

Resolving basal lamiid phylogeny and the circumscription of Icacinaceae with a plastome-scale data set¹

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PREMISE OF THE STUDY: Major relationships within *Lamiidae*, an asterid clade with ~40 000 species, have largely eluded resolution despite two decades of intensive study. The phylogenetic positions of Icacinaceae and other early-diverging lamiid clades (Garryales, Metteniusaceae, and Oncothecaceae) have been particularly problematic, hindering classification and impeding our understanding of early lamiid (and euasterid) character evolution.

METHODS: To resolve basal lamiid phylogeny, we sequenced 50 plastid genomes using the Illumina sequencing platform and combined these with available asterid plastome sequence data for more comprehensive phylogenetic analyses.

KEY RESULTS: Our analyses resolved basal lamiid relationships with strong support, including the circumscription and phylogenetic position of the enigmatic Icacinaceae. This greatly improved basal lamiid phylogeny offers insight into character evolution and facilitates an updated classification for this clade, which we present here, including phylogenetic definitions for 10 new or converted clade names. We also offer recommendations for applying this classification to the Angiosperm Phylogeny Group (APG) system, including the recognition of a reduced Icacinaceae, an expanded Metteniusaceae, and two orders new to APG: Icacinales (Icacinaceae + Oncothecaceae) and Metteniusales (Metteniusaceae).

CONCLUSIONS: The lamiids possibly radiated from an ancestry of tropical trees with inconspicuous flowers and large, drupaceous fruits, given that these morphological characters are distributed across a grade of lineages (Icacinaceae, Oncothecaceae, Metteniusaceae) subtending the core lamiid clade (Boraginales, Gentianales, Lamiales, Solanales, *Vahlia*). Furthermore, the presence of similar morphological features among members of Aquifoliales suggests these characters might be ancestral for the *Gentianidae* (euasterids) as a whole.

KEY WORDS Garryales; Icacinaceae; *Lamiidae*; Metteniusaceae; Oncothecaceae; phylogenetic nomenclature; phylogenomics; plastid genome

Lamiidae are a major clade of asterid angiosperms, including ~40 000 species, or ~15% of angiosperm species richness (Refulio-Rodriguez and Olmstead, 2014). Earlier studies have referred to this clade informally as asterids I (Chase et al., 1993), euasterids I (APG, 1998; Soltis et al., 1999a; APG II, 2003), or lamiids (Bremer et al., 2002; Judd and Olmstead, 2004; APG III, 2009). Although Cantino et al. (2007) applied the name *Garryidae* to this clade, it has more commonly been called *Lamiidae* (Refulio-Rodriguez and Olmstead,

2014), and this treatment will be followed in the Companion Volume of the PhyloCode (R. G. Olmstead, University of Washington, personal communication; note that all clade names are in italics following Cantino et al., 2007).

Lamiids are generally characterized by superior ovaries and corollas with late sympetaly (Erbar and Leins, 1996; Judd et al., 2008), but considerable variation in morphology occurs across the clade, and nonmolecular synapomorphies are unclear (Stevens, 2001 onward; Judd and Olmstead, 2004). Phylogenetic studies over the past 20 years (e.g., Olmstead et al., 1992, 1993, 2000; Chase et al., 1993; Soltis et al., 1999a, 2000, 2011; Savolainen, 2000a, b; Albach et al., 2001; Bremer et al., 2002; Refulio-Rodriguez and Olmstead, 2014) have clarified the composition of *Lamiidae*. However, relationships among the major lineages have largely eluded resolution. Most lamiid diversity falls into a clade informally known as the “core lamiids” (Refulio-Rodriguez and Olmstead, 2014; the formal

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name *Lamianae* will be applied to this clade in the upcoming Companion Volume to the PhyloCode, R. G. Olmstead, University of Washington, personal communication), which includes four species-rich groups—Boraginales, Gentianales, Lamiales, and Solanales—as well as the small, phylogenetically isolated genus *Vahlia* Thunb. Relationships among these five groups have varied across studies and rarely received strong bootstrap support (Olmstead et al., 1992, 1993, 2000; Soltis et al., 1999a, 2000, 2011; Savolainen, 2000a, b; Albach et al., 2001; Bremer et al., 2002), although a recent study by Refulio-Rodriguez and Olmstead (2014) found moderate support for (Gentianales, ((Solanales + Vahliaceae), (Boraginales + Lamiales))).

Subtending the core lamiids are multiple phylogenetically isolated lineages of uncertain placement, which we informally call the “basal lamiids” because they possibly comprise a grade at the base of the lamiid tree. These include Garryales (Eucommiaceae and Garryaceae), Icacinaceae, and the monogeneric families Metteniusaceae and Oncothecaceae. Relationships among the basal lamiids are poorly known largely because previous studies (e.g., Kårehed, 2001; González et al., 2007; Lens et al., 2008; Soltis et al., 2011; Byng et al., 2014; Refulio-Rodriguez and Olmstead, 2014) have included an insufficient sampling of characters and/or taxa to investigate this phylogenetic problem comprehensively.

The Icacinaceae, with ~34 genera and 200 species (Kårehed, 2001; Byng et al., 2014), constitute the most diverse basal lamiid family. They are of particular importance for understanding basal lamiid phylogeny because the family, as currently recognized (Kårehed, 2001), is likely not monophyletic, with some members considered probably more closely related to other basal lamiid groups (Lens et al., 2008; Byng et al., 2014). The traditional circumscription of the family (referred to henceforth as Icacinaceae s.l.) has included ~54 genera and 400 species, united largely by the presence of superior, unilocular ovaries with two pendant ovules, only one of which matures (Engler, 1893; Howard, 1940; Sleumer, 1942, 1969, 1971; Kårehed, 2001). The family was previously considered a rosoid group and was placed with Celastraceae and Aquifoliaceae (Miers, 1852, 1864), although earlier authors (de Candolle, 1824; Bentham, 1841, 1862) had associated members of Icacinaceae s.l. with Olacaceae of Santalales (a superasterid; Soltis et al., 2011).

The most widely adopted infrafamilial classification of Icacinaceae s.l. was that of Engler (1893) and Sleumer (1942), who divided the family into four tribes—Icacineae (including >30 genera), Iodeae (*Hosiea* Hemsley & E. H. Wilson, *Iodes* Blume, *Mappianthus* Hand.-Mazz., *Natsiatopsis* Kurz, *Natsiatum* Buch.-Ham. ex Arn., and *Polyporandra* Becc.), Sarcostigmateae (*Sarcostigma* Wight & Arn.), and Phytocreneae (*Chlamydocarya* Baill., *Miquelia* Meisn., *Phytocrene* Wall., *Polycephalium* Engl., *Pyrenacantha* Wight, and *Stachyanthus* Engl.)—based largely on wood anatomical characters. However, various lines of morphological evidence—e.g., pollen (Dahl, 1952; Lobreau-Callen, 1972, 1973), leaf epidermal characters (van Staveren and Baas, 1973), nodal anatomy (Bailey and Howard, 1941a), and wood anatomy (Bailey and Howard, 1941b–d)—suggested that the aforementioned tribes, and especially Icacinaceae, might not be monophyletic. Bailey and Howard (1941b) instead organized the family into three informal groups based on vessel characters of the primary and secondary xylem and nodal anatomy (i.e., the presence of uni- vs. trilacunar nodes). Group I included ~13 genera from the Icacinaceae characterized by trilacunar nodes and vessels of both the primary and secondary xylem with scalariform perforations. Group II included ~10 genera

from the Icacinaceae characterized by trilacunar nodes and vessels of the secondary xylem with scalariform-porous perforations. Group III included ~23 genera, representing all four tribes, characterized by unilacunar nodes and vessels of the secondary xylem with simple perforations (Bailey and Howard, 1941a, b).

Multiple phylogenetic studies have shown Icacinaceae s.l. to be highly polyphyletic (Soltis et al., 1999a, 2000; Savolainen et al., 2000b; Kårehed, 2001), with members falling near the base of either the lamiids or the campanulids (=euasterids II). Kårehed (2001) conducted the first family-wide phylogenetic investigation of Icacinaceae, based primarily on *ndhF* sequences—although a sparse sampling of several other loci (*rbcL*, *atpB*, and 18S rDNA) was also included—and ~70 morphological characters across 45 of the ~54 traditional genera. As a result, Kårehed (2001) transferred ~18 genera to the campanulid families Cardiopteridaceae (Aquifoliales), Pennantiaceae (Apiales), and Stemonuraceae (Aquifoliales). The remaining 34 genera (only 16 of which were sampled for molecular characters) were provisionally retained in Icacinaceae by Kårehed (2001), although it was evident that these genera might not constitute a monophyletic group. The 34 genera appeared to comprise four clades—which Kårehed (2001) informally called the *Apodytes*, *Cassinopsis*, *Emmotum*, and *Icacina* groups—but the relationships among these groups and the other basal lamiid lineages were unclear.

More recent studies of Icacinaceae have included greater sampling of morphological/anatomical characters (Lens et al., 2008) or additional genera not included in previous studies (Angulo et al., 2013; Byng et al., 2014). However, these studies were still unable to clarify the circumscription of the family and relationships among basal lamiids. Nevertheless, Byng et al. (2014) confirmed the placement of *Dendrobangia* Rusby among the basal lamiids—as opposed to in Cardiopteridaceae (Aquifoliales), where it had been placed previously (Kårehed, 2001)—and resolved some relationships within the *Icacina* group.

Resolving lamiid relationships, particularly toward the base of the tree, is critical for establishing an improved classification system for this clade. It is also essential for interpreting patterns of character evolution across not only the lamiids but also the whole of the core asterids (Stevens, 2001 onward; Endress and Rapini, 2014), i.e., the clade comprising the lamiids + campanulids (~80 000 species). The core asterids have been referred to informally as euasterids (e.g., APG I, 1998) and formally as the *Gentianidae* (Cantino et al., 2007); we will use this latter name throughout the paper. In many respects, the basal lamiids differ morphologically from the core lamiid clade. For example, the core lamiids are variable in habitat and habit and characterized by showy, sympetalous flowers with epipetalous stamens and distinctly two-carpellate/loculate gynoecea; the fruits are variable but usually contain multiple relatively small seeds (Stevens, 2001 onward; Judd et al., 2008). In contrast, the basal lamiids are strictly woody (trees, shrubs, or lianas) and generally occur in tropical rainforest (Sleumer, 1971; Carpenter and Dickison, 1976; González et al., 2007; Hua and Howard, 2008). The flowers are small, with petal apices often inflexed in bud, varying degrees of perianth connation, and unilocular gynoecea (Sleumer, 1942; Howard, 1942b, d; González and Rudall, 2010). The fruits are large and fleshy, generally drupaceous, usually containing one large seed (Sleumer, 1942; Howard, 1942b, d; González and Rudall, 2010). If the basal lamiids do indeed form a grade leading to the core clade, this topology would parsimoniously suggest that the aforementioned characters are ancestral for

the lamiids. Furthermore, many of these characters are also shared by members of Aquifoliales (Howard, 1942c, d, 1943a–c; Stevens, 2001 onward), which are sister to the rest of the campanulids (Soltis et al., 2011), suggesting that these morphological features might represent ancestral states for *Gentianidae* as a whole (see also Endress and Rapini, 2014).

To resolve basal lamiid phylogeny, we sequenced 50 plastid genomes across the core asterids, focusing on the basal lamiid genera, and combined these with publically available asterid plastome data for comprehensive phylogenetic analyses. The resulting data matrix comprised 112 accessions, including all families and 36 of the 38 currently recognized genera of basal lamiids. On the basis of our results, we present a phylogenetic classification for Icacinae and the basal lamiids, providing formal definitions for 10 clade names following the PhyloCode version 4c (Cantino and de Queiroz, 2010; <http://www.ohio.edu/phylocode/toc.html>). This treatment includes the conversion of six names already recognized under the International Code of Nomenclature for Algae, Fungi, and Plants (ICN; McNeill et al., 2012) (e.g., Icacinae Miers) and four new clade names. We also offer suggestions for the application of these clade names under a rank-based system (namely, the Angiosperm Phylogeny Group). Finally, we discuss the general implications of our results for understanding patterns of character evolution across *Gentianidae*.

MATERIALS AND METHODS

Taxon sampling—We included 112 accessions (=109 species) across *Asteridae* with a sampling emphasis on the basal lamiid lineages (Appendix S1, see Supplemental Data with online version of this article). Plastomes of 50 species were newly sequenced for this study (with the majority being basal lamiids; one campanulid species, *Discophora guianensis* Miers, was also sequenced). Voucher information for the newly sequenced taxa is presented in Table 1. Data for the other species included were obtained from GenBank or the 1KP Project (<http://onekp.com/>).

The basal lamiids comprise 38 genera: *Aucuba*, *Eucommia*, *Garrya* (Garryales), *Metteniusa* (Metteniusaceae), *Oncotheca* (Oncothecaceae), and the 33 genera of Icacinae sensu Kårehed (2001; see table 4). This generic tally for Icacinae accounts for the newly described genus *Sleumeria* Utteridge, Nagam. & Teo (Utteridge et al., 2005), the synonymization of three genera (*Chlamydocarya* Baill. and *Polycephalum* Engl. = *Pyrenacantha* Wight; *Polyporandra* Becc. = *Iodes* Blume; Byng et al., 2014), and the position of *Dendrobangia* in/near the *Apodytes* group (Byng et al., 2014). We sampled 47 basal lamiid species, representing all currently recognized families and 36/38 genera (with *Sleumeria* and *Natsiatopsis* Kurz being the only two missing genera). In general, we sampled only one species per genus, but in the cases of *Iodes* and *Pyrenacantha*, multiple species were sampled to provide an initial assessment of the monophyly of these diverse and widespread taxa.

We sampled 51 representatives from the core lamiids (Refulio-Rodriguez and Olmstead, 2014), including multiple representatives from each of the four recognized orders (Boraginales: 9 spp., Gentianaales: 16 spp., Lamiales: 13 spp., Solanales: 12 spp.), as well as a species from the phylogenetically isolated genus *Vahlia*. For outgroups, we included 10 representatives of the *Campanulidae*, as well as *Cornus* and *Rhododendron*, which represent the successive sister groups (Cornales and Ericales) to the core asterids (Soltis et al., 2011).

DNA isolation and sequencing—We used a modified CTAB protocol (Doyle and Doyle, 1987) to obtain genomic DNA from either herbarium-sampled or silica-dried leaf tissue (Table 1). To build genomic libraries for next-generation sequencing, we followed the procedure of Stull et al. (2013), using insert sizes in the range of 200–400 bp. Following library construction, the samples were divided among three separate sequencing runs. The first two involved no plastid enrichment. One of these included 12 samples (11 for this project) multiplexed on one lane of the Illumina GAIIx (2 × 100 bp; Interdisciplinary Center for Biotechnology Research, University of Florida). The other included 13 samples (8 for this project) multiplexed on one lane of the Illumina MiSeq (2 × 150 bp; Biotechnology Center, University of Wisconsin-Madison). The third sequencing run involved enrichment of the plastid genome using the probe set and method of Stull et al. (2013) before multiplex sequencing on one lane of the Illumina HiSeq (1 × 100 bp; Biotechnology Center, University of Wisconsin-Madison). For this run, 95 samples were multiplexed in total, but only 31 of these were included for this study. Table 1 presents the number of reads obtained from each sample, as well as other pertinent sequencing information. The raw reads generated from these sequencing runs were submitted to the Sequence Read Archive (SRP063611).

Plastome assembly and alignment—After sequencing, the reads were barcode-sorted and trimmed using the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/download.html). The reads were then quality filtered using the FASTX-Toolkit or Sickle (Joshi and Fass, 2011; <https://github.com/najoshi/sickle>). We used two different approaches to assemble the reads for subsequent phylogenetic analyses. In the first, we conducted de novo assemblies using Velvet 1.2 (Zerbino and Birney, 2008), followed by reference-guided assembly of the contigs using Geneious 6.0.4 (www.geneious.com) to obtain complete to nearly complete plastomes. A complete plastid genome of *Aucuba japonica* (M. J. Moore, Oberlin College, unpublished data; individual genes, however, were analyzed and published by Moore et al. [2010]) was used as the initial reference for this approach. The resulting plastomes were then uploaded to DOGMA (<http://dogma.cccb.utexas.edu/>; Wyman et al., 2004) to extract the protein-coding regions for phylogenetic analysis. We also used these extracted regions as references for the second assembly approach, employing the program Assembly by Reduced Complexity (<http://ibest.github.io/ARC/>; Hunter et al., 2015), a hybrid mapping/de novo assembly method for targeted loci. The gene sequences assembled under both approaches were then sorted together by gene to create individual files for alignment and subsequent phylogenetic analyses.

The final data set included 73 protein-coding genes (Appendix S1). We excluded the other six protein-coding plastid regions of the plastome (*petG*, *psbZ*, *rps7*, *rps12*, *ycf1*, and *ycf3*) due to poor assembly from our Illumina data and/or their absence from the 1KP data set.

Multiple sequence alignment was performed on each of the 73 genes individually using MAFFT v.7.220 (Katoh and Standley, 2013), followed by manual inspection in Geneious. Using Geneious, we translated the alignments and made adjustments as needed to ensure that each of the coding regions was in the correct reading frame.

After concatenation of the individual loci, the combined data set had an aligned length of 59 113 bp, with 23.4% missing data across all loci (Appendix S1). The aligned lengths of the individual loci are

TABLE 1. Species sequenced for this study, including voucher and sequencing information. In the “Voucher” column, the herbarium locations for each voucher are noted in parentheses, using the herbarium codes from the Index Herbariorum (<http://sweetgum.nybg.org/ih/>). The samples noted with an asterisk in the “Sequencer: read info” column were plastid-enriched prior to sequencing.

Clade/family	Taxon	Voucher	Tissue source	Sequencer: read info	No. reads obtained
Basal lamiids					
<i>Garryaceae</i>	<i>Garrya flavescens</i>	Heany 2158 (FLAS)	Silica	GAllx: 2 × 100	5 721 128
<i>Icacinaeaceae</i>	<i>Alsodeiopsis poggei</i>	Stone et al., 3237 (MO)	Silica	GAllx: 2 × 100	5 744 996
	<i>Casimirella guaranitica</i>	Zardini 43662 (MO)	Silica	HiSeq: 1 × 100*	690 688
	<i>Cassinopsis madagascariensis</i>	Lowry 5162 (MO)	Herbarium	MiSeq: 2 × 150	2 357 614
	<i>Desmostachys planchoniana</i>	Rabevohitra et al., 4419 (MO)	Silica	GAllx: 2 × 100	7 454 176
	<i>Hosiea japonica</i>	Sugawara s.n. (MO: 4020073)	Herbarium	MiSeq: 2 × 150	1 967 318
	<i>Icacina mannii</i>	Leeuwenberg 2708 (UC)	Herbarium	GAllx: 2 × 100	5 977 218
	<i>lodes cirrhosa</i>	Nihn 11463 (L)	Herbarium	HiSeq: 1 × 100*	2 775 225
	<i>lodes klaineana</i>	Wieringa 6280 (WAG)	Silica	HiSeq: 1 × 100*	2 441 786
	<i>lodes liberica</i>	Jongkind 9410 (WAG)	Herbarium	HiSeq: 1 × 100*	2 705 201
	<i>lodes perrieri</i>	Jongkind 3693 (MO)	Herbarium	MiSeq: 2 × 150	2 075 838
	<i>lodes (Polyporandra) scandens</i>	Takeuchi 9320 (MO)	Herbarium	MiSeq: 2 × 150	1 822 700
	<i>lodes seretii</i>	Wieringa 4427 (WAG)	Herbarium	HiSeq: 1 × 100*	2 321 195
	<i>Lavigeria macrocarpa</i>	Wieringa 5840 (WAG)	Silica	HiSeq: 1 × 100*	1 745 017
	<i>Leretia cordata</i>	Stull 125 (LPB)	Silica	HiSeq: 1 × 100*	2 647 287
	<i>Mappia mexicana</i>	Gonzalez-Medrano 7265 (MEXU)	Herbarium	MiSeq: 2 × 150	2 025 392
	<i>Mappianthus iodoides</i>	McClure 8569 (UC)	Herbarium	GAllx: 2 × 100	4 907 828
	<i>Merrilliodendron megacarpum</i>	Moran 4718 (UC)	Herbarium	GAllx: 2 × 100	6 456 008
	<i>Miquelia caudata</i>	Beaman 7504 (L)	Herbarium	HiSeq: 1 × 100*	3 621 133
	<i>Natsiatum herpeticum</i>	Chand 8312 (L)	Herbarium	HiSeq: 1 × 100*	1 152 495
	<i>Nothapodytes montana</i>	Beusekom 48 (L)	Herbarium	HiSeq: 1 × 100*	935 827
	<i>Phytocrene borneensis</i>	Ambri W885 (L)	Herbarium	HiSeq: 1 × 100*	2 035 139
	<i>Phytocrene racemosa</i>	Hotta 12687 (L)	Herbarium	HiSeq: 1 × 100*	2 287 487
	<i>Pleurisanthes flava</i>	Hoffman 2806 (L)	Herbarium	HiSeq: 1 × 100*	1 349 029
	<i>Pyrenacantha acuminata</i>	Wieringa 5017 (WAG)	Silica	HiSeq: 1 × 100*	2 710 015
	<i>Pyrenacantha gabonica</i>	Breteler 15281 (WAG)	Herbarium	HiSeq: 1 × 100*	1 454 094
	<i>Pyrenacantha (Polycephalium) lobata</i>	Wieringa 4409 (WAG)	Herbarium	HiSeq: 1 × 100*	2 420 522
	<i>Pyrenacantha malvifolia</i>	Wilde 7281 (MO)	Herbarium	HiSeq: 1 × 100*	4 208 917
	<i>Pyrenacantha rakotazafyi</i>	Barthelat 1764 (MO)	Herbarium	MiSeq: 2 × 150	2 052 890
	<i>Pyrenacantha (Chlamydocarya) thomsoniana</i>	Wieringa 6295 (WAG)	Silica	HiSeq: 1 × 100*	7 977 710
	<i>Rhyticaryum macrocarpum</i>	Katik 46856 (L)	Herbarium	HiSeq: 1 × 100*	1 649 132
	<i>Sarcostigma paniculata</i>	Beaman 7056 (L)	Herbarium	HiSeq: 1 × 100*	984 900
	<i>Stachyanthus zenkeri</i>	Cheek 5009 (MO)	Herbarium	HiSeq: 1 × 100*	2 133 225
<i>Metteniusaceae</i>	<i>Apodytes dimidiata</i>	Rabevohitra et al., 4533 (MO)	Silica	GAllx: 2 × 100	8 640 592
	<i>Calatola mollis</i>	Ambrosio 346 (MO)	Herbarium	HiSeq: 1 × 100*	1 113 992
	<i>Emmotum nitens</i>	Thomas et al., 12893 (CICY)	Herbarium	GAllx: 2 × 100	3 981 300
	<i>Metteniusa tessmanniana</i>	Lewis et al., 3447 (MO)	Herbarium	HiSeq: 1 × 100*	1 054 884
	<i>Oecopetalum mexicanum</i>	Lascurain 360 (CICY)	Herbarium	GAllx: 2 × 100	5 324 216
	<i>Ottoschulzia rhodoxylon</i>	Trego s.n. (CICY)	Silica	GAllx: 2 × 100	4 116 382
	<i>Pittosporopsis kerrii</i>	Stull 132 (FLAS)	Silica	MiSeq: 2 × 150	4 903 968
	<i>Platea parviflora</i>	Averyanov et al. VH4429 (MO)	Silica	MiSeq: 2 × 150	1 734 998
	<i>Rhaphiostylis ferruginea</i>	Merello et al., 1645 (MO)	Silica	GAllx: 2 × 100	5 809 890
<i>Oncothecaceae</i>	<i>Oncotheca balansae</i>	Carlquist 15610 (MO)	Herbarium	HiSeq: 1 × 100*	1 495 155
Core lamiids					
<i>Boraginaceae</i>	<i>Borago officinalis</i>	Davis 1516 (FLAS)	Herbarium	HiSeq: 1 × 100*	2 626 335
<i>Boraginaceae</i>	<i>Cordia sebastina</i>	Stull 128 (FLAS)	Silica	HiSeq: 1 × 100*	1 338 956
<i>Hydroleaceae</i>	<i>Hydrolea coymbosa</i>	Brockington 379 (FLAS)	Herbarium	HiSeq: 1 × 100*	2 660 144
<i>Sphenocleaceae</i>	<i>Sphenoclea zeylanica</i>	Ionta 352 (FLAS)	Herbarium	HiSeq: 1 × 100*	1 058 401
<i>Tetrachondraceae</i>	<i>Polypremum procumbens</i>	Davis 406 (FLAS)	Herbarium	HiSeq: 1 × 100*	1 283 557
<i>Vahliaceae</i>	<i>Vahlia capensis</i>	s.n.	Unknown	HiSeq: 1 × 100*	1 254 388
Campanulids					
<i>Stemonuraceae</i>	<i>Discophora guianensis</i>	Kajekai 240 (CICY)	Herbarium	HiSeq: 1 × 100*	9 649 463

listed in online Appendix S2. Thirteen accessions had >50% missing data, and most of these accessions were core lamiids from the 1KP project; only four were basal lamiids. *Nothapodytes montana* and *Lavigeria macrocarpa* had 53% and 80% missing data, respectively, due to poor assembly of the plastome from the Illumina reads; *Dendrobangia* and *Poraqueiba* Aubl. each had 94.7% missing data because we were unable to sequence these taxa for this study

and instead included them based on five plastid loci from GenBank (*matK*, *psbA*, *rbcl*, *rpoB*, and *rpoCl*). Appendix S1 shows the distribution of missing data via a taxon-by-gene table. The data matrix analyzed for this paper is available on TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S18118>) and Dryad (<http://datadryad.org/resource/doi:10.5061/dryad.v7g2k>). The individual gene sequences assembled and analyzed for this study were submitted to GenBank

(see online Appendix S3 for accession numbers). References for the previously generated plastome data used in this study are presented in Appendix S4.

Molecular sampling rationale—We limited our analyses to the protein-coding regions of the plastome for several reasons. It has been demonstrated that numerous, slowly evolving characters represent the best data for resolving ancient rapid radiations because slowly evolving characters are often less prone to homoplasy/signal saturation over long periods of evolutionary time (e.g., Wortley et al., 2005; Jian et al., 2008). However, we note that some such nucleotide positions may be highly constrained by selection and therefore are themselves prone to homoplasy (e.g., Olmstead et al., 1998; P. S. Soltis and D. E. Soltis, 1998; Soltis et al., 1999b). Given that the basal lamiids (and perhaps also core lamiids) seem to be the product of an ancient rapid radiation (Bremer et al., 2004), we reasoned that the protein-coding regions of the plastome—which are slowly evolving, yet collectively comprise a wealth of character data—would provide an excellent source of information for resolving these problematic relationships. Furthermore, numerous studies have already demonstrated the utility of the plastome coding regions for resolving ancient rapid radiations within the angiosperms (e.g., Moore et al., 2010; eudicots; Jian et al., 2008; Saxifragales; Wang et al., 2009; rosids; Xi et al., 2012; Malpighiales). Also, limiting our analyses to the protein-coding regions of the plastome maximized compatibility with already available data sets of plastome coding sequences (e.g., Moore et al., 2010; Ruhfel et al., 2014).

The plastome effectively represents a single gene tree (Doyle, 1992) and therefore might not accurately track the pattern of lamiid species diversification. However, the attributes mentioned above make the plastome well suited for this particular phylogenetic problem, and the results should serve as a robust hypothesis to be examined against future studies employing numerous nuclear loci. Furthermore, we note that plastome-based studies of broad-scale angiosperm phylogeny (e.g., Jansen et al., 2007; Moore et al., 2007; Moore et al., 2010; Ruhfel et al., 2014) have generally been corroborated by studies employing nuclear or mitochondrial data (e.g., Qiu et al., 2010; Soltis et al., 2011; Xi et al., 2014; Wickett et al., 2014), suggesting that the plastome effectively tracks major angiosperm relationships in most cases, with important exceptions (e.g., Sun et al., 2015).

Phylogenetic analysis—We analyzed the data under both maximum likelihood (ML) and Bayesian frameworks using the programs RAxML v 8.1.12 (Stamatakis, 2014) and MrBayes v 3.2.1 (Huelsenbeck and Ronquist, 2001; Ronquist et al., 2012), respectively. Because the plastid genome is uniparentally inherited and does not undergo recombination, its constituent genes should thus track the same evolutionary history (D. E. Soltis and P. S. Soltis, 1998). This means that plastid genes can be safely concatenated for phylogenetic analyses without concern about strongly conflicting phylogenetic signals (Olmstead and Sweere, 1994). We therefore concatenated the 73 plastid genes for all analyses.

The ML and Bayesian analyses included four model partitioning strategies, using the GTR+ Γ model for each partition: (1) no partitioning, (2) partitioning by codon position (three partitions), (3) partitioning by each gene (73 partitions), and (4) partitioning by each codon position within each gene (219 partitions). The RAxML analyses included 1000 bootstrap replicates in addition to a search for the best-scoring ML tree. The Bayesian analyses included 15

million generations with four chains sampling the posterior every 1000 generations. To evaluate the convergence of the analyses and determine the burn-in, we visually inspected the parameter outputs using the program Tracer v 1.5 (Rambaut and Drummond, 2009). We removed the burn-in (usually around 10%) before sampling the trees from the posterior distribution.

RESULTS

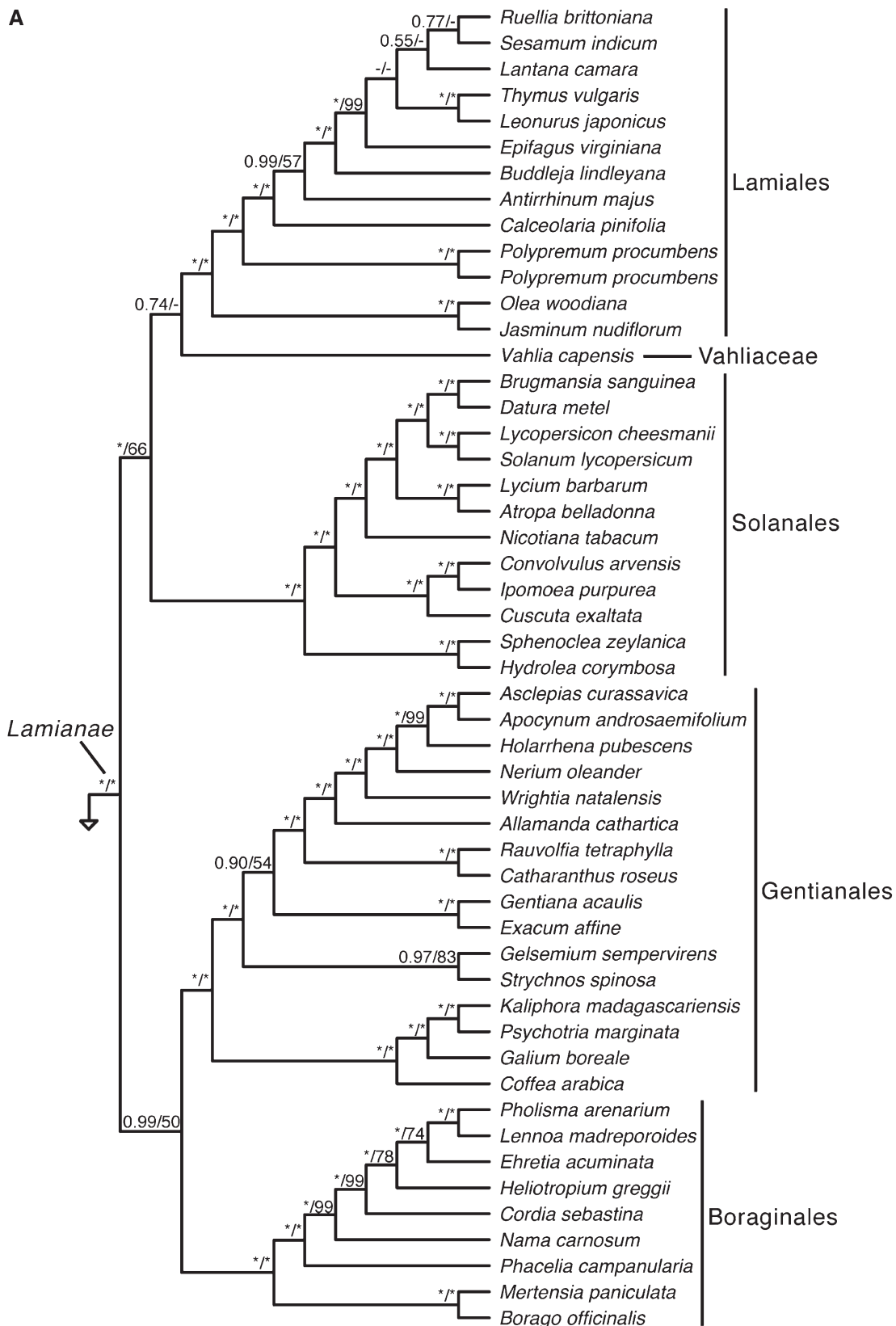
Of the 59 113 DNA characters analyzed, 31 051 were constant and 28 062 were variable. We recovered nearly identical relationships across all analyses (both ML and Bayesian), with differences in topology restricted to areas of poor support—there were no strongly supported conflicting relationships among the major lineages. Of the four partitioning schemes in the ML analyses, the gene-codon scheme (219 partitions) had the highest likelihood score ($-\ln L = 598\,491.98$) and is depicted in Figs. 1 and 2 and discussed in the text. The $-\ln L$ scores of the other ML analyses were 613 007.34 (no partition), 609 229.67 (codon partition), and 607 903.56 (gene partition). The best $-\ln L$ score of the gene-codon partitioned Bayesian analysis was 596 805.40; the other partitioning schemes did not reach stationarity. The posterior probabilities of the gene-codon partitioned Bayesian analysis are mapped onto the best ML tree in Figs. 1 and 2 and discussed in the text. All trees resulting from these analyses (except those already shown in the text) are available in the online supplemental files (online Appendices S5–S11).

The overall topology recovered is as follows (Figs. 1 and 2). *Lamiidae* are strongly supported as monophyletic. The core lamiids form a well-supported clade (BS 100/PP 1.0), but relationships among the major core lineages (i.e., Boraginales, Gentianales, Lamiales, Solanales, and *Vahlia*) are more poorly supported given their modest/low ML bootstrap values. However, the Bayesian posterior probability values are generally strong. Boraginales were recovered as sister to Gentianales (BS 50/PP 0.99), with these together being sister to a clade of Lamiales, Solanales, and *Vahlia* (BS 66/PP 1.0). *Vahlia* was recovered as sister to Lamiales but with very weak support (BS <50/PP 0.74).

The 33 genera comprising Icacinaceae sensu Kårehed (noted with asterisks in Fig. 1) form two distinct clades: one includes 21 genera and corresponds to the *Icacina* and *Cassinopsis* groups of Kårehed (2001); the other clade comprises 11 genera, including 10 icacinaceous genera, primarily from the *Apodytes* and *Emmotum* groups of Kårehed (2001), as well as *Metteniusa* embedded well within the clade, sister to *Ottoschulzia* Urb. The first Icacinaceae clade (including 21 genera) and Oncothecaceae form a well-supported clade (BS 100/PP 1.0) sister to the rest of the lamiids. The second clade of Icacinaceae (including *Metteniusa*) is placed with strong support (BS 90/PP 1.0) as sister to the remaining lamiids, with Garryales highly supported as sister to the core lamiids (BS 98/PP 1.0).

Within the first Icacinaceae clade, *Cassinopsis* Sond. is sister to a clade comprising most of the genera of the *Icacina* group (BS 78/PP 0.99). The *Icacina* group, in turn, includes four well-supported clades (Fig. 3), which are discussed in more detail below; in general, relationships are well resolved and well supported across the entire clade. Within the second major clade of Icacinaceae sensu Kårehed, *Platea* Blume + *Calatola* Standl. are strongly supported (BS 100/PP 1.0) as sister to a clade of genera from the *Emmotum* and *Apodytes*

A



(Continued)

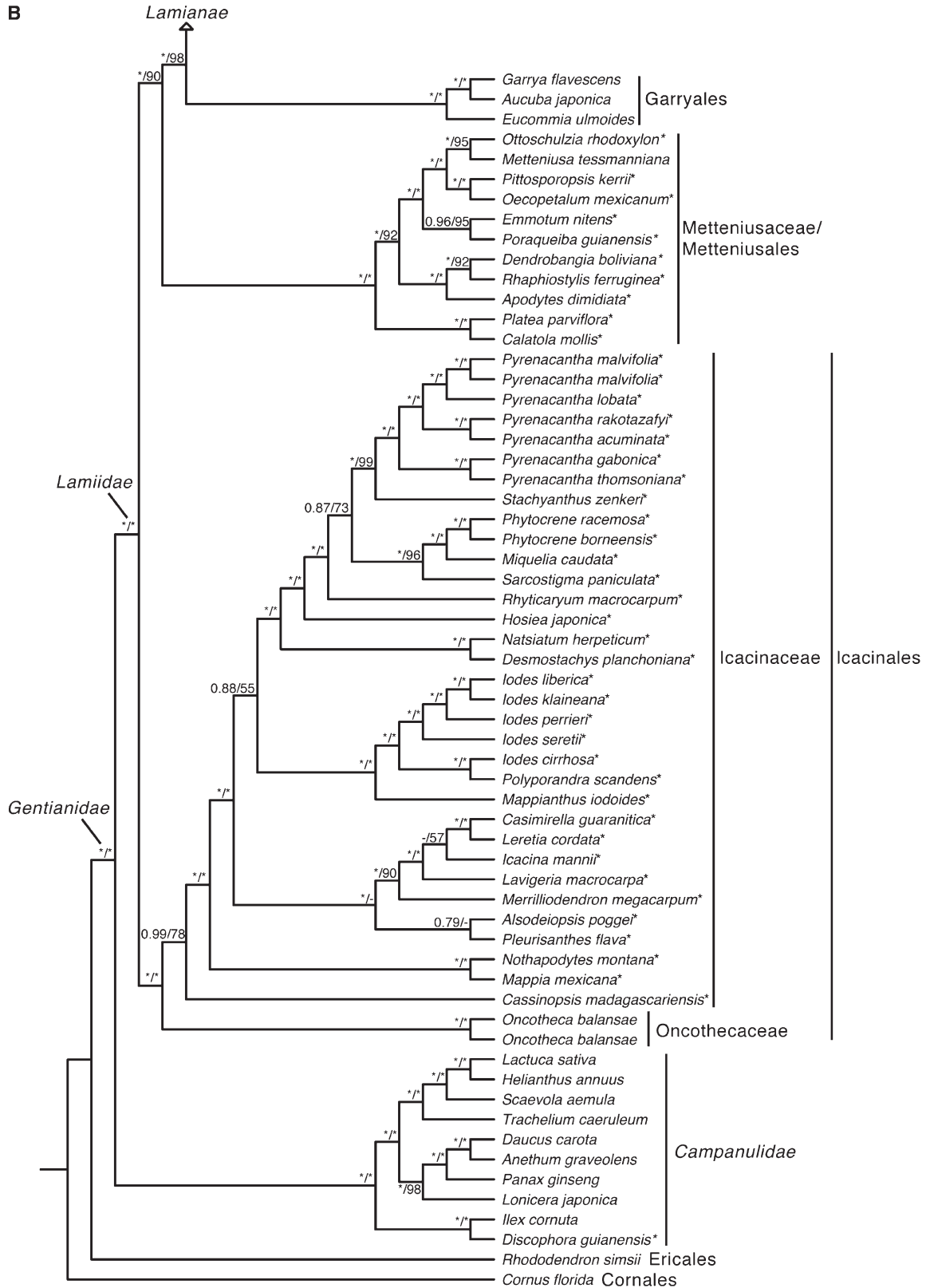


FIGURE 1 The best tree obtained from the gene-codon partitioned maximum likelihood (ML) analysis of 73 plastid genes. Accessions denoted with an asterisk are members of Icacinaceae sensu Kårehed (2001). Numbers above the branches are Bayesian posterior probability/ML bootstrap values from the gene-codon partitioned analyses. An asterisk indicates a posterior probability of 1.0 or ML bootstrap value of 100%. A dash indicates that a given branch was either (1) not obtained in the Bayesian analysis or (2) received <0.50 Bayesian or 50% ML support.

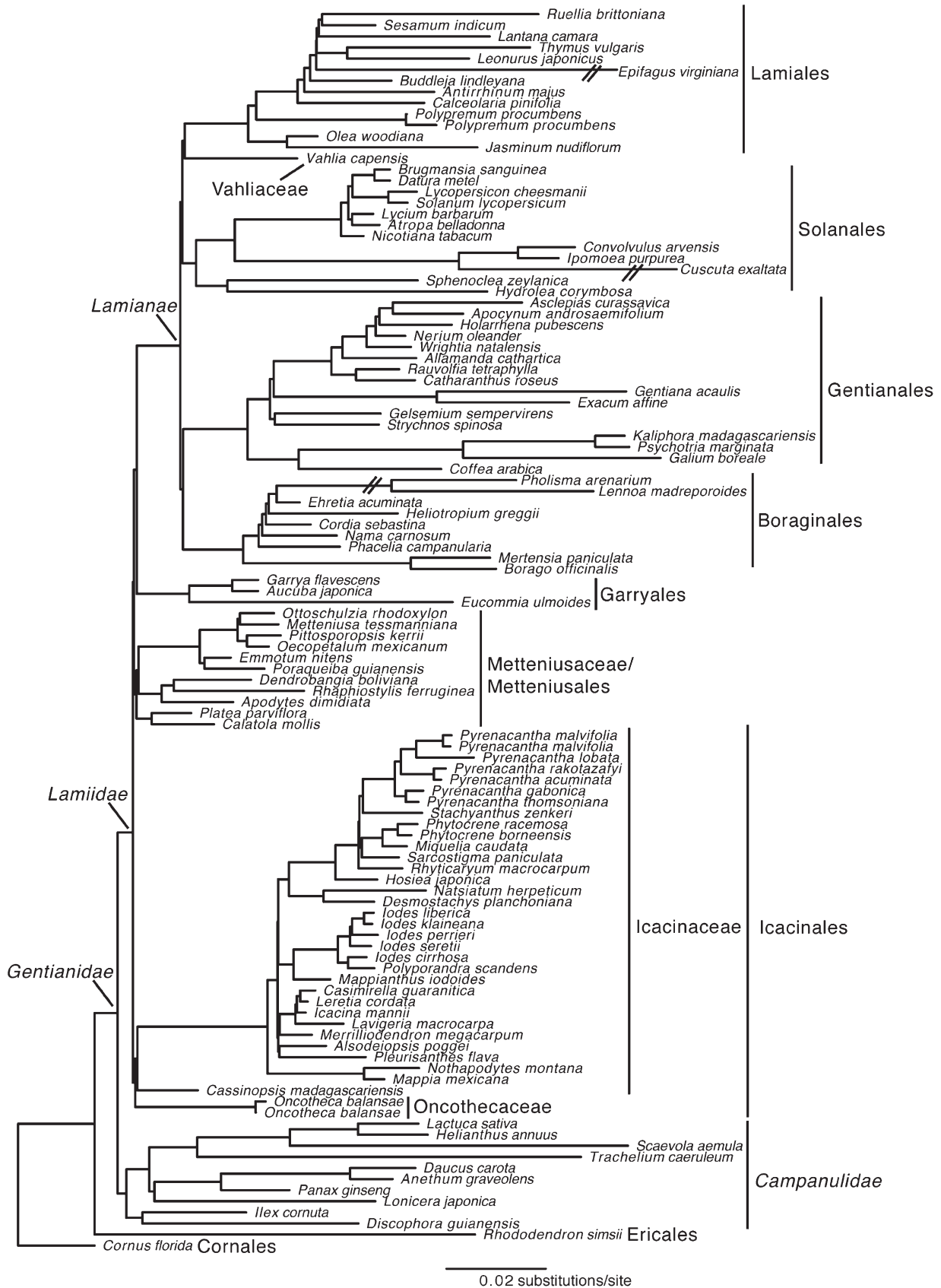


FIGURE 2 The best tree, with branch lengths, obtained from the gene-codon partitioned maximum likelihood analysis of 73 plastid genes. Note the short internal branch lengths along the lamiid backbone and within the core lamiid clade. Support for these relationships is shown in Fig. 1.

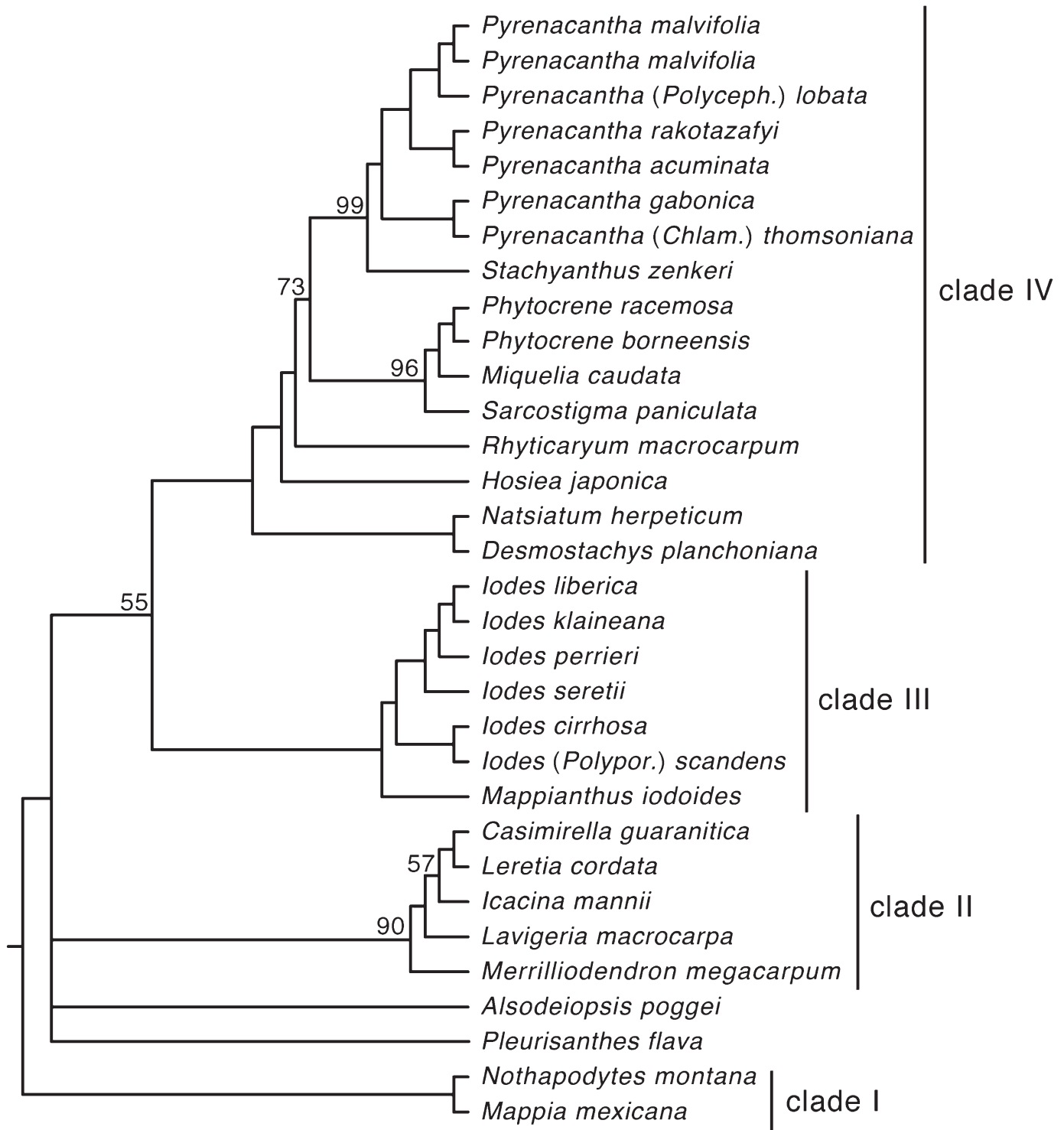


FIGURE 3 Summary of *Icacinoideae* relationships obtained from the maximum likelihood analyses. Branches receiving <50% bootstrap support are collapsed. All other branches are fully supported except where noted (bootstrap values are from the gene-codon partitioned analysis). Four major well-supported clades are indicated on the tree. The morphology of these clades is discussed in the text.

groups. However, *Pittosporopsis* Craib (which, until now, had never been included in a molecular phylogenetic analysis) and *Metteniusa* (Metteniusaceae) are nested in the *Emmotum* group, and *Dendrobangia* is nested in the *Apodytes* group, in all cases with strong support (Fig. 1).

DISCUSSION

Major lamiid clades—Our results show with strong support that Garryales are the immediate sister of the core lamiids (Fig. 1). Several studies have suggested this relationship, albeit with bootstrap

support <50% (Bremer et al., 2002; Refulio-Rodríguez and Olmstead, 2014), while other studies have recovered other basal lamiid groups as sister to the core lamiids—e.g., Oncothecaceae (Soltis et al., 2011) or Metteniusaceae (González et al., 2007)—with Garryales sister to all other lamiids. We apply the name *Garryidae* R. G. Olmstead, W. S. Judd, and P. D. Cantino [G. W. Stull, D. E. Soltis, and P. S. Soltis] to this clade (i.e., Garryales + *Lamianae*), which represents a slight modification to its former circumscription (see Phylogenetic Classification section for definitions of clade names mentioned throughout the Discussion). Originally, the name *Garryidae* was used for the lamiids/euasterids I as a whole, with the name *Lamiidae* comprising a more exclusive clade within *Garryidae* (Cantino et al., 2007). However, Refulio-Rodríguez and Olmstead (2014) apply the name *Lamiidae* to the entire lamiid/euasterid I clade (including Garryales and all other basal lamiid lineages), following common usage of the name and priority, and this practice will be followed in the upcoming Companion Volume to the PhyloCode (R. G. Olmstead, University of Washington, personal communication). Thus, the name *Garryidae* is available for this more exclusive clade of lamiids.

Our results also show with strong support that *Metteniusaceae* (as here circumscribed/defined; see below) are sister to *Garryidae* (as here defined). This major clade had not been recovered in previous studies (González et al., 2007; Byng et al., 2014; Refulio-Rodríguez and Olmstead, 2014), which placed *Metteniusa* and the other basal lamiids in various different configurations in relation to the core lamiids, generally with poor support. We establish a new name under the PhyloCode for this major clade: *Metteniusidae* G. W. Stull, D. E. Soltis, and P. S. Soltis. Finally, *Icacinaeae* (as here circumscribed; see below) and Oncothecaceae form a clade sister to the rest of the lamiids (or *Metteniusidae*). We adopt the name *Icacinales* Tiegh. ex Reveal [G. W. Stull] specifically for this clade—i.e., *Icacinaeae* (as here defined) + Oncothecaceae. The relationships and morphology of *Icacinaeae* and Oncothecaceae are discussed in more detail below.

Core lamiid relationships—Although numerous studies have investigated lamiid phylogeny, considerable uncertainty still surrounds relationships among the core lamiid lineages (i.e., Boraginales, Gentianales, Lamiales, Solanales, and *Vahlia*). This represents one of the largest gaps in our current understanding of broad-scale angiosperm phylogeny (Stevens, 2001 onward; Soltis et al., 2011). Practically every previous major study of lamiid or angiosperm phylogeny has uncovered unique relationships among these groups, but never with strong support (e.g., Olmstead et al., 1992, 1993, 2000; Chase et al., 1993; Soltis et al., 1999a; Savolainen, 2000a, b; Soltis et al., 2000, 2011; Albach et al., 2001; Bremer et al., 2002; Moore et al., 2010; Qiu et al., 2010; Ruhfel et al., 2014). The most comprehensive study of lamiid phylogeny to date (Refulio-Rodríguez and Olmstead, 2014) recovered (Gentianales, ((Solanales + *Vahlia*)), (Boraginales + Lamiales))). Although Bayesian support for this tree was relatively strong, the ML bootstrap values, which are generally considered a more conservative measure of support (Suzuki et al., 2002; Erixon et al., 2003), were only moderate (i.e., in the range of 50–70%). Our results differ considerably from those of Refulio-Rodríguez and Olmstead (2014), as well as from other previous studies of lamiid phylogeny. We found two major clades—((Boraginales + Gentianales), (Lamiales + Solanales + *Vahlia*))—representing another unique topology compared to previous studies of lamiid phylogeny. However, ML support for the

core lamiid relationships presented here is not strong, despite the large number of characters included in the analyses, indicating that there is still uncertainty surrounding some core lamiid relationships. Although the Bayesian support for these relationships is generally strong (Fig. 1), posterior probability values are often inflated compared to ML bootstrap values (e.g., Suzuki et al., 2002; Cummings et al., 2003; Erixon et al., 2003), as noted already.

Difficulty in resolving core lamiid relationships might be due to an ancient rapid radiation (Bremer et al., 2004), reflecting the short internal branch lengths connecting the core lamiid lineages in Fig. 2. Such radiations pose numerous problems for phylogeny reconstruction—e.g., very few unambiguous characters supporting the “true” relationships, homoplasy, and saturation of sites (e.g., Whitfield and Lockhart, 2007). Hopefully, accumulation of additional molecular characters, especially from the nuclear genome (and, less likely, the more slowly evolving mitochondrial genome), will facilitate resolution of core lamiid phylogeny, but we have nearly exhausted the use of the plastid genome.

Circumscription and relationships of *Icacinaeae*—Our results provide a greatly improved understanding of the circumscription and relationships of *Icacinaeae*. The *Apodytes* and *Emmotum* groups, and several other genera of *Icacinaeae* s.l., are more closely related to *Metteniusa* and therefore should be excluded from *Icacinaeae* (Figs. 1, 2). The remaining 21 genera of *Icacinaeae* s.l. included in our analyses (which comprise ~160 spp.) form a well-supported clade, which we formally name under the PhyloCode using the converted clade name *Icacinaeae* Miers [G. W. Stull]. This clade should be treated as a family under the APG system (as shown in Fig. 1 and Table 2).

Icacinaeae includes *Cassinopsis* as well as most of the genera of the *Icacina* group. Here, we adopt the subfamily name *Icacinoideae* Engl. [G. W. Stull] for the clade that essentially corresponds to the *Icacina* group (Fig. 3); we use this clade name henceforth. Although there are no clear morphological synapomorphies of *Icacinaeae*, all members of *Icacinoideae* possess unilacunar nodes and simple perforation plates (Bailey and Howard, 1941a, b). Furthermore, members of *Icacinoideae* show a strong tendency to produce alternate (rather than opposite or scalariform) intervessel pits, short vessel elements and fibers, and vasicentric and/or banded axial parenchyma (Lens et al., 2008).

Previous studies have offered insights into relationships among members of *Icacinoideae* (Kårehed, 2001; Lens et al., 2008; Angulo et al., 2013; Byng et al., 2014)—for example, the sister relationship of *Mappia* Jacq. + *Nothapodytes* Blume, the sister relationship of *Miquelia* + *Phytocrene*, the nested position of *Polyporandra* within *Iodes*, and the nested positions of *Polycephalium* and *Chlamydocarya* within *Pyrenacantha*. However, the major clades within *Icacinoideae* and the positions of numerous genera (e.g., *Alsodeiopsis* Oliv., *Desmostachys* Planch., *Hosiea*, *Merrilliodendron* Kanehira, *Mappianthus*, *Natsiatum*, *Natsiatopsis*, *Pleurisanthes* Baill., *Rhyticaryum* Becc., *Stachyanthus*) have been unclear. Our results indicate that *Icacinoideae* consists of four major clades (Fig. 3). The following discussion highlights the general geographic distributions and a few distinct morphological features of these clades; however, formal reconstructions of numerous morphological characters will be necessary to determine unambiguous synapomorphies of each.

The first clade, comprising the Neotropical genus *Mappia* (4 spp.) and the Asiatic genus *Nothapodytes* (~8 spp.), is sister to the

TABLE 2. Rank-based classification of Icacinaceae s.l. All genera listed were formerly included in Icacinaceae s.l. (Engler, 1893; Sleumer, 1942), although *Cardiopteris* and *Metteniusa* have typically been excluded from the family (Howard, 1940; Bailey and Howard, 1941a). Of the ~54 genera traditionally included in Icacinaceae s.l., only 23 are retained in the circumscription recognized here. Note that *Polyporandra* Becc. was recently subsumed in *Iodes* Blume and that *Chlamydocarya* Baill. and *Polycephalum* Engl. were recently subsumed in *Pyrenacantha* Wight (Byng et al., 2014). Furthermore, *Sleumeria* Utteridge, Nagam. & Teo, included here, was only recently described (Utteridge et al., 2005). A broader Metteniusaceae (which formerly included only *Metteniusa* H. Karst.) is here recognized, including 10 generic segregates of Icacinaceae s.l. Kårehed (2001) broadened the circumscription of Cardiopteridaceae (which formerly included only *Cardiopteris* Wall. ex Royle) to include four generic segregates of Icacinaceae s.l. He additionally established the family Stemonuraceae for 12 generic segregates of Icacinaceae s.l. and recognized Pennantiaceae J. Agardh to accommodate the phylogenetically isolated genus *Pennantia*.

Family	Constituent genera	Order/major clade
Icacinaceae Miers (23 genera/160 spp.)	<i>Alsodeiopsis</i> , <i>Casimirella</i> , <i>Cassinopsis</i> , <i>Desmostachys</i> , <i>Hosiea</i> , <i>Icacina</i> , <i>Iodes</i> , <i>Lavigeria</i> , <i>Leretia</i> , <i>Mappia</i> , <i>Mappianthus</i> , <i>Merrilliodendron</i> , <i>Miquelia</i> , <i>Natsiatopsis</i> , <i>Natsiatum</i> , <i>Nothapodytes</i> , <i>Phytocrene</i> , <i>Pleurisanthes</i> , <i>Pyrenacantha</i> , <i>Rhyticaryum</i> , <i>Sarcostigma</i> , <i>Sleumeria</i> , <i>Stachyanthus</i>	Icaginales/Lamiidae
Metteniusaceae H. Karst. (11/59)	<i>Apodytes</i> , <i>Calatola</i> , <i>Dendrobangia</i> , <i>Emmotum</i> , <i>Metteniusa</i> , <i>Oecopetalum</i> , <i>Ottoschulzia</i> , <i>Pittosporopsis</i> , <i>Platea</i> , <i>Poraqueiba</i> , <i>Rhaphiostylis</i>	Metteniusales/Lamiidae
Cardiopteridaceae Blume (5/43)	<i>Cardiopteris</i> , <i>Citronella</i> , <i>Gonocaryum</i> , <i>Leptaulus</i> , <i>Pseudobotrys</i>	Aquifoliales/Campanulidae
Stemonuraceae Kårehed (12/95)	<i>Cantleya</i> , <i>Codiocarpus</i> , <i>Discophora</i> , <i>Gastrolepis</i> , <i>Gomphandra</i> , <i>Grisollea</i> , <i>Hartleya</i> , <i>Irvingbaileya</i> , <i>Lasianthera</i> , <i>Medusanthera</i> , <i>Stemonurus</i> , <i>Whitmorea</i>	Aquifoliales/Campanulidae
Pennantiaceae J. Agardh (1/4)	<i>Pennantia</i>	Apiales/Campanulidae

rest of *Icacinoideae* and is characterized by an erect habit (trees or shrubs) with elongate, symmetrical styles and a fleshy foliaceous disk at the base of the ovary (Howard, 1942a). The second clade is pantropical and consists of five genera (~15 spp.) of the traditional tribe Icacineae (*Casimirella* Hassl., *Icacina*, *Merrilliodendron*, *Lavigeria* Pierre, and *Leretia* Vell.). This clade consists of climbers with colporate pollen with foveolate (i.e., finely pitted) to reticulate ornamentation (Lobreau-Callen, 1972, 1973), determinate inflorescences (Howard, 1942a, 1942c, 1992), and bisexual flowers, except for *Merrilliodendron*, which is a monotypic genus of trees with echinate colporate pollen. The third clade comprises the Paleotropical genus *Iodes* (~16 spp.) and the small Asiatic genus *Mappianthus* (2 spp.). Consistent with Byng et al. (2014), we found the monotypic genus *Polyporandra* to be nested within *Iodes*, and our tree reflects their new combination: *Iodes scandens* (Becc.) Utteridge & Byng (basonym: *Polyporandra scandens* Becc.). This clade comprises climbers with opposite leaves, extra-axillary tendrils, cymose inflorescences, and unisexual flowers (plants dioecious) (Sleumer, 1971; Hua and Howard, 2008).

The fourth clade is the largest (with nine genera included in our analyses, but probably 11 total with *Sleumeria* and *Natsiatopsis*, and ~75 species) and most morphologically heterogeneous, including genera from all four of the traditional tribes. This clade is also paleotropical and appears to be characterized by indeterminate inflorescences (Hutchinson and Dalziel, 1958; Lucas, 1968; Sleumer, 1971; Villiers, 1973), with the exception of *Hosiea*, which has cymes (Hua and Howard, 2008). It also consists almost entirely of climbers, with the exception of *Rhyticaryum* (trees and shrubs) and *Desmostachys* (trees and shrubs, occasionally scandent) (Hutchinson and Dalziel, 1958; Sleumer, 1971). Within this fourth clade, the genera of the traditional Phytocreneae form a subclade (including *Sarcostigma*) characterized by pitted endocarps (Sleumer, 1971; Villiers, 1973; Stull et al., 2012) and generally furrowed xylem (Lens et al., 2008). Consistent with Byng et al. (2014), we found *Chlamydocarya* and *Polycephalum* to be nested within *Pyrenacantha*. The trees we present reflect their new combinations: *Pyrenacantha thomsoniana* (Baill.) Byng & Utteridge (basonym: *Chlamydocarya thomsoniana* Baill.) and *Pyrenacantha*

lobata (Pierre) Byng & Utteridge (basonym: *Polycephalum lobatum* (Pierre) Pierre ex Engl.).

Our analyses consistently found a sister relationship between the two major Paleotropical clades (clades III and IV, Fig. 3). Although this relationship was not strongly supported based on our molecular data, most members of these clades have porate pollen with echinate ornamentation (Lobreau-Callen, 1973). These taxa also generally have unisexual flowers (plants dioecious), with the exception of *Desmostachys* and *Hosiea* (Hutchinson and Dalziel, 1958; Hua and Howard, 2008). Furthermore, they tend to have highly specialized wood anatomy associated with their predominantly climbing habit (Bailey and Howard, 1941b–d; Lens et al., 2008).

Our analyses failed to place *Alsodeiopsis* and *Pleurisanthes* among the four major clades of *Icacinoideae* with strong support (Fig. 3). They both exhibit unique combinations of morphological characters, making it difficult to predict their phylogenetic positions. *Alsodeiopsis* has bisexual flowers and determinate inflorescences, like clades I and II, but is distinct in having tetracolporate pollen (Dahl, 1952). *Pleurisanthes* has bisexual flowers and indeterminate inflorescences (Howard, 1942c) like *Desmostachys*, but is similar to clade II in having foveolate/reticulate colporate pollen (Dahl, 1952; Lobreau-Callen, 1972, 1973). It is possible that these taxa occupy isolated phylogenetic positions. *Pleurisanthes* was placed in its own family by van Tieghem (1897), highlighting its morphological distinctness from other members of Icacinaceae. Additional molecular data will be necessary to resolve the placements of these genera.

Of the traditional tribes of Engler (1893) and Sleumer (1942), none is strictly monophyletic. Icacineae are massively polyphyletic, with 12 genera retained within *Icacinaceae* and the ~27 others now in different groups: Cardiopteridaceae, Metteniusaceae (as here defined), Pennantiaceae, and Stemonuraceae). Iodeae, Phytocreneae, and Sarcostigmatae all fall within *Icacinaceae*. However, members of Iodeae—i.e., *Hosiea*, *Iodes* (incl. *Polyporandra*), *Mappianthus*, *Natsiatopsis*, *Natsiatum*—form two distinct clades, and the monogeneric tribe Sarcostigmatae is nested within Phytocreneae. Although two of Bailey and Howard's (1941b) groups are polyphyletic, their third group (characterized by simple perforation plates) is monophyletic, corresponding more or less to *Icacinoideae*.

As noted, two genera of Icacinaceae s.l., *Natsiatopsis* and *Sleumeria*, were not included in our analyses. However, Byng et al. (2014) found *Sleumeria* to be nested within the *Icacina* group, providing support for its inclusion in our *Icacinoideae*. Furthermore, Utteridge et al. (2005) highlighted the morphological similarities of *Sleumeria* to multiple Malesian genera of *Icacinaceae*, particularly *Phytocrene* and *Sarcostigma*, with which it shares successive cambia, for example. *Natsiatopsis* has yet to be included in a phylogenetic study of Icacinaceae, but it is morphologically very similar to *Natsiatum*, which is nested well within this clade. Both *Natsiatopsis* and *Natsiatum* are scandent shrubs/climbers, with long petioles, densely pubescent cordate leaves with toothed margins, and unisexual flowers (dioecious) in racemes (Hua and Howard, 2008). *Natsiatopsis* also has unilacunar nodes (G. W. Stull, personal observation) like all other members of *Icacinoideae*. Therefore, we are confident that *Natsiatopsis* belongs to this clade.

Phylogenetic position of Oncothecaceae—*Oncotheca*, long considered phylogenetically isolated (Carpenter and Dickison, 1976; Cameron, 2003), includes two species endemic to New Caledonia (Baillon, 1891; McPherson et al., 1981). The genus has generally been recognized as the sole constituent of its own family (Airy Shaw, 1965). Based on morphology, *Oncotheca* has variously been associated with Aquifoliaceae (Baillon, 1891, 1892), Ebenaceae (Guillaumin, 1938, 1948), and Theaceae (Takhtajan, 1969, 1997; Cronquist, 1981). Although molecular analyses have shown that *Oncotheca* occupies a basal branch of lamiids (e.g., Soltis et al., 1999a, 2000, 2011; Savolainen et al., 2000a; Bremer et al., 2002; Refulio-Rodriguez and Olmstead, 2014), its precise placement has been ambiguous. Multiple studies (Bremer et al., 2002; Lens et al., 2008; Byng et al., 2014) have recovered *Oncotheca* sister to *Apodytes* or the *Apodytes* group. Soltis et al. (2011) found *Oncotheca* sister to the core lamiids, while Refulio-Rodriguez and Olmstead (2014) found a sister relationship between *Metteniusa* and *Oncotheca*. However, in all previous cases, the position of *Oncotheca* was weakly supported.

Our results provide strong support for a sister relationship between Oncothecaceae and *Icacinaceae* as here circumscribed, which is a novel finding. Other studies perhaps failed to recover this relationship due to insufficient sampling of characters (e.g., Byng et al., 2014; Lens et al., 2008), taxa (e.g., Soltis et al., 2011; Refulio-Rodriguez and Olmstead, 2014), or both. Morphological features uniting Oncothecaceae and *Icacinaceae* are unclear, and in several respects, Oncothecaceae are more morphologically similar to *Metteniusa*, presumably due to either convergence or the retention of plesiomorphies. For example, both have pentalacunar nodes, epipetalous stamens, and five-carpellate fruits (Carpenter and Dickison, 1976; González et al., 2007; González and Rudall, 2010). However, as noted, the basal lamiids seem to be the product of an ancient rapid radiation, with very long branches leading to the crown clades. Given the phylogenetic isolation of *Icacinaceae* and Oncothecaceae, it is therefore not surprising that they are morphologically distinct, even if they do represent sister taxa.

Circumscription and relationships of Metteniusaceae—The systematic position of *Metteniusa* has been ambiguous since its initial description (Karsten, 1860). It has been treated as an unusual member of Icacinaceae (e.g., Sleumer, 1942; Cronquist, 1981; Thorne, 2000), the sole constituent of its own family (Karsten, 1860), a member of Olacaceae (Sleumer, 1934), a member of Opiliaceae

(Sleumer, 1936), or a member/near relative of Alangiaceae (Watson and Dallwitz, 1992 onward). Takhtajan (1997) placed *Metteniusa* in its own family and order (Metteniusaceae/Metteniusales), which he included in the superorder Celastranae (Rosidae) along with Icacinaceae and five other orders. In the APG III (2009) system, *Metteniusa* is recognized as the sole member of Metteniusaceae, within lamiids but unplaced to order.

Metteniusa was recently shown to be an early-diverging lamiid (González et al., 2007), although sampling of Icacinaceae s.l. was limited, and so the precise placement of *Metteniusa*/Metteniusaceae remained ambiguous. However, a relationship with *Oncotheca* has been suggested (González et al., 2007; González and Rudall, 2010), given that both genera share pentalacunar nodes, epipetalous stamens, and five-carpellate gynoecia (with this latter character somewhat obscured in *Metteniusa* due to its pseudomonormery; González and Rudall, 2010; Endress and Rapini, 2014). More recently, Refulio-Rodriguez and Olmstead (2014) also recovered *Metteniusa* sister to *Oncotheca*, while another recent study (Byng et al., 2014), with a greater sampling of Icacinaceae s.l., found *Metteniusa* nested within the *Emmotum* group, with *Oncotheca* sister to the *Apodytes* group. However, neither of these positions of *Metteniusa* was well supported. *Metteniusa* was not included in the phylogenetic study of Lens et al. (2008), and although it was included in Kårehed's (2001) study, which placed it within Cardiopoteridaceae (Aquifoliales), this placement was based on morphology alone.

Our results provide strong support that *Metteniusa* is nested within the *Emmotum* group of Kårehed (2001), sister to *Ottoschulzia*. We also recovered the Asiatic genus *Pittosporopsis* in this group. Our study is the first to include *Pittosporopsis* in a molecular phylogenetic analysis; Kårehed (2001) tentatively placed it within the *Icacina* group based on morphological analyses. A morphological feature potentially supporting the relationship of both *Metteniusa* and *Pittosporopsis* within the *Emmotum* group is the presence of anther connectives protruding beyond the anther sacs (Howard, 1942b, c; Hua and Howard, 2008; González and Rudall, 2010; Duno de Stefano et al., 2007). These genera also tend to have fleshy petals (Kårehed, 2001) and fruits with persistent styles (Hua and Howard, 2008; González and Rudall, 2010), but additional work will be necessary to document unequivocal synapomorphies for this clade.

We also found strong support for the sister relationship of the *Emmotum* and *Apodytes* groups (with the latter including *Dendrobangia*). Whereas Kårehed (2001) tentatively placed *Calatola* and *Platea* in the *Emmotum* group, we recovered these together as sister to the *Emmotum* + *Apodytes* groups. *Calatola* and *Platea* are both dioecious trees with indeterminate inflorescences (Howard, 1942c; Hua and Howard, 2008). Morphological synapomorphies for this larger clade of *Metteniusa* plus 10 genera from Icacinaceae s.l. are unclear. These genera possess a similar pollen type (i.e., colpate grains with foveolate to reticulate ornamentation), but this shared feature might represent a symplesiomorphy given that other basal lamiids and campanulids show similar pollen types (Lobreau-Callen, 1972, 1973). Nevertheless, molecular support for this group is maximal. Because this clade is phylogenetically isolated from *Icacinaceae* (as circumscribed here), under a rank-based system it must be recognized as not only a separate family but also a separate order. Here, we formally establish *Metteniusaceae* H. Karst. ex Schnizl. [G. W. Stull] as the converted name for this clade, comprising *Metteniusa* plus 10 genera from Icacinaceae s.l.

Under the APG system, this clade should be treated as a family and the sole constituent of the order Metteniusales. We also establish new names for the three major clades within *Metteniusaceae*: *Apodytoideae* G. W. Stull, *Metteniusoideae* G. W. Stull, and *Plateoideae* G. W. Stull.

Asterid character evolution—Our understanding of lamiid and euasterid (= *Gentianidae*) character evolution has been obscured both by poor resolution of basal lamiid relationships and a limited understanding of morphology (especially floral morphology) across *Icacinaeae* s.l. (Endress and Rapini, 2014). The relationships recovered here provide a solid framework for future investigations of character evolution across both the *Lamiidae* and *Gentianidae* as a whole. Given that members of *Icacinaeae* s.l. are scattered along the basal branches of both the lamiids and campanulids, many of their morphological features possibly represent ancestral conditions for the *Gentianidae* (whether symplesiomorphies or synapomorphies of this clade). For example, *Icacinaeae* s.l. are evergreen woody plants, mostly trees (although most *Icacinaeae* as here circumscribed are climbers); their flowers are small, with fused sepals and free or basally connate petals, which often possess an adaxial ridge and apices inflexed in bud; the stamens are usually alternate with the petals and equal in number; each carpel contains two apical, pendant ovules; the fruits are drupes with a single seed (Howard, 1940, 1942a–d, 1943a–c; Sleumer, 1971; Stevens, 2001 onward; González and Rudall, 2010; Endress and Rapini, 2014). Recent studies have revealed pentamerous gynoecea in *Metteniusa* (with one fertile locule; Gonzalez and Rudall, 2010) and *Emmotum* (with three fertile carpels; Endress and Rapini, 2014). It has long been assumed that other *Icacinaeae* s.l. also have pseudomonomerous gynoecea composed

of two or three carpels (e.g., Engler, 1893). *Oncotheca*, however, is distinctly five-carpellate (Dickison, 1986).

A more thorough investigation of morphological features across basal lamiids (*Icacinaeae*, *Oncothecaceae*, *Metteniusaceae*, *Garryales*) and basal campanulids (*Aquifoliales*) would provide a much-improved understanding of asterid morphological evolution. Furthermore, detailed developmental and morphological studies might also reveal synapomorphies for the newly resolved lamiid clades (e.g., *Icacinales* and *Metteniusaceae*). Nevertheless, it seems possible based on our phylogenetic results that the core lamiids, and their characteristic morphological and ecological diversity, radiated from an ancestry of tropical trees with inconspicuous flowers and large, drupaceous (often single-seeded) fruits.

PHYLOGENETIC CLASSIFICATION

Our results provide a well-resolved and strongly supported hypothesis of basal lamiid relationships. This improved phylogenetic framework offers an excellent opportunity to revise the classification of basal lamiids. Here we present phylogenetic definitions following the PhyloCode version 4c (Cantino and de Queiroz, 2010; <http://www.ohio.edu/phylocode/toc.html>), including the recognition of four new clade names, as well as the conversion of six names already recognized under the ICN. The clades named here are highlighted in boldface in Fig. 4, and the definitions for the clades are presented below. Following the definitions, we offer suggestions for the application of these clade names within the Angiosperm Phylogeny Group system, including a scheme of families and orders building on previous studies (e.g., Kårehed, 2001; Byng et al., 2014).

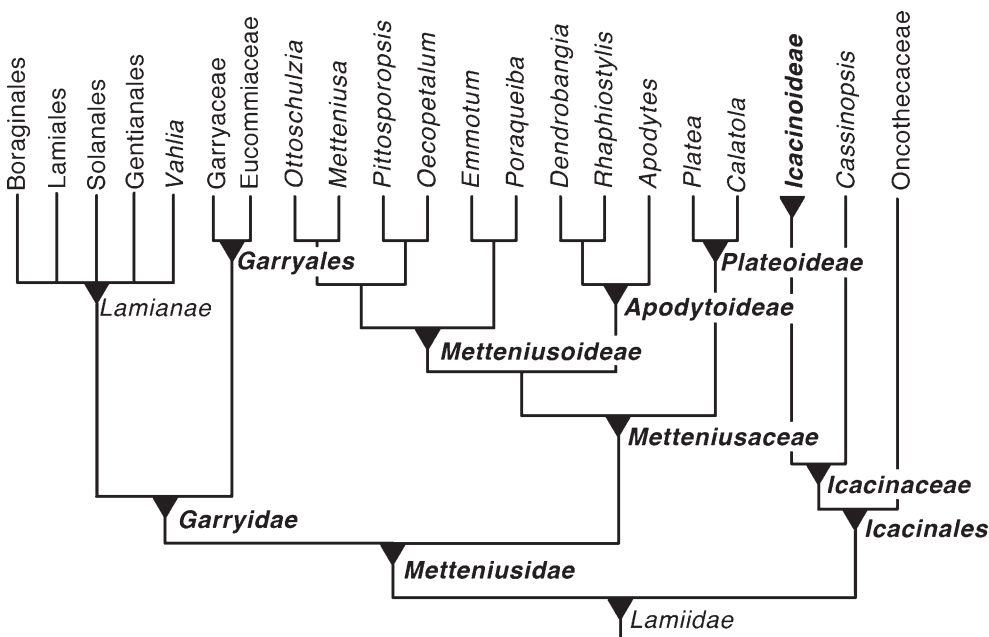


FIGURE 4 Phylogenetic classification of the *Lamiidae*, based on the results from this paper. Phylogenetic names newly proposed in this paper are indicated in boldface. Definitions for these new names are presented in the text. The names *Lamiidae* [R. G. Olmstead, W. S. Judd, and P. D. Cantino] and *Lamianae* [R. G. Olmstead and W. S. Judd] will be established in the upcoming Companion Volume to the PhyloCode (R. G. Olmstead, University of Washington, personal communication).

***Metteniusidae* G. W. Stull, D. E. Soltis, and P. S. Soltis, new clade name.**

Definition: The least-inclusive clade containing *Metteniusa edulis* H. Karst. 1860 (*Metteniusaceae*), *Garrya elliptica* Douglas ex Lindl. 1834 (*Garryales*), and *Gentiana acaulis* L. 1753 (*Lamianae*). This is a node-based definition in which all the specifiers are extant. Abbreviated definition: < *Metteniusa edulis* H. Karst. 1860 & *Garrya elliptica* Douglas ex Lindl. 1834 & *Gentiana acaulis* L. 1753.

Etymology: Derived from *Metteniusa* (name of an included genus), established by Hermann Karsten in honor of German botanist Georg Heinrich Mettenius.

Reference phylogeny: This paper is the primary reference; see Figs. 1, 2, and 4. See also Burge (2011).

Composition: *Metteniusaceae*, *Garryales*, and *Lamianae* (core lamiids: Boraginales, Gentianales, Lamiales, Solanales, and *Vahlia*).

Diagnostic apomorphies: No non-DNA synapomorphies are currently known.

Synonyms: None.

Comments: This is a newly discovered clade, lacking a pre-existing name. The composition of *Metteniusaceae* (discussed more below) and the positions of *Metteniusaceae* and *Garryales* in relation to the *Lamianae* were poorly supported or unresolved in previous studies (e.g., Soltis et al., 2011; Byng et al., 2014; Refulio-Rodriguez and Olmstead, 2014). Our results provide strong support that *Metteniusaceae* (as here circumscribed) and *Garryales* are successively sister to *Lamianae*. However, if upon further study *Metteniusaceae* is found to be sister to *Icacinales*, the name *Metteniusidae* would become a synonym with the prior name *Lamiidae*. Morphological synapomorphies for *Metteniusidae* are currently unknown.

We chose *Metteniusa edulis* as an internal specifier because it is the type of *Metteniusa*, which is the basis of the name *Metteniusidae*. Although we did not include this species in our analyses—instead, we included *Metteniusa tessmanniana* Sleum. (Sleum.)—the monophyly of *Metteniusa* is supported by numerous morphological features (Lozano-Contreras and de Lozano, 1988). We chose *Garrya elliptica* as an internal specifier because it is the type of the genus. Although we did not include *G. elliptica* in this study, a recent study (Burge, 2011) demonstrated that *Garrya* is monophyletic and that *G. elliptica* is closely related to *G. flavescens*, which we included in our analyses. The last internal specifier, *Gentiana acaulis*, was included in our analyses and occupies a highly nested position in the phylogeny of *Metteniusidae*.

***Garryidae* R. G. Olmstead, W. S. Judd, and P. D. Cantino 2007: 836 [G. W. Stull, D. E. Soltis, and P. S. Soltis], converted clade name.**

Definition: The least-inclusive clade containing *Garrya elliptica* Douglas ex Lindl. 1834 (*Garryales*) and *Gentiana acaulis* L. 1753 (*Lamianae*). This is a node-based definition in which all the specifiers are extant. Abbreviated definition: < *Garrya elliptica* Douglas ex Lindl. 1834 & *Gentiana acaulis* L. 1753.

Etymology: Derived from the included genus *Garrya* Douglas ex Lindl.

Reference phylogeny: This paper is the primary reference; see Figs. 1, 2, and 4. See also Bremer et al. (2002) and Refulio-Rodriguez and Olmstead (2014).

Composition: *Garryales* and *Lamianae* (core lamiids: Boraginales, Gentianales, Lamiales, Solanales, and *Vahlia*).

Diagnostic apomorphies: No non-DNA synapomorphies are currently known.

Synonyms: None.

Comments: The name *Garryidae* was originally applied to the lamiid/euasterid I clade as a whole (Cantino et al., 2007). However, Refulio-Rodriguez and Olmstead (2014) instead applied the name *Lamiidae* to the lamiid/euasterid I clade, and this procedure will be followed in the Companion Volume to the PhyloCode (R. G. Olmstead, University of Washington, personal communication). We therefore apply the name *Garryidae* to a less inclusive clade of lamiids, i.e., *Garryales* + *Lamianae*. Several previous studies recovered *Garryales* sister to the core lamiids, albeit generally with weak support (Bremer et al., 2002; Refulio-Rodriguez and Olmstead, 2014). Our analyses place *Garryales* sister to the core lamiids with strong ML and Bayesian support. Morphological synapomorphies for this clade, however, are currently unknown.

Garrya elliptica, one of the internal specifiers, represents the type of the genus *Garrya*, and it is closely related to the species of *Garrya* (*G. flavescens*) that we included in our phylogenetic analyses

(Burge, 2011). The other internal specifier, *Gentiana acaulis*, was included in our phylogenetic analyses.

***Garryales* Lindl. 1835: 16 [G. W. Stull, D. E. Soltis, and P. S. Soltis], converted clade name.**

Definition: The least-inclusive clade containing *Garrya elliptica* Douglas ex Lindl. 1834 (*Garryaceae*) and *Eucommia ulmoides* Oliv. 1890 (*Eucommiaceae*). This is a node-based definition in which all the specifiers are extant. Abbreviated definition: < *Garrya elliptica* Douglas ex Lindl. 1834 & *Eucommia ulmoides* Oliv. 1890.

Etymology: Derived from the included genus *Garrya* Douglas ex Lindl.

Reference phylogeny: Soltis et al. (1999a: supplemental fig. 11A) is the primary reference phylogeny. See also Soltis et al. (2000, 2011), Bremer et al. (2002), Savolainen et al. (2000a), Refulio-Rodriguez and Olmstead (2014), and this paper.

Composition: *Garryaceae*, *Eucommiaceae*.

Diagnostic apomorphies: Production of the latex gutta percha and dioecy.

Synonyms: None.

Comments: Numerous phylogenetic studies show that *Aucuba* (*Garryaceae* or sometimes treated in its own family, *Aucubaceae* Bercht. & J. Presl), *Garrya* (*Garryaceae*), and *Eucommia* (*Eucommiaceae*) form a well-supported clade (e.g., Soltis et al., 1999a, 2000, 2011; Refulio-Rodriguez and Olmstead, 2014; this paper). Although APG I (1998) recognized *Garryales* as including *Aucubaceae*, *Eucommiaceae*, *Garryaceae*, and *Oncothecaceae*, in APG II (2003) *Oncothecaceae* were excluded from the order and *Aucubaceae* were included in *Garryaceae*. This procedure was followed in APG III (2009). We apply the converted clade name *Garryales* to this same circumscription of taxa: *Eucommiaceae* (*Eucommia*) + *Garryaceae* (*Garrya* + *Aucuba*). The internal specifiers selected—*Garrya elliptica* and *Eucommia ulmoides*—are the type species of their respective genera. The production of the latex gutta percha appears to constitute a synapomorphy of *Garryales*. Another possible synapomorphy of this clade is dioecy.

***Icacinales* Tiegh. ex Reveal 1993: 175 [G. W. Stull], converted clade name.**

Definition: The least-inclusive crown clade containing *Icacina oliviformis* (Poir.) J. Raynal 1975 (*Icacinaceae*) and *Oncotheca balansae* Baill. 1891 (*Oncothecaceae*). This is a node-based definition in which all the specifiers are extant. Abbreviated definition: < *Icacina oliviformis* (Poir.) J. Raynal 1975 & *Oncotheca balansae* Baill. 1891.

Etymology: Derived from *Icacina* (name of an included genus), which refers to the resemblance of the type species, *Icacina oliviformis* (Poir.) J. Raynal (= *Icacina senegalensis* A. Juss.), to *Chrysobalanus icaco* L. of *Chrysobalanaceae* (Jussieu, 1823).

Reference phylogeny: This paper is the primary reference; see Figs. 1, 2, and 4.

Composition: *Icacinaceae* and *Oncothecaceae*.

Diagnostic apomorphies: No non-DNA synapomorphies are currently known.

Synonyms: None.

Comments: The sister relationship of *Icacinaceae*, as circumscribed here, and *Oncothecaceae* is a novel result, not found in previous studies of lamiid phylogeny (e.g., Bremer et al., 2002; González et al., 2007; Lens et al., 2008; Soltis et al., 2011; Refulio-Rodriguez and Olmstead, 2014; Byng et al., 2014), which have

generally placed *Oncotheca* with various genera of *Metteniusaceae*, as circumscribed here, although never with strong support.

The use of *Icacinales* for the clade comprising *Icacinaceae* (as circumscribed here) and *Oncothecaceae* is a novel application of the name. Van Tieghem (1897) first proposed *Icacinales* as a new order, but this name was not validly published until more recently (Reveal, 1993). Van Tieghem's circumscription of *Icacinales* more or less corresponded to *Icacinaceae* s.l., which he divided into multiple families (Emmotaceae, Iodaceae, *Icacinaceae*, Leptaulaceae, Phytocrenaceae, Pleurisanthaceae, and Sarcostigmataceae). Takhtajan's (1997) circumscription of *Icacinales* included *Icacinaceae* s.l. and three other families (Aquifoliaceae, Phellinaceae, and Sphenostemonaceae), which are now recognized as distantly related from *Icacinaceae* (Soltis et al., 2011). *Oncothecaceae* had generally been placed in Theales (Cronquist, 1981; Takhtajan, 1997).

Icacina oliviformis, the type species of *Icacina*, is synonymous with *Icacina senegalensis* A. Juss. 1823, which is the original name upon which the genus *Icacina* was based. The epithet "*oliviformis*" (originally treated as *Hirtella olivaeformis* Poir. 1813) takes priority over "*senegalensis*" as it is the older name. Although *Icacina oliviformis*, one of the internal specifiers, was not included in our analyses, we did include *Icacina mannii*, which Byng et al. (2014) found to be sister to *Icacina oliviformis* with strong support. Note, however, that in Byng et al. (2014), *I. oliviformis* is listed under the synonym *I. senegalensis*. Previous studies (Kårehed, 2001; Bremer et al., 2002) including *Icacina oliviformis* (listed under the synonym *Icacina senegalensis*) also found this species to be related to other members of *Icacinaceae* as here circumscribed. *Oncotheca balansae*, which was selected as the other internal specifier for *Icacinales*, is the type of *Oncotheca* and was included in our phylogenetic analyses.

***Icacinaceae* Miers 1851: 174 [G. W. Stull], converted clade name.**

Definition: The most-inclusive crown clade containing *Icacina oliviformis* (Poir.) J. Raynal 1975, *Mappia racemosa* Jacq. 1797, and *Pyrenacantha malvifolia* Engl. 1893 but not *Oncotheca balansae* Baill. 1891 (*Oncothecaceae*). This is a branch-modified node-based definition. Abbreviated definition: $>\nabla$ *Icacina oliviformis* (Poir.) J. Raynal 1975 & *Mappia racemosa* Jacq. 1797 & *Pyrenacantha malvifolia* Engl. 1893 ~ *Oncotheca balansae* Baill. 1891.

Etymology: Derived from *Icacina* (name of an included genus), which refers to the resemblance of the type species, *Icacina oliviformis* (Poir.) J. Raynal (= *Icacina senegalensis* A. Juss.), to *Chrysobalanus icaco* L. of *Chrysobalanaceae* (Jussieu, 1823).

Reference phylogeny: This paper is the primary reference; see Figs. 1, 2, and 4. See also Kårehed (2001), Bremer et al. (2002), Angulo et al. (2013), Byng et al. (2014).

Composition: *Alsodeiopsis*, *Casimirella*, *Cassinopsis*, *Desmostachys*, *Hosiea*, *Icacina*, *Iodes*, *Lavigeria*, *Leretia*, *Mappia*, *Mappianthus*, *Merrilliodendron*, *Miquelia*, *Natsiatopsis*, *Natsiatum*, *Nothapodytes*, *Phytocrene*, *Pleurisanthes*, *Pyrenacantha*, *Rhytiacaryum*, *Sarcostigma*, *Sleumeria*, and *Stachyanthus*.

Diagnostic apomorphies: No non-DNA synapomorphies are currently known.

Synonyms: None.

Comments: The name *Icacinaceae* was first proposed by Miers (1851) for ~13 genera formerly treated as the *Icacineae* tribe of *Olacaceae*. Others (e.g., Engler, 1893; Bailey and Howard, 1941a; Sleumer, 1942) later applied the name *Icacinaceae* to a much larger

assemblage of genera (~54) and species (~400). Based on molecular-morphological phylogenetic analyses, Kårehed (2001) recognized a much-reduced circumscription of *Icacinaceae* including ~34 genera and 200 species. None of the aforementioned circumscriptions were monophyletic. We apply the name *Icacinaceae* to a clade of 23 genera. This represents the most-inclusive monophyletic assemblage of genera from *Icacinaceae* s.l. including *Icacina*.

Because the position of *Cassinopsis* sister to the rest of *Icacinaceae* is not fully supported, we did not choose this species as an internal specifier. Instead we adopted a branch-modified node-based definition with three other species as internal specifiers, affording flexibility to include/exclude *Cassinopsis* upon further analyses. If it becomes sister to *Oncotheca*, for example, *Icacinaceae* (as defined above) would still exist; its composition would simply change slightly, in that *Cassinopsis* would be excluded. This situation would render *Icacinaceae* and *Icacinoideae* synonyms, with *Icacinaceae* taking priority. The internal specifiers *Mappia racemosa* and *Icacina oliviformis* (=the type of *Icacina* and thus ultimately *Icacinaceae*) represent the two clades successively sister to the rest of the family (after *Cassinopsis*). The third internal specifier, *Pyrenacantha malvifolia*, occupies a highly nested position in the phylogeny.

***Icacinoideae* Engl. 1893: 242 [G. W. Stull], converted clade name.**

Definition: The least-inclusive clade containing *Icacina oliviformis* (Poir.) J. Raynal 1975, *Mappia racemosa* Jacq. 1797, and *Pyrenacantha malvifolia* Engl. 1893. This is a node-based definition in which all of the specifiers are extant. Abbreviated definition: $<$ *Icacina oliviformis* (Poir.) J. Raynal 1975 & *Mappia racemosa* Jacq. 1797 & *Pyrenacantha malvifolia* Engl. 1893.

Etymology: Derived from *Icacina* (name of an included genus), which refers to the resemblance of the type species, *Icacina oliviformis* (Poir.) J. Raynal (= *Icacina senegalensis* A. Juss.), to *Chrysobalanus icaco* L. of *Chrysobalanaceae* (Jussieu, 1823).

Reference phylogeny: This paper is the primary reference; see Figs. 1–4. See also Kårehed (2001), Lens et al. (2008), Angulo et al. (2013), and Byng et al. (2014).

Composition: All genera of *Icacinaceae* except *Cassinopsis*.

Diagnostic apomorphies: Unilacunar nodes and simple perforation plates.

Synonyms: No formal synonyms exist, although several informally named groups constitute close approximations to *Icacinoideae*: the *Icacina* group of Kårehed (2001) and group III of Bailey and Howard (1941b).

Comments: Engler (1893) used the name *Icacinoideae* for one of three subfamilies of *Icacinaceae*, the other subfamilies being the monogeneric *Cardiopteridoideae* and *Lophopyxidoideae*. Engler (1893) recognized 36 genera in *Icacinoideae*, which he divided among four tribes: *Icacineae*, *Iodeae*, *Phytocreneae*, and *Sarcostigmataeae*. Subsequently, the circumscription of *Icacinoideae* was expanded to include >50 genera, while *Cardiopteris* Wall. ex Royle (*Cardiopteridoideae*) and *Lophopyxis* Hook.f. (*Lophopyxidoideae*) were recognized as dubious members of *Icacinaceae* (Sleumer, 1942; Bailey and Howard, 1941a). The above circumscriptions of *Icacinoideae* were shown to be polyphyletic by numerous phylogenetic studies (Savolainen et al., 2000a, b; Soltis et al., 2000; Kårehed, 2001).

We apply the name *Icacinoideae* to a clade comprising 22 genera. No formal name with a closer correspondence to this clade exists in the literature. *Icacinoideae* as here circumscribed includes 11

genera from the traditional Icacinaceae tribe and all genera of the traditional Iodeae, Phytocreneae, and Sarcostigmataeae tribes, plus the newly described genus *Sleumeria* (Utteridge et al., 2005). All the genera comprising *Icacinoideae* were included in our analyses except *Sleumeria* and *Natsiatopsis*. However, Byng et al. (2014) confirmed the placement of *Sleumeria* in this clade based on phylogenetic analyses of the plastid loci *matK*, *ndhF*, and *rbcl* (although *Sleumeria* was only represented by *ndhF* in the analyses), and multiple lines of morphological evidence (mentioned in this paper) suggest that *Natsiatopsis* falls within this clade close to *Natsiatum*. *Icacinoideae* corresponds closely to several informal groupings suggested in earlier studies—i.e., the *Icacina* group of Kårehed (2001) and group III of Bailey and Howard (1941b). The internal specifiers *Mappia racemosa* and *Icacina oliviformis* represent the two clades successively sister to the rest of *Icacinoideae*. The third internal specifier, *Pyrenacantha malvifolia*, occupies a highly nested position in the phylogeny. This clade is diagnosed by unilacunar nodes and vessels with simple perforation plates. *Cassinopsis*, which is sister to *Icacinoideae*, is distinguished by having trilacunar nodes and vessels with sclariform perforation plates.

Metteniusaceae H. Karst. ex Schnizl. 1860: 142 [G. W. Stull], converted clade name.

Definition: The least-inclusive clade containing *Apodytes dimidiata* E. Mey. ex Arn. 1840, *Metteniusa edulis* H. Karst. 1860, and *Platea parviflora* A. O. Dahl 1952. This is a node-based definition in which all of the specifiers are extant. **Abbreviated definition:** < *Apodytes dimidiata* E. Mey. ex Arn. 1840 & *Metteniusa edulis* H. Karst. 1860 & *Platea parviflora* A. O. Dahl 1952.

Etymology: Derived from *Metteniusa* (name of an included genus), established by Hermann Karsten in honor of German botanist Georg Heinrich Mettenius.

Reference phylogeny: This paper is the primary reference; see Figs. 1, 2, and 4.

Composition: *Apodytes*, *Calatola*, *Dendrobangia*, *Emmotum*, *Metteniusa*, *Oecopetalum*, *Ottoschulzia*, *Pittosporopsis*, *Platea*, *Poraqueiba*, and *Rhaphiostylis*.

Diagnostic apomorphies: No non-DNA synapomorphies are currently known.

Synonyms: None.

Comments: *Metteniusa* has generally been treated as the sole constituent of its own family, *Metteniusaceae*. González et al. (2007) showed that *Metteniusa* represents a basal lamiid lineage, but their sampling was too skeletal to place the genus precisely among the basal lamiids. Phylogenetic analyses by Byng et al. (2014) suggested that *Metteniusa* is closely related to several genera of the *Emmotum* group of Icacinaceae sensu Kårehed (2001): *Emmotum*, *Ottoschulzia*, *Oecopetalum*, and *Poraqueiba*. Our results are consistent with Byng et al. (2014) in that *Metteniusa* is nested in the *Emmotum* group and further show that this clade is related to multiple additional genera of Icacinaceae s.l.: *Apodytes*, *Calatola*, *Dendrobangia*, *Platea*, and *Rhaphiostylis*. The name *Metteniusaceae* was selected for this clade (comprising 11 genera) because it is the oldest family name associated with this clade's constituent genera. Morphological synapomorphies of this clade are currently unknown. The internal specifiers used in this definition were all included in our phylogenetic analyses, except *Metteniusa edulis*, the type of *Metteniusa*. Since the clade name *Metteniusaceae* is based on the genus name *Metteniusa*, the type of the genus must be included in the definition (Cantino et al., 2007). The monophyly of *Metteniusa* is

supported by numerous morphological features (Lozano-Contreras and de Lozano, 1988), suggesting that *Metteniusa edulis* is likely closely related to *Metteniusa tessmanniana*, which was included in our analyses.

Plateoideae G. W. Stull, new clade name.

Definition: The least-inclusive clade including *Calatola mollis* Standl. 1923 and *Platea latifolia* Blume 1826. This is a node-based definition in which all of the specifiers are extant. **Abbreviated definition:** < *Calatola mollis* Standl. 1923 & *Platea latifolia* Blume 1826.

Etymology: Derived from the included genus *Platea* Blume.

Reference phylogeny: This paper is the primary reference; see Figs. 1, 2, and 4. See also Kårehed (2001) and Byng et al. (2014).

Composition: *Calatola* and *Platea*.

Diagnostic apomorphies: Possible synapomorphies for this clade include unisexual flowers and indeterminate inflorescences.

Synonymy: None.

Comments: Kårehed (2001) recovered a sister relationship between *Calatola* and *Platea* based on morphological phylogenetic analyses. Based on three plastid loci, Byng et al. (2014) also recovered this relationship with moderate support. The results in this paper corroborate these earlier analyses with strong support. *Calatola mollis* and *Platea latifolia* were selected as internal specifiers because the former was included in our phylogenetic analyses and the latter represents the type of *Platea*, the basis of the name *Plateoideae*.

Apodytoideae G. W. Stull, new clade name.

Definition: The least-inclusive clade including *Apodytes dimidiata* E. Mey. ex Arn. 1840, *Dendrobangia boliviana* Rusby 1896, and *Rhaphiostylis ferruginea* Engl. 1909. This is a node-based definition in which all of the specifiers are extant. **Abbreviated definition:** < *Apodytes dimidiata* E. Mey. ex Arn. 1840 & *Dendrobangia boliviana* Rusby 1896 & *Rhaphiostylis ferruginea* Engl. 1909.

Etymology: Derived from the included genus *Apodytes* E. Mey. ex Arn.

Reference phylogeny: This paper is the primary reference; see Figs. 1, 2, and 4. See also Kårehed (2001) and Byng et al. (2014).

Composition: *Apodytes*, *Dendrobangia*, and *Rhaphiostylis*.

Diagnostic apomorphies: No non-DNA synapomorphies are currently known.

Synonymy: None.

Comments: Several studies (Kårehed, 2001; Lens et al., 2008) have recovered a sister relationship between *Apodytes* and *Rhaphiostylis*, but these analyses did not include the Neotropical genus *Dendrobangia*. Byng et al. (2014) recovered *Dendrobangia* as sister to *Apodytes* + *Rhaphiostylis* with weak Bayesian support. Our analyses recovered *Apodytes* sister to *Dendrobangia* + *Rhaphiostylis* with strong ML and Bayesian support. Although our results differ from those of Byng et al. (2014) in the placement of *Dendrobangia*, both indicate that these three genera form a clade. Morphological synapomorphies for this clade are not currently known. The internal specifiers selected were included in our phylogenetic analyses. *Apodytes dimidiata* and *Dendrobangia boliviana* also constitute the type species of their respective genera.

Metteniusoideae G. W. Stull, new clade name.

Definition: The least-inclusive clade including *Emmotum fagifolium* Desv. ex Ham. 1825 and *Metteniusa edulis* H. Karst. 1860.

Abbreviated definition: < *Emmotum fagifolium* Desv. ex Ham. 1825 & *Metteniusa edulis* H. Karst. 1860.

Etymology: Derived from *Metteniusa* (name of an included genus), established by Hermann Karsten in honor of German botanist Georg Heinrich Mettenius.

Reference phylogeny: This paper is the primary reference; see Figs. 1, 2, and 4. See also Duno de Stefano and Fernández-Concha (2011) and Byng et al. (2014).

Composition: *Emmotum*, *Metteniusa*, *Oecopetalum*, *Ottoschulzia*, *Pittosporopsis*, and *Poraqueiba*.

Diagnostic apomorphies: Possible synapomorphies for this clade include stamen connectives extending beyond the anther sacs, fleshy petals, and fruits with persistent styles.

Synonymy: None.

Comments: In his combined molecular-morphological analyses, Kårehed (2001) recovered relationships among *Emmotum*, *Oecopetalum*, *Ottoschulzia*, and *Poraqueiba*, which he informally called the *Emmotum* group. Kårehed's (2001) analyses, however, did not include *Pittosporopsis* or *Metteniusa*. A recent paper by Byng et al. (2014) found moderate support for a clade including the *Emmotum* group plus *Metteniusa*, but *Pittosporopsis* was not included in their analyses. Our analyses corroborate this general result and further show, with strong support, that *Pittosporopsis* belongs in this clade.

The internal specifier *Emmotum fagifolium* represents the type of the genus *Emmotum*. Although we did not include this species in our phylogenetic analyses—instead, we included *Emmotum nitens* (Benth.) Miers—a morphology-based phylogeny of the genus (Duno de Stefano and Fernández-Concha, 2011) found numerous morphological synapomorphies supporting its monophyly. The other internal specifier, *Metteniusa edulis*, represents the type of *Metteniusa*, the monophyly of which is supported by numerous morphological features (Lozano-Contreras and de Lozano, 1988).

Recommendations for APG—We synthesized our results with previous studies (e.g., Kårehed, 2001; Lens et al., 2008; Byng et al., 2014) to provide a familial and ordinal classification of genera formerly included in Icacinaceae s.l. (Table 2). We recommend that the next edition of APG adopt this classification. Compared with the most recent APG system (APG III, 2009), this classification includes a reduced circumscription of Icacinaceae (23 genera), an expanded circumscription of Metteniusaceae (11 genera), and the recognition of two orders new to APG: Icaciniales Tiegh. ex Reveal (including Icacinaceae and Oncothecaceae) and Metteniusales Takht. (including Metteniusaceae). Garryales Lindl. should be restricted to the families Eucommiaceae Engl. and Garryaceae Lindl. These changes are based largely on the results from this paper, and in all cases they are strongly supported by both ML bootstrap and Bayesian posterior probability values (Fig. 1). Although recognizing Metteniusales to include a single family is taxonomically redundant, it is necessary under a rank-based system given the isolated phylogenetic position of Metteniusaceae; were Metteniusaceae included in any other order, it would render that order nonmonophyletic.

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