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Phylogeny of Celastraceae subfamily Hippocrateoideae inferred from morphological characters and nuclear and plastid loci

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ABSTRACT

The phylogeny of Celastraceae subfamily Hippocrateoideae (~100 species and 19 genera in the Old and New World tropics) was inferred using morphological characters together with plastid (*matK*, *trnL-F*) and nuclear (ITS and 26S rDNA) genes. The subfamily is easily recognized by the synapomorphies of transversely flattened, deeply lobed capsules and seeds with membranous basal wings or narrow stipes together with bisexual, 5-merous flowers that generally have an extrastaminal disk and three stamens. Hippocrateoideae, like Salacioideae, are inferred to have an Old World origin. The narrow stipes of Neotropical species that are water-dispersed are inferred to be derived within the subfamily from ancestral species with wind-dispersed winged seeds. *Helictonema*, a monotypic genus endemic to tropical Africa, has a small, white, spongy aril that is located at the base of the seed wing and appears to be unique within Hippocrateoideae. Our inference that *Helictonema* is sister to the remaining members of the subfamily, considered in the context of *Sarawakodendron* being sister to Salacioideae, suggests that small arils and capsular fruit were primitive within both subfamilies. The aril became dramatically enlarged within Salacioideae, in which the fruits are berries, and lost entirely within Hippocrateoideae, in which the fruits are transversely flattened capsules. All five Old World taxa of *Prionostemma* and all eight currently recognized species within *Simirestis* are transferred to *Pristimera*, one South African variety of *Pristimera* is raised to species level, and all three taxa in *Pristimera* subgenus *Trochantha* are transferred to the new genus *Trochantha*.

1. Introduction

Celastraceae subfamily Hippocrateoideae consist of ~100 species in the Old and New World tropics (Simmons, 2004a). The subfamily is easily recognized by the synapomorphies of transversely flattened, deeply lobed capsules and seeds with membranous basal wings or narrow stipes together with bisexual, 5-merous flowers that generally have an extrastaminal disk and three stamens. Bark from stems and/or roots of at least four species [*Apodostigma pallens, Hippocratea myriantha* Oliv., *Loeseneriella africana* (Willd.) R.Wilczek, and *Reissantia indica* (Willd.) N.Hallé] has been reported to be used in traditional medicine in Africa for a variety of applications, including pain relief and treatment of skin infections (Burkill, 1985). Likewise, bark from *Semialarium mexicanum* is variously used as an anti-inflammatory, gastroprotective, and louse killer in Mexico (Perez et al., 1995; Navarrete et al., 2002) and contains a high concentration of gutta (Palacios et al., 1989).

Hallé (1962), who performed extensive monographic studies on Old World members of the family, recognized two subfamilies in Hippocrateaceae (now recognized as nested within Celastraceae sensu stricto [s.s.]; e.g., Robson et al., 1994; Savolainen et al., 1997; Takhtajan, 1997): Hippocrateoideae and Salacioideae. Although many more species of Salacioideae are recognized (~265 species in six genera), Hippocrateoideae have been more finely partitioned into separate genera (~100 species in 19 genera), including six that are monotypic (*Anthodon, Apodostigma*, *Bequaertia*, *Helictonema*, *Plagiopteron*, *Simicratea*; Table 1). Most of these 19 genera have restricted distributions, though six are widespread across multiple continents (*Cuervea, Elachyptera, Hippocratea, Loeseneriella, Prionostemma, Reissantia*; Table 1). Although some workers have supported recognition of these many finely partitioned genera (e.g., Smith, 1940; Hallé, 1986), others have recognized just two (*Campylostemon* and *Hippocratea*; Bentham and Hooker, 1862; Robson, 1965, 1989) or three (*Campylostemon, Hippocratea*, and *Tristemonanthus*; Loesener, 1942a,b) genera.

Within Hippocrateoideae, Hallé (1962, 1986, 1990) recognized three tribes: Campylostemoneae, Helictonemateae, and Hippocrateeae (see also Simmons and Hedin, 1999, p. 724). *Helictonema velutinum* is the sole member of Helictonemateae; Campylostemoneae are composed of *Bequaertia, Campylostemon*, and *Tristemonanthus*; and Hippocrateeae contain ten other genera [Hallé (1962, 1986,

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Table 1
Number of species and distribution of genera currently recognized in Hippocrateoideae.

Genus	Species #	Distribution
Anthodon Ruiz & Pav.	1	C. & S. America
Apodostigma R. Wilczek	1	Africa, Madagascar
Arnicratea N. Hallé	3	India, S.E. Asia, Macronesia
Bequaertia R. Wilczek	1	Africa
Campylostemon Welw.	⩾8	Africa
Cuervea Triana ex Miers	5	C. & S. America, W. Indies, Africa
Elachyptera A.C.Sm.	8	C. & S. America, Africa, Madagascar
Helictonema Pierre	1	Africa
Hippocratea L.	3	Americas, W. Indies, Africa
Hylenaea Miers	3	C. & S. America
Loeseneriella A.C.Sm.	16	Africa, Madagascar, S.E. Asia, Macronesia, Australia
Plagiopteron Griff.	1	S.E. Asia
Prionostemma Miers	5	C. & S. America, Africa, India
Pristimera Miers	25	Old and New World
Reissantia N. Hallé	6	Africa, Madagascar, India, SE Asia
Semialarium N. Hallé	2	New World
Simicratea N. Hallé	1	Africa
Simirestis N. Hallé	8	Africa
Tristemonanthus Loes.	2	Africa

1990) did not specifically address *Anthodon, Arnicratea, Hylenaea, Plagiopteron*, or *Semialarium*, which are not included in the geographic ranges covered by his floras]. Simmons and Hedin (1999) inferred that Helictonemateae and Campylostemoneae are nested within Hippocrateeae, albeit with weak Bremer (1988) support. Likewise, Simmons et al. (2001b) inferred that *Campylostemon* is nested within Hippocrateeae, albeit with low bootstrap support (BS; Felsenstein, 1985). Simmons et al. (2001b) did not infer any intergeneric relationships within Hippocrateoideae with >50% BS.

The winged seeds of Hippocrateoideae and *Kokoona* are autogyros, which are expected to have greater dispersal potential than the rolling autogyros of *Lophopetalum* (Green, 1980; Augspurger, 1986). Ridley (1930) suggested that some East Asian species of Hippocrateoideae that grow along streams may be water-dispersed while other species that grow on open hillsides are wind-dispersed. Several Neotropical species [*Anthodon decussatum, Cuervea crenulata* Mennega, *Elachyptera festiva* (Miers) A.C.Sm., *E. floribunda, E. micrantha* (Cambess.) A.C.Sm., *Hippocratea volubilis* L., *Prionostemma aspera, Pristimera celastroides, P. nervosa* (Miers) A.C.Sm., *P. verrucosa* Miers, *Semialarium mexicanum, S. paniculatum* (Mart. ex Schultes) N.Hallé] are large lianas that grow both along borders and inside forests where wind may or may not help with dispersal (J.A.L., pers. obs.). Other Neotropical species (*Cuervea kappleriana, Hylenaea comosa, H. praecelsa*, and probably *Pristimera tenuiflora*), are water-dispersed and have been reported from riparian and floodplain (varzea) forests (J.A.L., pers. obs.). The first three of those four species have large embryos with corky testa and vestigial wings rather than the well developed wings that are typical of Hippocrateoideae. Hallé (1983) suggested that these seed types are derived within the subfamily.

Helictonema velutinum has a small (<0.5 mm thick and <2 mm wide), white, spongy aril that is located at the base of the seed wing (Hallé, 1983; see also Coughenour et al., 2010). This aril appears to be unique to *H. velutinum* within Hippocrateoideae (Corner, 1976; Espinosa-Osornio and Engleman, 1993, 1994; J.A.L., pers. obs.). This small, whitish aril might be attractive to ants such that the seeds are dispersed by diplochory (Vander Wall and Longland, 2004) in which the first stage is wind dispersal and the second stage is myrmecochory. But the seeds of *Helictonema* are fairly large (nearly 5 cm long; Hallé, 1962) and heavy, perhaps too heavy for most species of African ants to move effectively. For example, Pizo and Oliveira (2000) reported that large ponerine ants in the Atlantic Forest of southeastern Brazil will only move diaspores of less than one gram to their nests. As such, the aril may simply be vestigial, as noted by Hallé (1983). Hallé's (1986, 1990) recognition of *Helictonema* as the sole member of Helictonemateae and the aril as vestigial would be supported if the genus is sister to the remaining extant Hippocrateoideae.

The four primary goals of this study were to infer intergeneric relationships within Hippocrateoideae; test the monophyly of genera within the subfamily; infer the biogeographic origin of Hippocrateoideae; and infer the pattern of diversification of morphological characters within this lineage, with a focus on the aril of *Helictonema*. To address these goals, we used the taxon and character sampling from Coughenour et al. (2010) as a basis with which to substantially increase our taxon sampling for Hippocrateoideae, from which only three species were sampled in that study. Sequence data were generated from two nuclear gene regions (26S rDNA and the internal transcribed spacers [ITS of rDNA]), and two plastid loci (maturase K [*matK*] and *trnL-F*). These data were analyzed together with morphological characters and phytochrome B (*phyB*) sequences generated by Simmons et al. (2001a).

2. Materials and methods

2.1. Taxon sampling

Ninety taxa were sampled (Appendix A; see also Islam et al., 2006; Simmons et al., 2001a,b, 2008; Zhang and Simmons, 2006; Coughenour et al., 2010 for vouchers and GenBank accession numbers for taxa and sequences sampled from those studies) including 17 of the 19 genera within Hippocrateoideae. Material permitting, at least two species were sampled from each non-monotypic genus of Hippocrateoideae to test generic monophyly. Two or three accessions were sampled from some species for a total of 104 terminals included in the simultaneous analyses (Kluge, 1989; Nixon and Carpenter, 1996).

Preliminary parsimony tree searches based on the taxon sampling used by Coughenour et al. (2010), which included members of Lepidobotryaceae and Parnassiaceae as outgroups, indicated that all members of Hippocrateoideae are a monophyletic group sister to the clade of *Sarawakodendron* + Salacioideae. Therefore, to speed tree searches and help decrease alignment ambiguity caused by inclusion of divergent sequences, our ingroup sampling was limited to Hippocrateoideae, *Sarawakodendron*, Salacioideae and the sister group of this clade as inferred by Simmons et al. (2008) and Coughenour et al. (2010): *Brexia* + *Elaeodendron* + *Pleurostylia* + *Polycardia* + *Pseudocatha*. The ingroup clade received 89% parsimony jackknife support (JK; Farris et al., 1996) and 72% likelihood BS in the simultaneous analysis of Simmons et al. (2008) and 99% JK/99% BS in the simultaneous analysis of Coughenour et al. (2010). The trees were rooted using three species of *Salaciopsis*, which was inferred to be closely related to the ingroup clade by Simmons et al. (2008) and Coughenour et al. (2010).

Two voucher-identification errors from previous studies were discovered. First, the identification of *M.W. Chase 2471* (K), from Kew's living collection, was changed from *Reissantia indica* (Willd.) N.Hallé to *Loeseneriella barbata* (F.Muell.) C.T.White. This accession, with the original identification, was sampled by Simmons et al. (2001a,b). The plant is sterile and was originally collected from the Gatton area of Queensland, Australia by seed in 1985 (L. Csiba, pers. comm., 2009). *Loeseneriella barbata* is the only species of Hippocrateoideae reported from Australia (Jessup, 1984). Second, the identification of *M.W. Chase 2095* (K), from the Bogor Botanical Gardens, was changed from *Reissantia* sp. to *Loeseneriella* sp. The voucher specimen is sterile and is morphometrically consistent with both *Loeseneriella* and *Reissantia*. Sequences obtained from this sample were unambiguously supported as nested within *Loeseneriella* (Fig. 1). This accession, with the original identification,

was sampled by Savolainen et al. (2000) and Simmons et al. (2001b). Taxonomic updates to these GenBank accessions were made on 28 April 2010.

2.2. Morphological characters

Morphological characters were derived from matrices previously published by Simmons and Hedin (1999), Simmons et al. (2001a,b, 2008), Islam et al. (2006), and Coughenour et al. (2010). For the 90 taxa sampled in this study, 33 characters are parsimony informative, representing variation in vegetative and floral morphology, leaf and seed anatomy, and pollen morphology. To the degree possible, characters were scored using reductive coding rather than composite coding (Wilkinson, 1995; Simmons and Freudenstein, 2002). The codings for most morphological characters are described in detail by Simmons and Hedin (1999, pp. 746–751). All morphological characters, including both character and character-state definitions, are included as part of the

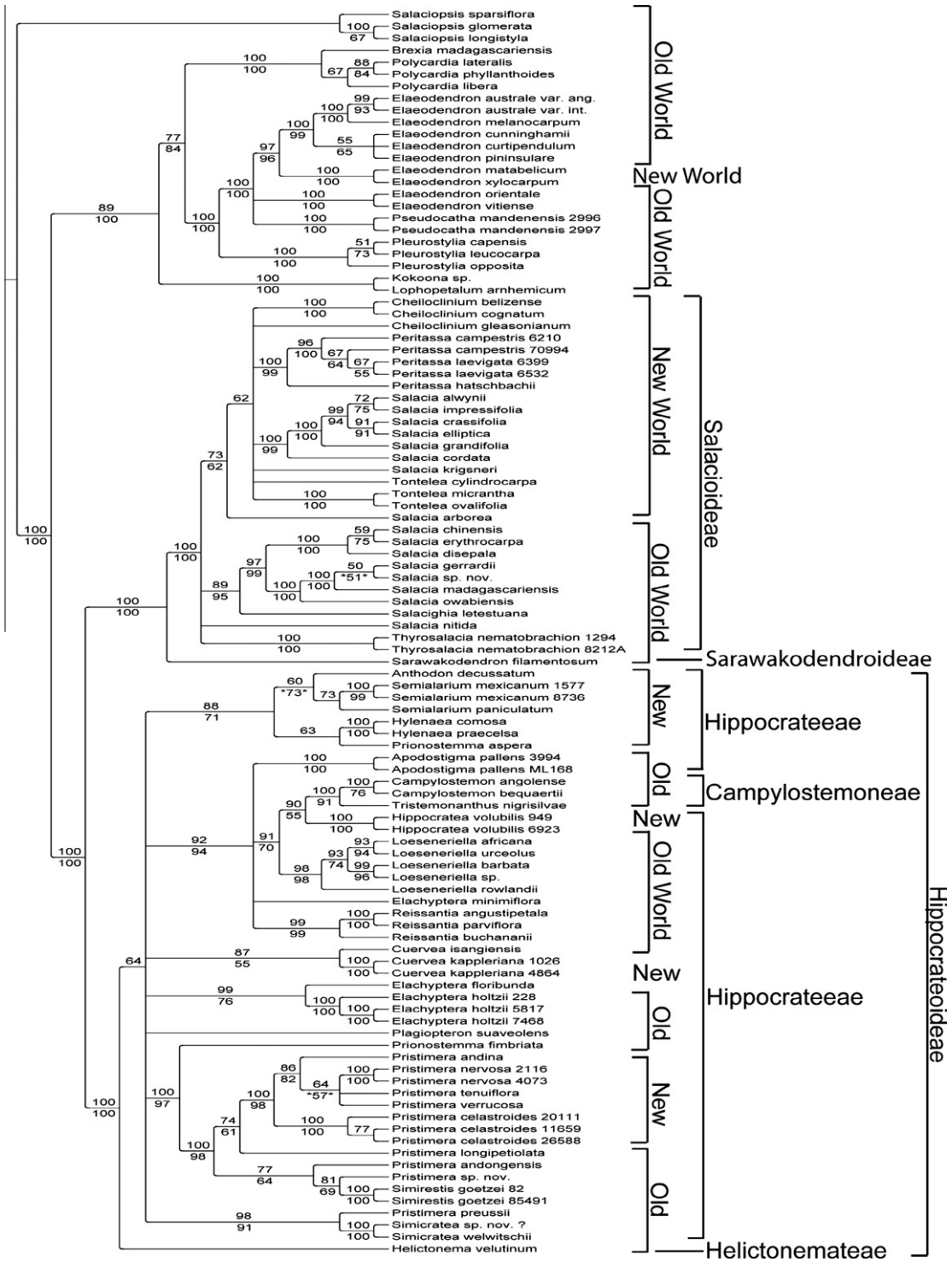

Fig. 1. Simultaneous-analysis parsimony JK tree of all morphological and molecular characters with parsimony JK values above each branch, and likelihood BS values (for the matrix of all nucleotide characters) below each branch. Clades in the parsimony JK tree that were contradicted by clades in the likelihood BS tree are indicated by ∗XX∗, with BS support for the highest contradictory likelihood clade listed.

Table 2
Data matrix and tree statistics for each of the analyses.

Matrix	# terminals	# characters analyzed	# of parsimony-informative characters	% missing/ inapplicable	Most parsimonious tree length	# of most parsimonious trees	# of jackknife/ bootstrap clades ⩾50%	Average jackknife/bootstrap support (%)	CI[a]	RI[b]
26S rDNA	103	947	132	7.4	679	770	50/54	84.6/78.6	0.31	0.79
ITS rDNA	95	658	291	14.1	1994	190	71/72	86.1/85.7	0.31	0.73
rDNA (26S, ITS)	104	1605	423	13.7	2704	3362	80/80	88.3/86.7	0.30	0.74
phyB	16	1123	135	5.4	404	2	12/10	84.7/82.5	0.68	0.79
matK	97	1352	182	12.4	481	14,780	41/46	81.8/82.5	0.66	0.92
trnL-F	97	1248	163	28.0	477	10,340	49/50	77.4/78.4	0.67	0.92
Plastid (*matK, trnL-F*)	99	2600	345	21.5	973	11,650	60/59	83.9/84.1	0.65	0.91
Morphology only	90	56	33	22.6	106	170	11	66.1	0.44	0.86
All molecular	104	5328	901	34.7	4116	4457	82/84	90.5/86.8	0.39	0.78
Simultaneous parsimony	104	5384	934	34.6	4260	741	83	90.3	0.39	0.78

[a] "CI" = ensemble consistency index on the most parsimonious tree(s) for the parsimony-informative characters.
[b] "RI" = ensemble retention index.

simultaneous-analysis data matrix that has been posted as online Supplementary data on the journal's website.

The morphological characters represent just 3.5% of the 934 parsimony informative characters included in the simultaneous analysis (Table 2), and might be considered to be "swamped" by the sequence-based characters and unhelpful for phylogenetic inference (e.g., Hedges and Maxson, 1996; Scotland et al., 2003). Yet we know of no compelling theoretical reason not to analyze these data partitions together (Barrett et al., 1991), the number of characters in each data partition may be less relevant than the pattern and distribution of homoplasy (Donoghue and Sanderson, 1992), and the ensemble consistency (CI; Kluge and Farris, 1969) and retention indices (RI; Farris, 1989) for the morphological-characters-only analysis are higher than those in the molecular-characters-only analysis (Table 2).

2.3. Molecular methods

Total genomic DNA was extracted from herbarium specimens and fresh, silica gel- and sodium chloride/CTAB-preserved (Chase and Hills, 1991; Rogstad, 1992) leaves and/or stems using DNeasy Plant Mini Kits (Qiagen Inc., Valencia, CA) or the protocol described by Alexander et al. (2006). New sequences for two loci from the plastid genome (*matK* and *trnL-F*) and two gene regions from the nuclear genome (ITS and 26S rDNA) were generated for this project. All four gene regions were amplified with the following PCR protocol: an initial denaturation of 96° preceding 10 cycles denaturation (96° for 45 s), annealing (50–53° for 30 s), and extension (72° for 2 min), followed by 25 cycles of denaturation (96° for 20 s), annealing (50–53° for 30 s), and extension (72° for 2 min).

Most amplifications of the *matK* locus were split into two reactions, one using the primer combination *trnK*-710 (Johnson and Soltis, 1995) and *matK*-R1 (Yokoyama et al., 2000) for the 5′ end and the second reaction using *matK*-F1 (Yokoyama et al., 2000) and *matK*-8R (Steele and Vilgalys, 1994) for the 3′ end. When one or both of the above combinations did not amplify or produced poor electropherogram reads, alternate primers were used. The primer *matK*-441R (Zhang et al., 2006) was used instead of *matK*-R1, and *matK*-F3 (Yokoyama et al., 2000) was used instead of *matK*-F1. Where *matK*-441F was used, the combination of *matK*-F1 and *matK*-R1 was used to amplify the central region of the *matK* locus.

The *trnL* intron and the *trnL-F* intergenic spacer were amplified in one reaction using primers 'c' and 'f' or in two reactions using the combinations 'c' and 'd' for the 5′ end and 'e' and 'f' for the 3′ end (Taberlet et al., 1991). The ITS region (ITS1-5.8S-ITS2) was amplified with either the primer combination ITSA and ITSB (Blattner, 1999), or ITS5 with ITS4 (White et al., 1990; Rauscher et al., 2004). Most amplifications of 26S rDNA were amplified using the primers 26S1 and 950rev, or in two reactions using 26S1 with 641rev for the 5′ end and 26S2 with 950rev for the 3′ end (Kuzoff et al., 1998). When those primer combinations were unsuccessful, the region was amplified in three reactions using 26S1 with 268rev, 26S2 with 641rev, and 26S3 with 950rev. Amplified products were purified using the QIAquick Gel Extraction Kit or the Qiagen PCR Purification Kit. Purified PCR products were sequenced by Macrogen (Seoul, Korea) or the University of Chicago Cancer Research Center DNA Sequencing Facility using automated fluorescent sequencing with ABI DNA Analyzers. The same primers used for amplification were also used for sequencing except for 26S rDNA where some taxa were sequenced with 268rev and/or 26S3 (Kuzoff et al., 1998). All new sequences generated in this study have been deposited in GenBank under accession numbers HM230065 to HM230291 (Appendix A).

GenBank sequences available for one additional nuclear locus (*phyB*) were also included in the study. In addition to those species sampled by Coughenour et al. (2010), consensus sequences used by Simmons et al. (2001a) were added for *Campylostemon angolense, Cuervea integrifolia, C. kappleriana, Pristimera andina, Reissantia* sp., *Reissantia indica, Semialarium paniculatum* (incorrectly identified as *Hippocratea volubilis* in Simmons et al., 2001a), and *Simicratea welwitschii*. GenBank sequences for 18S rDNA, *atpB*, and *rbcL* were not included in this study because they were only available for four, four, and five, respectively, of the species we sampled here and the relationships among those taxa [*Brexia madagascariensis* Thouars ex Ker-Gawl., *Elaeodendron orientale* Jacq., *Loeseneriella africana* (Willd.) Wilczek ex N. Hallé, *Plagiopteron suaveolens* Griff., *and Reissantia* sp.] were well supported in other gene trees.

2.4. Data analysis

Preliminary nucleotide alignments were obtained independently for each locus using the default alignment parameters in MUSCLE ver. 3.6 (Edgar, 2004). Manual adjustments to the MUSCLE alignments were performed in MacClade ver. 4.03 (Maddison and Maddison, 2001) using the procedure outlined by Simmons (2004b) following Zurawski and Clegg (1987), though a single position was added to the *trnL-F* MUSCLE alignment. No manual adjustments were necessary for *phyB*. We observed some ambiguously-aligned regions where one or more sequences had a duplicate insertion (or the others had a deletion of one of two repeats) and the character-state distribution among the characters in the ambiguously-aligned region was identical for those sequences that have both repeats such that the character-state distribution among the positions in question would be identical for either of the alternative alignments. In these cases, the ambiguously-aligned regions

were kept in the analysis following Davis et al. (1998). A total of 357 ambiguously-aligned positions were excluded from the analyses (26S rDNA: 11 positions from one region; ITS: 266 positions from 17 regions; *trnL-F*: 80 positions from two regions). Ambiguously-aligned nucleotides of individual sequences in regions that could not be unambiguously aligned with the remaining sequences were scored as ambiguous ("?").

Gap characters, whose inclusion often affects the inferred tree topology and increase branch-support values (Simmons et al., 2001c), were scored using modified complex-indel-coding (Simmons and Ochoterena, 2000; Müller, 2006). Only parsimony-informative complex-indel-coding gap characters were scored from unambiguously-aligned regions. A total of 43 gap characters were scored (26S rDNA: 4; ITS: 18; *matK*: 3; *trnL-F*: 18) for inclusion in the parsimony analyses.

As a means of data exploration, several alternative potential process partitions (Bull et al., 1993) of the characters were analyzed, although actual delimitation of process partitions is often arbitrary (Siddall, 1997). Each of the five gene regions was analyzed independently of one another to resolve their respective gene trees. Putative coalescent genes (Hudson, 1990; Doyle, 1995) were then analyzed and their trees compared to check for well supported, contradictory signal that may have been caused by lineage sorting, introgression, and/or unrecognized paralogy (Doyle, 1992). As such, gene trees for the adjacent rDNA gene regions and the plastid loci were analyzed independently of one another to check for potential introgression of the plastid genome or rDNA (Doyle, 1992; Wendel et al., 1995) or unrecognized paralogy problems with rDNA (Álvarez and Wendel, 2003; Bailey et al., 2003). An analysis of all molecular characters was then performed, followed by a simultaneous analysis of all morphological and molecular characters (using parsimony only), which was conducted as the primary basis for phylogenetic inference. The simultaneous-analysis data matrix has been posted as online Supplementary data on the journal's website.

Equally weighted parsimony tree searches were conducted for each data matrix using 2000 random addition tree-bisection-reconnection (TBR) searches in PAUP* ver. 4.0b10 (Swofford, 2001) with a maximum of 10 trees held per replicate. Parsimony JK analyses were conducted using PAUP* with the removal probability set to approximately e^{-1} (36.7879%), and "jac" resampling emulated. Two-thousand JK replicates were performed with 100 random addition TBR searches (each with a maximum of ten trees held) per replicate.

jModeltest ver. 0.1.1 (Posada, 2008) was used to select the best-fit likelihood model for each data matrix using the Akaike Information Criterion (AIC; Akaike, 1974). Following Yang (2006) and Stamatakis (2008), invariant-site models (Reeves, 1992) were not considered because models that incorporated the gamma distribution (Yang, 1993) were considered. The models selected all incorporated the gamma distribution. The Q-matrices selected were all variants of TIM, TVM, or GTR.

Maximum likelihood (Felsenstein, 1973) analyses of nucleotide characters from each of the molecular data matrices were performed as (not infallible; Gaut and Lewis, 1995; Siddall, 1998; Sanderson and Kim, 2000) tests for long-branch attraction (Felsenstein, 1978). Likelihood analyses were conducted using RAxML ver. 7.03 (Stamatakis, 2006). Given that RAxML only implements GTR Q-matrices for nucleotide characters, more restrictive variants of the GTR matrix were not used when selected by the AIC. Optimal likelihood trees were searched for using 1000 independent searches starting from randomized parsimony trees with the GTRGAMMA model and four discrete rate categories. Likelihood BS analyses were conducted with at least 2000 replicates with ten searches per replicate using the "–f i" option, which "refine[s] the final BS tree under GAMMA and a more exhaustive algorithm" (Stamatakis, 2008:9).

3. Results

The simultaneous analysis parsimony JK tree of all five gene regions and morphological characters is presented in Fig. 1 with BS values below each branch given for likelihood analysis of all five gene regions. The parsimony JK trees with JK values above each branch and likelihood BS values below each branch for each of the five gene regions, the three remaining combined analyses and the parsimony JK morphology tree are presented in Figs. S1–S10 as online Supplementary data. These trees were created using TreeGraph 2 (Stöver and Müller, 2010). Data matrix and tree statistics for all analyses are presented in Table 2. No mutually well supported ($\geqslant$70% JK and BS) contradictory clades were identified in any of the parsimony and likelihood analyses.

Of the five gene regions, only *phyB* was found to exhibit significant nucleotide-frequency heterogeneity for the parsimony-informative nucleotide characters among different terminals, as determined by the chi-square test implemented in PAUP* (which ignores phylogenetic correlations). Specifically, members of the Hippocrateoideae were found to have a high AC content relative to the other taxa sampled.

3.1. Process partitions

One mutually well supported ($\geqslant$70% JK) incongruence was observed when comparing the rDNA and plastid gene trees (Figs. S3 and S7). *Elachyptera floribunda* was resolved as sister to the three accessions of *Elachyptera holtzii* sampled in the rDNA gene tree (as well as the separate 26S rDNA and ITS gene trees; Figs. S1 and S2), whereas *E. floribunda* was resolved as more closely related to *Simicratea welwitschii* on the plastid gene tree (72% JK/58% BS, 76% JK/66% BS, and 61% JK/58% BS on successive branches; Fig. S7).

We were unable to amplify *E. floribunda* for *trnL-F*, so the plastid resolution is based on the *matK* sequence. Two people independently generated *matK* sequences for *E. floribunda* and obtained the same sequence. The *matK* synapomorphies uniting *E. floribunda* with *S. welwitschii* are scattered across the entire region of *matK* amplified (as two separate PCR products) rather than being restricted to the 5′ or 3′ ends. Finally, the *matK* sequences of *E. floribunda* and *S. welwitschii* are not identical. Based on these three factors, we do not believe that the *matK* sequence of *E. floribunda* is an artifact.

E. floribunda is native to Central America, whereas *S. welwitschii* and the two species of *Pristimera* that are closely related to it are from Africa. The 26S rDNA and ITS gene trees are congruent with each other as well as the current taxonomy in resolving *E. floribunda* as sister to *E. holtzii* (from Africa). Given this taxonomic congruence in contrast with the plastid gene-tree topology (for which we could not identify any morphological synapomorphies of *E. floribunda* and *S. welwitschii* that are not also shared by *E. holtzii*), we believe that the rDNA gene tree is tracking the phylogenetic signal of *E. floribunda* whereas the *matK* sequence in the plastid gene tree is not, perhaps due to introgression of the plastid genome via hybridization (Rieseberg and Soltis, 1991). Therefore, following Lecointre and Deleporte (2005), the *E. floribunda* sequence was excluded from the combined molecular and simultaneous analyses.

The *phyB* gene tree (Fig. S4) clearly conflicts with all other gene trees in resolving the Hippocrateoideae as more closely related to *Brexia* and *Elaeodendron* than to Salacioideae (73% and 53% JK on successive clades), and *Elaeodendron* as more closely related to Hippocrateoideae than it is to *Brexia* (53% JK/63% BS). Other incongruencies were noted within Hippocrateoideae including *Loeseneriella* being more closely related to *Simicratea* (64% JK/50% BS) than it is to *Campylostemon*. Because of the multiple incongruencies, the combined molecular parsimony jackknife analysis was re-run after excluding *phyB*. No contradictory clades were resolved,

but the support for the clade of Hippocrateeae and Campylostemoneae was raised from 71% to 84% JK, one clade with 62% JK was lost (not present in the simultaneous analysis either) and two additional clades were resolved with 54% and 67% JK on successive branches in which *Plagiopteron* was resolved as sister to the clade of *Pristimera preussii* + *Simicratea.* sp. nov.? + *S. welwitschii* (Figs. S9–S10).

4. Discussion

Based on the general congruence between the parsimony and likelihood trees for each process partition as well as between process partitions (except as noted in the Results section), the simultaneous-analysis tree (Fig. 1) was used as the best estimate of the phylogeny and is the focus of the Discussion section. Conflict between *phyB* and other gene regions may in part be caused by unrecognized paralogs that have been differentially sampled in various taxa (Doyle, 1992), which is particularly problematic for polyploids, which are known to occur in Hippocrateoideae (e.g., Mangenot and Mangenot, 1957). Another possible factor is the low terminal sampling for *phyB* relative to all other gene regions (16 vs. 95–103) causing long-branch attraction. Although *phyB* was the only locus found to exhibit significant nucleotide-frequency heterogeneity for the parsimony-informative nucleotide characters among different terminals, this was not inferred to have occurred in a convergent manner and hence should not have negatively affected the *phyB* gene-tree topology (Lockhart et al., 1992). Unless otherwise noted, morphological and indel synapomorphies described below were unambiguously optimized onto the simultaneous-analysis tree topology (Fig. 1) using Fitch (1971) optimization while taking into account all possible resolutions of polytomies.

The Hippocrateoideae are an unambiguously supported clade (100% JK/100% BS) for which synapomorphies include presence of an annulus on the pollen grains (Lobreau-Callen, 1977), capsular fruits that are strongly parted among carpels, presence of a non-arillate basal seed wing, a 1-bp deletion at position 688 in 26S rDNA, and a 19-bp deletion from positions 68–86 in the *trnL-F* intergenic spacer. Potential wood-anatomy synapomorphies for Hippocrateoideae, which were not included in this study and would be ambiguously optimized due to large amounts of missing data, are some rays greater than ten cells wide and loss of parenchyma-like bands of thin-walled septate wood fibers (Simmons and Hedin, 1999). This resolution of Hippocrateoideae is consistent with all previous phylogenetic analyses that have tested monophyly of Hippocrateoideae (Simmons and Hedin, 1999; Savolainen et al., 2000; Simmons et al., 2001a,b).

Based on Fitch (1971) optimization, the Hippocrateoideae appear to have had an Old World origin followed by at least 3–5 successful radiations within the New World (Fig. 1). This inference is supported regardless of how the large polytomy within Hippocrateoideae is resolved. There is no indication of dispersals from the New World back to the Old World. The Old World origin of Hippocrateoideae is consistent with the Old World origin of Salacioideae inferred by Coughenour et al. (2010). Five genera of Hippocrateoideae are native to Madagascar (Hallé, 1978), and one species of each genus was sampled in this study: *Apodostigma pallens*, *Elachyptera minimiflora*, *Loeseneriella urceolus*, *Pristimera* sp. nov., and *Reissantia angustipetala*. These five genera represent at least four independent colonizations of Madagascar, all of which appear to have an African origin.

4.1. Tribes within Hippocrateoideae

Helictonema was supported as sister to all other members of Hippocrateoideae in the combined rDNA tree (85% JK/43% BS; Fig. S3), combined molecular tree (71% JK/45% BS; Fig. S9), and the simultaneous analysis (64% BS; Fig. 1). A morphological synapomorphy for all Hippocrateoideae other than *Helictonema* is loss of the aril. This resolution supports Hallé's (1962, 1986, 1990) recognition of Helictonemateae as distinct from Campylostemoneae and Hippocrateeae.

The Campylostemoneae, from which two of the three genera were sampled in this study (*Campylostemon* and *Tristemonanthus*; *Bequaertia* was not sampled) are strongly supported (100% JK/91% BS) as a monophyletic group for which a morphological synapomorphy is absence (loss) of the nectary disk. *Hippocratea* is supported as the sister group of Campylostemoneae (90% JK/55% BS) and a morphological synapomorphy for this broader clade is pollen grains aggregated into tetrads or polyads, which are also convergently derived in *Hylenaea*. Given that *Bequaertia* shares these two morphological synapomorphies, we believe all three genera of Campylostemoneae are a monophyletic group. Having five, rather than three, stamens per flower is a unique reversal for the genus *Campylostemon* within the Celastraceae as a whole. Neither *Campylostemon* nor *Tristemonanthus* are transitional between Celastraceae sensu stricto and the former Hippocrateaceae, contra Loesener (1942a,b) and Görts-van Rijn and Mennega (1994). Rather, they are derived genera nested within Hippocrateoideae as asserted by Robson (1965).

The strong support for Campylostemoneae as nested within Hippocrateeae (92% JK/94% BS, 91% JK/70% BS, and 90% JK/55% BS on successive clades) precludes maintenance of Campylostemoneae as distinct from Hippocrateeae. As such, we propose that Campylostemoneae be treated as a synonym of Hippocrateeae, and continue to maintain Helictonemateae.

4.2. Intergeneric relationships

The monophyly of several genera (*Campylostemon*, *Cuervea*, *Hylenaea*, *Loeseneriella*, *Reissantia*, and *Semialarium*) was supported based on our current taxon sampling, but not all genera for which multiple species were sampled were resolved as monophyletic groups. *Elachyptera* and *Prionostemma* were resolved as polyphyletic, *Simirestis* was resolved as nested within *Pristimera*, and two species of *Pristimera* were resolved as more closely related to *Simirestis* than they were to the other species of *Pristimera* sampled. Despite these taxonomic problems, we do not believe that the Hippocrateoideae should be reduced to just two genera (*Campylostemon* and *Hippocratea*) as asserted by Robson (1965, 1989), especially given that *Campylostemon* is nested within *Hippocratea* sensu lato (i.e., all genera of Hippocrateoideae other than *Campylostemon*).

Anthodon and *Semialarium* are unique within the Hippocrateoideae in having connate mericarps, and this is a synapomorphy for the clade in the simultaneous-analysis tree (60% JK; Fig. 1), though the clade is not resolved in the combined molecular analyses (Fig. S9).

The clade of *Apodostigma* + *Campylostemon* + *Elachyptera minimiflora* + *Hippocratea* + *Loeseneriella* + *Reissantia* + *Tristemonanthus* was strongly supported as a monophyletic group (92% JK/94% BS), though we were unable to identify any unambiguously optimized synapomorphies for this clade. This resolution is consistent with Smith's (1941) inference that *Hippocratea* is closely related to *Loeseneriella* though the two lineages are distinct, Wilczek's (1956) inference that *Apodostigma* is closely related to *Loeseneriella*, and Hedin's (1999) inference that *Apodostigma* is closely related to Campylostemoneae. Yet this resolution contradicts Hedin's (1999) inference that *Hippocratea* and *Pristimera* are sister groups.

Elachyptera minimiflora was resolved as part of a polytomy with *Apodostigma,* Campylostemoneae + *Hippocratea* + *Loeseneriella*, and

Reissantia rather than with the two other species of *Elachyptera* sampled (92% JK/94% BS; Fig. 1). *E. minimiflora* was originally described as a member of *Hippocratea* sensu lato by Perrier de la Bâthie in 1942 before Hallé (1978) transferred the species to *Elachyptera*. Although fully ripe fruits of *E. minimiflora* remain unknown, Hallé (1978) predicted that the seeds of this species are winged (and indeed they are based on available immature fruits from two specimens [*Ramirison 656*, G, P; *Archer 3789*, PRE]), which is known to be the case with the other Madagascan species that Hallé (1978) newly assigned to *Elachyptera* – *E. parvifolia* (Oliv.) N.Hallé. If Hallé's (1978) prediction is correct, then inclusion of *E. minimiflora* (and *E. parvifolia*) would require that *Elachyptera* be more broadly defined to include this species with its well developed seed wing. Yet our inferred phylogeny suggests that *E. minimiflora* is more closely related to *Reissantia* than it is to *Elachyptera* such that the circumscription of *Elachyptera* should not be increased to include the predicted winged seeds of *E. minimiflora*, or, by probable extension, the known winged seeds of *E. parvifolia*.

The New and Old World species of *Prionostemma* were unambiguously resolved as a polyphyletic group (63% JK/73% BS, 88% JK/71% BS, and 100% JK/97% BS on successive clades; Fig. 1). *Prionostemma* was originally described by Miers (1872) for six New World species, but was later reduced to a single species by Smith (1940). Hallé (1981, 1986) later expanded the genus to include five Old World species. We follow Smith's (1940) delimitation of *Prionostemma* and transfer the five Old World species to *Pristimera* (Appendix B).

Simirestis goetzei was strongly supported (77% JK/64% BS and 81% JK/69% BS on successive branches) as nested within *Pristimera*. Synapomorphies for the nesting of *Simiresits goetzei* within *Pristimera* included two ITS indels – a 1-bp deletion at position 201 and a 6-bp insertion at positions 801–806. Hallé (1962:42) hypothesized that *Simirestis* is ancestral to *Apodostigma*, *Bequaertia*, *Elachyptera*, *Hippocratea*, *Loeseneriella*, and *Reissantia*, but our results indicate that *Simirestis* is a derived lineage nested within *Pristimera*. Robson (1965) asserted that *Simirestis* cannot be distinguished from *Pristimera* and our results corroborate this. Hallé (1981) recognized the close relationship between *Prionostemma*, *Pristimera*, and *Simirestis* and transferred several species that he had previously assigned to *Simirestis* (Hallé, 1958) to *Pristimera*. This close relationship is corroborated in our inferred phylogeny (Fig. 1), and we reduce *Simirestis* to a synonym of *Pristimera* (Appendix B).

Pristimera preussii and a possible new species of *Simicratea* from Kenya are strongly supported (98% JK/91% BS) as more closely related to *Simicratea welwitschii* than they are to the other eight species of *Pristimera* sampled. The close relationship of *P. preussii* and *S. welwitschii* was previously identified by Simmons et al. (2001b), albeit with weak support (53% parsimony BS). *Simicratea* is not closely related to *Simirestis* (contra Hallé, 1983). David Harris (pers. comm., 2009) from the Royal Botanic Garden Edinburgh confirmed the identification of *D. Harris 4969* as *P. preussii*. Iain Darbyshire (pers. comm., 2009) from the Royal Botanic Gardens Kew noted that *Luke & Luke 4747* (here treated as *Simicratea* sp. nov.?) is vegetatively most similar to *Simicratea* though it lacks an androgynophore, which is one of the diagnostic character states for *S. welwitschii*. Being in young fruit, definite morphological identification of the specimen is problematic.

Hallé (1981) recognized the following three subgenera of *Pristimera*: *Beccariantha*, *Pristimera*, and *Trochantha*. Subgenus *Beccariantha* includes a single species from Malesia [*P. glaga* (Korth.) N.Hallé] and subgenus *Trochantha* includes two African species [*P. graciliflora* (Welw. ex Oliv.) N.Hallé and *P. preussii*]. Subgenus *Pristimera* includes all remaining species from the Americas, Africa, and Madagascar. Hallé (1981) distinguished subgenus *Trochantha* from the other two subgenera based on their completely rotate flowers (as opposed to urceolate or semi-rotate), orbicular petals that are slightly unguiculate (i.e., clawed; as opposed to suborbicular petals that are oval or oblong and ± sessile), and annular unlobed discs (as opposed to lobed or angled disks that are sometimes cupular). *Simicratea welwitschii* also has rotate flowers with annular unlobed discs, though the petals are ovate to oblong-elliptic and not unguiculate (Robson et al., 1994). Based on the strong support uniting *P. preussii* and a possible new species with *S. welwitschii*, we could transfer both species of *Pristimera* subgenus *Trochantha* to *Simicratea.* However, this would lead to a dilution of a well defined and morphologically distinctive monotypic genus in *Simicratea*. The second, and taxonomically less disruptive option followed here would be to raise *Pristimera* subgen. *Trochantha* to generic level (Appendix B).

4.3. Morphological characters

Most morphological characters that are variable and have alternative character states that are each present in many species of Hippocrateoideae were highly homoplasious when optimized onto the simultaneous-analysis tree topology (data not shown). Consequently, delimiting morphologically well defined genera within Hippocrateoideae is difficult. Having at least some terminal inflorescences present was inferred to be convergently derived at least six times from having strictly axillary inflorescences. Thyrsoid to racemose inflorescences were inferred to be convergently derived at least three times from cymose inflorescences. Cupular or columnar nectary disks were inferred to be convergently derived at least five times from annular or flat nectary disks. Pubescent disks were inferred to be convergently derived from glabrous disks in four separate lineages. Androgynophores were inferred to be convergently derived in *Helictonema*, *Loeseneriella*, *Simicratea*, and *Simirestis*. The number of ovules per locule that was ancestral within Hippocrateoideae is ambiguous, but there have been at least four shifts between having two or four ovules vs. having a variable number greater than four.

Hallé's (1983) hypothesis that seeds adapted to water dispersal rather than wind dispersal are derived within Hippocrateoideae was supported. Seeds with large embryos, corky testa, and vestigial wings are inferred to be convergently derived in *Cuervea kappleriana*, *Elachyptera holtzii*, and *Hylenaea*.

The resolution of *Helictonema* as sister to all other members of Hippocrateoideae supports Hallé's (1983) inference that its small aril is vestigial given that the presence of an aril is plesiomorphic within the study lineage (also present in *Polycardia*, Salacioideae, *Salaciopsis*, and *Sarawakodendron*). The unique aril of *Helictonema*, which is sister to all other members of Hippocrateoideae, recalls the unique aril of *Sarawakodendron*, which is sister to Salacioideae (Fig. 1; Coughenour et al., 2010). The arils of both *Helictonema* and *Sarawakodendron* are inferred to be derived from the more typical fleshy arils of Celastraceae that are present in genera such as *Celastrus*, *Euonymus*, *Gymnosporia*, *Maytenus*, and *Salaciopsis*.

In *Sarawakodendron* the aril has been elaborated into what Corner (1976:94) described as "Aril double, as a caruncle and as filaments..." Coughenour et al. (2010) hypothesized that the mucilagenous pulp of Salacioideae, which "... ultimately appear[s] as a network of crowded, white, spiral filaments" (Miers, 1872:324) is homologous to the filamentous aril of *Sarawakodendron*. If this hypothesis is corroborated by anatomical study, then the aril has been dramatically elaborated within the Salacioideae lineage, whereas it has been reduced and then entirely lost within the Hippocrateoideae lineage. These divergent evolutionary pathways for seed dispersal account for the radically different seed and fruit morphology of Hippocrateoideae and Salacioideae, which led Robson (1965, 1989) and Robson et al. (1994) to suggest that

Hippocrateoideae and Salacioideae are a polyphyletic group derived from independent lineages of Celastraceae sensu stricto.

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Appendix A

List of taxa sampled with taxonomic authorities, voucher information and GenBank accession numbers for new sequences generated for this study.

Anthodon decussatum Ruiz & Pav.—*G. Montgomery 19*, Panama (MO); 26S rDNA HM230068, ITS rDNA HM230113, *matK* HM230160, *trnL intron* HM230206, *trnL-F* spacer HM230251; ***Apodostigma pallens*** (Planch. ex Oliv.) R.Wilczek—*Friis* et al. *3994,* Ethiopia (K); 26S rDNA HM230069, ITS rDNA HM230114, *matK* HM230161, *trnL intron* HM230208, *trnL-F* spacer HM230252; ***Apodostigma pallens*** (Planch. ex Oliv.) R.Wilczek—*Sanogo* et al. *ML-168*, Mali (K); 26S rDNA HM230070, ITS rDNA HM230115, *matK* HM230162, *trnL intron* HM230207, *trnL-F* spacer HM230253; ***Campylostemon angolense*** Welw. ex Oliv.—*W.J.J.O. de Wilde* et al. *3754,* Cameroon (MO, P); 26S rDNA HM230071, ITS rDNA HM230116, *matK* HM230163, *trnL intron* HM230209, *trnL-F* spacer HM230254; ***Campylostemon bequaertii*** De Wild.—*J. Harris & J.M. Fay 565,* Cameroon (K); 26S rDNA HM230072, ITS rDNA HM230117; ***Cuervea isangiensis*** (De Wild.) N.Hallé— *L. White 1238,* Gabon (MO); 26S rDNA HM230073, ITS rDNA HM230118, *matK* HM230164, *trnL intron* HM230210, *trnL-F* spacer HM230255; ***Cuervea kappleriana*** (Miq.) A.C.Sm.—*N.C. Garwood & A. Gonzalez 1879A*, Panama (F); 26S rDNA HM230074, ITS rDNA HM230119, *matK* HM230165, *trnL intron* HM230211, *trnL-F* spacer HM230256; ***Cuervea kappleriana*** (Miq.) A.C.Sm.— *G.L. Sobel* et al. *4864,* Brazil (NY); ITS rDNA HM230120, *matK* HM230166, *trnL intron* HM230212, *trnL-F* spacer HM230257; ***Elachyptera floribunda*** (Benth.) A.C.Sm.— *E.M. Martinez* et al. *23410,* Guatemala (MO); 26S rDNA HM230075, ITS rDNA HM230121, *matK* HM230167; ***Elachyptera holtzii*** (Loes. ex Harms) R.Wilczek—*O.A. Kibure 228,* Tanzania (MO); 26S rDNA HM230076, ITS rDNA HM230122, *matK* HM230168, *trnL intron* HM230213, *trnL-F* spacer HM230258; ***Elachyptera holtzii*** (Loes. ex Harms) R.Wilczek—*Luke & Mbinda 5817*, Kenya (K); 26S rDNA HM230077, ITS rDNA HM230123, *matK* HM230169, *trnL intron* HM230214, *trnL-F* spacer HM230259; ***Elachyptera holtzii*** (Loes. ex Harms) R.Wilczek—*Luke & Luke 7468,* Kenya (K); 26S rDNA HM230078, ITS rDNA HM230124, *matK* HM230170, *trnL intron* HM230215, *trnL-F* spacer HM230260; ***Elachyptera minimiflora*** (H.Perrier) N.Hallé—*R.H. Archer* et al. *2934,* Madagascar (CS); 26S rDNA HM230079, ITS rDNA HM230125, *matK* HM230171, *trnL intron* HM230216, *trnL-F* spacer HM230261; ***Helictonema velutinum*** (Afzel.) Pierre ex N.Hallé—*T.B. Hart 1580,* Zaire (MO); ITS rDNA HM230126, *matK* HM230172, *trnL intron* HM230217, *trnL-F* spacer HM230262; ***Helictonema velutinum*** (Afzel.) Pierre ex N.Hallé—*R. Spichiger 6910,* Ivory Coast (MO); 26S rDNA HM230080; ***Hippocratea volubilis*** L.—*J.F. Castrejon* et al. *949*, Mexico (MO); ITS rDNA HM230127, *trnL intron* HM230218, *trnL-F* spacer HM230263; ***Hippocratea volubilis*** L.—*J.A. Lombardi 6923*, Brazil (HRCB); 26S rDNA HM230081, ITS rDNA HM230128, *matK* HM230173, *trnL intron* HM230219, *trnL-F* spacer HM230264; ***Hylenaea comosa*** (Sw.) Miers—*J.A. Lombardi 6400,* Brazil (HRCB); 26S rDNA HM230082, ITS rDNA HM230129, *matK* HM230174, *trnL intron* HM230220, *trnL-F* spacer HM230265; ***Hylenaea praecelsa*** (Miers) A.C.Sm.—*R. Foster 897*, Panama (MO); 26S rDNA HM230083, ITS rDNA HM230130, *matK* HM230175, *trnL intron* HM230221; ***Loeseneriella rowlandii*** (Loes.) N.Hallé—*D.K. Harder* et al. *2980,* Ghana (MO); 26S rDNA HM230084, ITS rDNA HM230131, *matK* HM230176, *trnL intron* HM230222, *trnL-F* spacer HM230266; ***Loeseneriella* sp.**—*M.W. Chase 2095,* cult. Bogor, Indonesia (K); ITS rDNA HM230132, *matK* HM230178, *trnL intron* HM230223, *trnL-F* spacer HM230267; ***Loeseneriella urceolus*** (Tul.) N.Hallé—*R.H. Archer* et al. *3002,* Madagascar (CS); 26S rDNA HM230085, ITS rDNA HM230133, *matK* HM230177, *trnL intron* HM230224, *trnL-F* spacer HM230268; ***Prionostemma aspera*** Miers—*Pires 1399,* Brazil (NY); 26S rDNA HM230087, ITS rDNA HM230134, *matK* HM230179, *trnL intron* HM230225, *trnL-F* spacer HM230269; ***Prionostemma fimbriata*** (Excell) N.Hallé—*G. McPherson 15681*, Gabon (MO); 26S rDNA HM230088, ITS rDNA HM230135, *matK* HM230180, *trnL intron* HM230226, *trnL-F* spacer HM230270; ***Pristimera andina*** Miers—*E. Zardini & P. Aquino 33474,* Paraguay (F); 26S rDNA HM230089, ITS rDNA HM230136, *matK* HM230181, *trnL intron* HM230227, *trnL-F* spacer HM230271; ***Pristimera andongensis*** (Welw. ex Oliv.) N.Hallé—*M.A. Mwangoka* et al. *1230*, Tanzania (MO); 26S rDNA HM230090, ITS rDNA HM230137, *matK* HM230182, *trnL intron* HM230228, *trnL-F* spacer HM230272; ***Pristimera celastroides*** (Kunth) A.C.Sm.— *T.S. Cochrane* et al. *11659*, Mexico (F); 26S rDNA HM230092, ITS rDNA HM230139, *matK* HM230184, *trnL intron* HM230230, *trnL-F* spacer HM230274; ***Pristimera celastroides*** (Kunth) A.C.Sm.—*B. Hammel* et al. *20111*, Costa Rica (F); 26S rDNA HM230091, ITS rDNA HM230138, *matK* HM230183, *trnL intron* HM230229, *trnL-F* spacer HM230273; ***Pristimera celastroides*** (Kunth) A.C.Sm.—*Nee & Taylor 26588,* Mexico (NY); ITS rDNA HM230140, *matK* HM230185, *trnL intron* HM230231, *trnL-F* spacer HM230275; ***Pristimera longipetiolata*** (Oliv.) N.Hallé—*R.H. Archer 2174,* South Africa (PRE); 26S rDNA HM230093, ITS rDNA HM230141, *matK* HM230186, *trnL intron* HM230232, *trnL-F* spacer HM230276; ***Pristimera nervosa*** (Miers) A.C.Sm.—*E. Gudiño* et al. *2116*, Ecuador (MO); 26S rDNA HM230095, ITS rDNA HM230143, *matK* HM230188, *trnL-F* spacer HM230278; ***Pristimera nervosa*** (Miers) A.C.Sm.—*J. Schunke 4073*, Peru (MO); 26S rDNA HM230096, ITS rDNA HM230144, *matK* HM230189, *trnL intron* HM230234, *trnL-F* spacer HM230279; ***Pristimera preussii*** (Loes.) N.Hallé—*D. Harris 4969,* Central African Republic (E); ITS rDNA HM230145, *matK* HM230190, *trnL intron* HM230235, *trnL-F* spacer HM230280; ***Pristimera* sp. nov.**—*R.H. Archer* et al. *2948,* Madagascar (CS); 26S rDNA HM230094, ITS rDNA HM230142, *matK* HM230187, *trnL intron* HM230233, *trnL-F* spacer HM230277; ***Pristimera tenuiflora*** (Mart. ex Peyr) A.C.Sm.—*C.A. Cid Ferreira* et al. *7998,* Brazil (F); 26S rDNA HM230097, ITS rDNA HM230146, *matK* HM230191, *trnL intron* HM230236, *trnL-F* spacer HM230281; ***Pristimera verrucosa*** Miers—*A. Gentry* et al. *78456*, Colombia (MO); 26S rDNA HM230098, ITS rDNA HM230147, *matK* HM230192, *trnL intron* HM230237, *trnL-F* spacer HM230282; ***Reissantia angustipetala*** (H.Perrier) N.Hallé—*R.H. Archer* et al. *2939,* Madagascar (CS); 26S rDNA HM230099, ITS rDNA HM230148, *matK* HM230193, *trnL intron* HM230238, *trnL-F* spacer HM230283; ***Reissantia buchananii*** (Loes.) N.Hallé—*W. Kindeketa* et al. *1430,* Tanzania (MO); 26S rDNA HM230100, ITS rDNA HM230149, *matK* HM230194, *trnL intron* HM230239, *trnL-F* spacer HM230284; ***Reissantia parviflora*** (Oliv.) N.Hallé—*E. Mboya* et al. *271,* Tanzania (MO); 26S rDNA HM230101, ITS rDNA HM230150, *matK* HM230195, *trnL intron* HM230240, *trnL-F* spacer HM230285; ***Salacia chinensis*** L.—*A.J. Ford 5550*, Australia (BRI);

26S rDNA HM230065, ITS rDNA HM230110, *matK* HM230157, *trnL intron* HM230203, *trnL-F* spacer HM230248; ***Salacia grandifolia*** (Mart. ex. Schult.) G.Don—*J.A. Lombardi 6851*, Brazil (HRCB); 26S rDNA HM230066, ITS rDNA HM230111, *matK* HM230158, *trnL intron* HM230204, *trnL-F* spacer HM230249; ***Salacia krigsneri*** Lombardi—*J.A. Lombardi 6687*, Brazil (HRCB); 26S rDNA HM230067, ITS rDNA HM230112, *matK* HM230159, *trnL intron* HM230205, *trnL-F* spacer HM230250; ***Semialarium mexicanum*** (Miers) Mennega—*J.F. Morales* et al. *1577*, Costa Rica (F, MO); 26S rDNA HM230102, ITS rDNA HM230151, *matK* HM230196, *trnL intron* HM230241, *trnL-F* spacer HM230286; ***Semialarium mexicanum*** (Miers) Mennega—*E. Cabrera & H. de Cabrera 8736,* Mexico (F); 26S rDNA HM230103, *matK* HM230197, *trnL intron* HM230242; ***Semialarium paniculatum*** (Mart. ex Schult.) N.Hallé—*E. Zardini & C. Benitez 3421*, Paraguay (MO); 26S rDNA HM230104, *trnL intron* HM230243; ***Simicratea* sp. nov. (?)**—*Luke & Luke 4747,* Kenya (K); 26S rDNA HM230105, ITS rDNA HM230152, *matK* HM230198, *trnL intron* HM230244, *trnL-F* spacer HM230287; ***Simicratea welwitschii*** (Oliv.) N.Hallé—*C.C.H. Jongkind & D.K. Abbiw 2127*, Ghana (MO); 26S rDNA HM230106, ITS rDNA HM230153, *matK* HM230199, *trnL intron* HM230245, *trnL-F* spacer HM230288; ***Simirestis goetzei*** (Loes.) N.Hallé ex R.Wilczek—*G. Simon & I.L. Mollel 82,* Tanzania (MO); 26S rDNA HM230107, ITS rDNA HM230154, *matK* HM230200, *trnL intron* HM230246, *trnL-F* spacer HM230289; ***Simirestis goetzei*** (Loes.) N.Hallé ex R.Wilczek—*Borhidi* et al. *85491,* Tanzania (K); 26S rDNA HM230108, ITS rDNA HM230155, *matK* HM230201, *trnL intron* HM230247, *trnL-F* spacer HM230290; ***Tristemonanthus nigrisilvae*** (N.Hallé) N.Hallé—*A.J.M. Leeuwenberg 3758*, Ivory Coast (L); 26S rDNA HM230109, ITS rDNA HM230156, *matK* HM230202, *trnL-F* spacer HM230291.

Appendix B

New binomials proposed in this study. All five Old World taxa of *Prionostemma* (Hallé, 1981) are hereby transferred to *Pristimera* as follows.

Pristimera arnottiana (Wight) R.H.Archer, comb. nov. *Hippocratea arnottiana* Wight, Ill. Ind. Bot. 1: 133 (1838). *Loeseneriella arnottiana* (Wight) A.C.Sm., J. Arnold Arbor. 26: 174 (1945). *Prionostemma arnottiana* (Wight) N.Hallé, Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér 4, 3: 7 (1981).— TYPES: India, Malabar, Wight 2445 E (E179081)!, lecto., designated here. Annotated by Wight 'Specimens figured -The others got long after and from a difft. part of the country'.

Pristimera delagoensis (Loes.) R.H.Archer, comb. nov. *Hippocratea delagoensis* Loes. Bot. Jahrb. Syst. 34: 119 (1904). *Simirestis delagoensis* (Loes.) N.Hallé, Bull. Mus. Nat. Hist. Nat., Sér 2, 30: 465 (1958). *Prionostemma delagoensis* (Loes.) N.Hallé, Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér 4, 3: 7 (1981).

—TYPE: Mozambique, Lourenço Marques, Schlechter 11517 (G, GRA!, K!, P!, PRE!, iso.).

Pristimera delagoensis (Loes.) R.H.Archer **var. ritschardii** (R. Wilczek) R.H.Archer, comb. nov. *Loeseneriella ritschardii* R.Wilczek, Bull. Jard. Bot. État Bruxelles 26: 406 (1956). *Simirestis ritschardii* (R.Wilczek) N.Hallé, Bull. Mus. Nat. Hist. Nat., Sér 2, 30: 465 (1958). *Hippocratea ritschardii* (R.Wilczek) N.Robson, Bol. Soc. Brot. Sér 2, 39: 49 (1965). *Prionostemma delagoensis* (Loes.) N.Hallé var. ritschardii (Wilczek) N.Hallé, Fl. Gabon 29: 232 (1986).—TYPE: Democratic republic of the Congo, Haut-Katanga Distr., route Likasi, Ritschard 1705 (BR, holo., K!, iso.).

Pristimera fimbriata (Exell) R.H.Archer, comb. nov. *Hippocratea fimbriata* Exell, J. Bot. 65 (Suppl. 1): 79 (1927). *Simirestis fimbriata* (Exell) N.Hallé, Bull. Mus. Nat. Hist. Nat., Sér 2, 30: 465 (1958). *Prionostemma fimbriata* (Exell) N.Hallé, Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér 4, 3: 7 (1981).—TYPE: Angola, Chiuango, Gossweiler 6292 (BM, holo., K!, LISC, iso.).

Pristimera unguiculata (Loes.) R.H.Archer, comb. nov. *Hippocratea unguiculata* Loes., Bot. Jahrb. Syst. 34: 118 (1904). *Simirestis unguiculata* (Loes.) N.Hallé, Bull. Mus. Nat. Hist. Nat., Sér 2, 30: 465 (1958). *Prionostemma unguiculata* (Loes.) N.Hallé, Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér 4, 3: 7 (1981).—TYPE: Cameroun, Bipendi, Urwaldgebiet, Zenker 2358 G, L, K!, P!, iso.).

All eight currently recognized species within *Simirestis* (Hallé, 1984; Robson et al., 1994) are hereby transferred to *Pristimera* as follows.

***Pristimera atractaspis* (N.Hallé) R.H.Archer,** comb. nov. *Simirestis atractaspis* N.Hallé, Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér 4, 6: 10 (1984).—TYPE: Ghana, Berekuso, J.B. Hall 47010 (P, holo.).

***Pristimera brianii* (N.Hallé) R.H.Archer,** comb. nov. *Simirestis brianii* N.Hallé, Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér 4, 6: 10 (1984).—TYPE: Kenya, N. Kavirondo Distr., Malaba Forest near Kakamega, Tweedie 3264 (K, holo.!).

***Pristimera dewildemaniana* (N.Hallé) R.H.Archer,** comb. nov. *Simirestis dewildemaniana* N.Hallé, Bull. Mus. Nat. Hist. Nat., Sér 2, 30: 465 (1958). *Hippocratea dewildemaniana* (N.Hallé) J.B. Hall, Kew. Bull. 35: 841 (1981). *Simirestis dewildemaniana* N.Hallé, Mem. Inst. Franc. Afr. Noire 64: 71 (1962). —TYPE: Democratic Republic of Congo, Central Forest, Lesse, Bequaert 4154 (BR, holo.). *Hippocratea affinis* De Wild., Pl. Bequaert 2: 61 (1923).

Pristimera goetzei (Loes.) R.H.Archer, comb. nov. *Hippocratea goetzei* Loes., Bot. Jahrb. Syst. 30: 346 (1902). *Simirestis goetzei* (Loes.) N.Hallé, Bull. Mus. Nat. Hist. Nat., Sér 2, 30: 465 (1958).—TYPE: Tanzania, Njombe Distr., Ukinga, Manganyema Mt., Goetze 1209 (B†, holo.).

***Pristimera klaineana* (N.Hallé) R.H.Archer,** comb. nov. *Simirestis klaineana* N.Hallé, Notul. Syst. (Paris) 16: 127 (1960).—TYPE: Gabon, Libreville, Klaine 2633 bis (P, holo.).

Pristimera scheffleri (Loes.) R.H.Archer, comb. nov. *Hippocratea scheffleri* Loes., Bot. Jahrb. Syst. 34: 115 (1904). *Simirestis scheffleri* (Loes.) N.Hallé, Bull. Mus. Nat. Hist. Nat., Sér 2, 30: 465 (1958). *Simirestis scheffleri* (Loes.) N.Hallé, Bull. Mus. Natl. Hist. Nat., B, Adansonia Sr 4, 3: 7 (1981).—TYPE: Tanzania, Derema, Usambara, Scheffler 197 (P!, K!, EA, iso.).

Pristimera staudtii (Loes.) R.H.Archer, comb. nov. *Hippocratea staudtii* Loes., Bot. Jahrb. Syst. 34: 113 (1904). *Simirestis staudtii* (Loes.) N.Hallé, Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér 4, 6: 8 (1984).—TYPE: Cameroun, Yaoundé, Zenker & Staudt 325 (K!, iso.).

***Pristimera tisserantii* (N.Hallé) R.H.Archer,** comb. nov. *Simirestis tisserantii* N.Hallé, Bull. Mus. Nat. Hist. Nat., Sér 2, 30: 465 (1958).—TYPE: Central African Republic, Boukoko, Tisserant 1268 (P!, holo.).

An additional, distinct South African taxon, incorrectly referred to as the Madagascar *Pristimera bojeri* (Tul.) N.Hallé (Robson, Fl. Zam. 2, 2: 408 (1966); Hallé, Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér 4, 3: 10 (1981), is hereby transferred to *Pristimera.*

Pristimera peglerae (Loes.) R.H.Archer, comb & stat.nov. *Hippocratea schlechteri* Loes. var. *peglerae* Loes., Feddes Rep. 49: 227 (1940).—TYPE: South Africa, Cape Province, Kentani, 14 Jan. fl. A. Pegler 914 (PRE!, lecto., designated here).

Both species and one subspecies of Hallé's (1981) *Pristimera* subgenus *Trochantha* are hereby transferred to the new genus *Trochantha* as follows:

***Trochantha* (N.Hallé)** R.H.Archer, gen. & stat. nov. *Pristimera* subg. *Trochantha* N.Hallé, Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér 4, 3: 12 (1981).—TYPE: *Trochantha graciliflora* (Welw. ex Oliv.) R.H.Archer.

Trochantha graciliflora (Welw. ex Oliv.) R.H.Archer, comb. nov. *Hippocratea graciliflora* Welw. ex Oliv., Fl. Trop. Afr. 1: 371 (1868).

Simirestis graciliflora (Welw. ex Oliv.) N.Hallé, Bull. Mus. Nat. Hist. Nat., Sér 2, 30: 465 (1958). *Pristimera graciliflora* (Welw. ex Oliv.) N.Hallé, Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér 4, 3: 12 (1981).—TYPE: Angola, Golungo Alto, Welwitsch 1332 (G, K!, P!, iso.).

Trochantha graciliflora (Welw. ex Oliv.) R.H.Archer **subsp. newalensis** (Blakelock) R.H.Archer, comb. nov. *Hippocratea graciliflora* Welw. ex Oliv. subsp. *newalensis* Blakelock, Kew Bull. 20: 295 (1966). *Pristimera graciliflora* (Welw. ex Oliv.) subsp. *newalensis* (Blakelock) N.Hallé, Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér. 4, 3: 12 (1981).—TYPE: Tanzania, Newala Distr., E. of Newala, Hay 35 (K!, holo.).

Trochantha preussii (Loes.) R.H.Archer, comb. nov. *Hippocratea preussii* Loes., Bot. Jahrb. Syst. 34: 112 (1904). *Simirestis preussii* (Loes.) N.Hallé, Bull. Mus. Nat. Hist. Nat., Sér 2, 30: 465 (1958). *Pristimera preussii* (Loes.) N.Hallé, Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér 4, 3: 12 (1981).—TYPE: Cameroon, Limbe, Preuss 1306 (K!, P!, iso).

Appendix C. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.02.017.

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