Cerro Tute. *Lao & Gentry* 531 (MO!, image seen).

Tresanthera condamineoides is found nested within *Rustia*. The two genera have primarily been distinguished by the position and number of pores of the anthers. An inclusion of *Tresanthera* in *Rustia* is supported by this study, and consequently, we propose the reduction of the former to synonymy.

Rustia Klotzsch in Hayne, *Getreue Darstell. Gew.* 14: t. 14, 15 (1846). Type: *Rustia formosa* (Cham. & Schldl.) Klotzsch.

Tresanthera H. Karst., *Fl. Columb.* 1: 37 (1859). Type: *Tresanthera condamineoides* H. Karst.

Shrubs to trees. Leaves lanceolate, elliptic-ovate, with pellucid glands. Stipules interpetiolar, free at base, entire, narrowly triangular to lanceolate, caducous. Inflorescence terminal, paniculate or thyrsoid. Flowers protandrous. Calyx cupular, truncate or with small lobes. Corolla tubular or campanulate, thin to fleshy, lobes valvate to valvate-reduplicate in bud. Anthers poricidal, opening by one lateral pore, or two apical pores. Ovary 2-locular, with numerous horizontally inserted ovules. Fruit a loculicidal capsule. Seeds minute, polymorphic, reticulate, not clearly winged.

Rustia condamineoides (H. Karst.) Kainul. & B. Bremer, comb. nov. Basionym: *Tresanthera condamineoides* H. Karst., *Fl. Columb.* 1: 37 (1859). Type: Venezuela, Aragua, Puerto Cabello, Cumbre de Valencia. *Karsten s.n.* (W; F!, fragment, image seen).

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APPENDIX 1. Continued.

| Species | Voucher | ITS | matK | ndhF | rps16 | trnH-psbA | trnS-G | trnT-L-F |
|--|-----------------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| <i>Pogonopus exsertus</i> (Oerst.) Oerst. | Dwyer 15265 (MO) | — | — | — | FJ884669* | FJ860197* | FJ948340* | FJ948409* |
| <i>Pogonopus spectosus</i> (Jacq.) K. Schum. | Meyer & Walter-Weisbeck 2548 (GB) | — | FJ905378* | FJ871964* | FJ884670* | FJ860198* | FJ948341* | FJ948410* |
| <i>Pogonopus tubulosus</i> (DC.) K. Schum. | Novara et al., 8939 (S) | FJ985003* | FJ905379* | FJ871965* | AF242996 ²² | FJ860199* | FJ948342* | FJ948411* ⁵ |
| <i>Rustia alba</i> Delprete | Delprete & Verduga 6414 (UPS) | — | FJ905380* | — | FJ884671* | FJ860200* | FJ948343* | FJ948412* |
| <i>Rustia bilcana</i> Delprete | Clarke 2979 (GB) | — | FJ905381* | FJ871966* | FJ884672* | FJ860201* | FJ948344* | FJ948413* |
| <i>Rustia formosa</i> Delprete | Kirkbride 5431 (NY) | — | FJ905382* | FJ871967* | FJ884673* | FJ860202* | FJ948345* | FJ948414* |
| <i>Rustia cf. occidentalis</i> (Benth.) Hemsli. | Tuberquia et al., 495 (GB) | FJ985004* | FJ905383* | FJ871968* | FJ884674* | FJ860203* | FJ948346* | FJ948415* |
| <i>Rustia rubra</i> Standl. ex D. A. Simpson | Stahl 2800 (GB) | FJ985005* | FJ905384* | — | AF243017 ²² | FJ860204* | FJ948347* | FJ948416* ⁶ |
| <i>Rustia thibaudoides</i> (H. Karst.) Delprete | Delprete 6378 (UPS) | FJ985006* | FJ905385* | AJ236310 ²¹ | FM204731 ²³ | FJ860205* | FM204777 ²³ | FM207138 ²³ |
| <i>Rustia viridiflora</i> Delprete | Stahl 1822 (GB) | — | FJ905386* | — | FJ884675* | FJ860206* | FJ948348* | FJ948417* |
| <i>Simira cordifolia</i> (Hook. f.) Steyerl. | Bremer et al., 3361 (UPS) | FJ985008* | FJ905388* | FJ871970* | FJ884677* | FJ860209* | FJ948350* | FJ948419* |
| <i>Simira corubensis</i> (Standl.) Steyerl. | Malmé 2733 (UPS) | FJ985009* | FJ905389* | — | — | FJ860210* | — | FJ948420* |
| <i>Simira glaziovii</i> (K. Schum.) Steyerl. | Andersson et al., 37060 (UPS) | FJ985010* | FJ905390* | — | — | FJ860211* | FJ948351* | FJ948421* |
| <i>Simira lancifolia</i> (Lundell) E. Martínez & Borhidi | Lundell & Contreras 19909 (S) | FJ985011* | FJ905391* | — | FJ884678* | FJ860212* | FJ948352* | FJ948422* |
| <i>Simira maxonii</i> (Standl.) Steyerl. | Santamaría S-949 (GB) | FJ985012* | FJ905392* | — | FJ884679* | FJ860213* | FJ948353* | FJ948423* |
| <i>Sommeria arborescens</i> Schlttdl. | Salomón Maya 2925 (BR) | — | FJ905393* | — | — | FJ860214* | — | FJ948424* |
| <i>Sommeria donnell-smithii</i> Standl. | Rova & Sundbaum 2407 (GB) | FJ985013* | FJ905394* | — | AF243025 ²² | FJ860215* | FJ948355* | FJ948425* |
| <i>Sommeria grandis</i> (Bartl. ex DC.) Standl. | Skutch 2422 (S) | FJ985014* | FJ905395* | FJ871971* | FJ884680* | FJ860216* | FJ948356* | FJ948426* |
| <i>Tamnia anomala</i> H. Karst. | Meier 11709 (MO) | — | FJ905396* | — | FJ884681* | — | — | — |
| <i>Tresantha condamineoides</i> H. Karst. | Webster et al., 9805 (S) | FJ985015* | FJ905397* | FJ871972* | FJ884682* | FJ860217* | FJ948357* | FJ948427* |
| <i>Warszewiczia coccinea</i> (Vahl) Klotzsch | Delprete 6437 (UPS) | AJ846884 ²⁴ | FJ905398* | — | FJ884683* | FJ860218* | FJ948358* | AJ847397 ²⁴ |
| <i>Warszewiczia cordata</i> Spruce ex K. Schum. | Woytkowski 5416 (S) | FJ985016* | — | AJ236314 ²¹ | FJ884684* | FJ860219* | — | FJ968793* |
| <i>Wittmannanthus stanleyanus</i> (R. H. Schomb.) Kuntze | Uribe 6648 (MO) | FJ985017* | FJ905399* | FJ871973* | FJ884685* | FJ860220* | FJ948357* | FJ948428* |
| <i>Alberta magna</i> E. Mey. | — | FM204677 ²³ | — | AJ236282 ²¹ | FM204702 ²³ | AM939389 ²³ | FM204742 ²³ | FM207110 ²³ |
| <i>Cephalanthus occidentalis</i> L. | — | — | AY538377 ²⁷ | AJ236288 ²¹ | AF004033 ²⁸ | — | — | AJ346955 ²⁹ |
| <i>Cinchona pubescens</i> Vahl. | — | — | ZF0197 ³⁰ | AJ235843 ³¹ | FM204714 ²³ | — | FM204758 ²³ | AJ346963 ²⁹ |
| <i>Coffea arabica</i> L. | — | AJ224846 ³² | EF044213 ³³ | EF044213 ³³ | EF044213 ³³ | — | EF044213 ³³ | EF044213 ³³ |
| <i>Crossopteryx febrifuga</i> (Afzel. ex G. Don) Benth. | Bremer 3097 (UPS) | FM204690 ²³ | FJ905329* | AM949851 ²³ | FM204717 ²³ | AM939406 ²³ | FM204760 ²³ | FM207123 ²³ |
| <i>Dendrospanea spigelioides</i> Ducke | — | — | AY538386 ²⁷ | AM117345 ³⁴ | FM204718 ²³ | — | FM204761 ²³ | FM207124 ²³ |
| <i>Guettarda speciosa</i> L. | Prance et al., 16199 (MO,NY,S) | AY555121 ³⁵ | FJ905324* | FJ871942* | FJ884627* | — | FJ948291* | FJ948360* |
| <i>Luculia gratissima</i> (Wall.) Sweet | Perrison 141 (GB) | — | AY538389 ²⁷ | FJ871942* | AF242964 ²² | — | FJ948289* | FJ948358* ⁷ |
| <i>Mussaenda erythrophylla</i> Schumacher & Thonn. | — | AJ224823 ³² | AJ429325 ³⁶ | AJ011987 ³⁷ | AJ431036 ³⁶ | — | FM204765 ²³ | AJ430911 ³⁶ |
| <i>Posoqueria latifolia</i> (Rudge) Roem. & Schult. | Gillis 10838 (FTG) | AJ224823 ³² | FJ905326* | AJ130836 ²¹ | FJ884628* | — | FJ948292* | AJ620116 ³⁸ |
| <i>Rondeletia odorata</i> Jacq. | Bremer & Andersson 3504 (UPS) | AJ224828 ³² | FJ905325* | AM949855 ²³ | FM204728 ²³ | — | FM204774 ²³ | FM207135 ²³ |
| <i>Sabicea aspera</i> Aubl. | Bremer et al., 1941 (GB) | — | — | AJ235845 ³¹ | AF243010 ²² | — | FJ948290* | FJ948359* ⁸ |
| <i>Scyphiphora hydrophyllacea</i> CF. Gaertn. | Andersson et al., 1941 (GB) | — | AY538420 ²⁷ | EUI45416 ²⁶ | AF004079 ²⁸ | — | FJ948293* | FJ948362* ⁹ |
| <i>Spanaea biflora</i> (L. f.) Cham. & Schltdl. | Bremer et al., 99 (S) | — | FJ905327* | AJ236311 ²¹ | EU817450 ³⁹ | — | FM204779 ²³ | EU817475 ³⁹ |
| <i>Steenisia pleurocarpa</i> (Airy Shaw) Bakh. f. | Puff BF 990619-1/4 (WU) | — | AY538421 ²⁷ | EUI45413 ²⁶ | AF004085 ²⁸ | — | FM204780 ²³ | FM207141 ²³ |
| | | | FJ905328* | — | FM204735 ²³ | — | FM204781 ²³ | FM207142 ²³ |

Notes: Combined sequence fragments: 1, combined with AF152629⁴⁰; 2, combined with AF152644⁴⁰; 3, combined with AJ847410⁴⁴; 4, combined with AF152628⁴⁰; 5, combined with AF152631⁴⁰; 6, combined with AF152630⁴⁰; 7, combined with AF152725⁴⁰; 8, combined with AF152741⁴⁰; 9, combined with AY538475⁷⁷; 10, combined with AF152675⁴⁰.

Differing voucher specimens: 11, Andersson et al. 2151 (GB); 12, Rova et al. 2252 (GB); 13, Andersson et al. 2008 (GB); 14, Bremer et al. 3357 (QCA, UPS); 15, Perrison & Grández 656 (GB); 16, Forbes s.n. (S); 17, Smith V10274 (MO); 18, Perrison & Gustafsson 210 (GB); 19, Bremer et al. 3333 (UPS); 20, Jansen-Jacobs et al. 4090 (GB);

Published sequences: 21, Bremer et al. (1999); 22, Rova (GenBank, unpublished); 23, Kainulainen et al. (2005); 24, Alejandro et al. (2005); 25, Novotny et al. (2002); 26, Rydin et al. (2009); 27, Andersson and Antonelli (2005); 28, Andersson and Rova (1999); 29, Razafimandimbison and Bremer (2002); 30, Endress et al. (1996); 31, Backlund et al. (2000); 32, Andersson et al. (1999); 33, Samson et al. (2007); 34, Bremer and Eriksson (2009); 35, Delprete and Cortés-B. (2004); 36, Bremer et al. (2002); 37, Oxelman et al. (1999); 38, Lantz and Bremer (2004); 39, Mouly et al. (2009); 40, Rova et al. (2002).