An updated tribal classification of Lamiaceae based on plastome phylogenomics

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Abstract

Background: A robust molecular phylogeny is fundamental for developing a stable classification and providing a solid framework to understand patterns of diversification, historical biogeography, and character evolution. As the sixth largest angiosperm family, Lamiaceae, or the mint family, constitutes a major source of aromatic oil, wood, ornamentals, and culinary and medicinal herbs, making it an exceptionally important group ecologically, ethnobotanically, and floristically. The lack of a reliable phylogenetic framework for this family has thus far hindered broad-scale biogeographic studies and our comprehension of diversification. Although significant progress has been made towards clarifying Lamiaceae relationships during the past three decades, the resolution of a phylogenetic backbone at the tribal level has remained one of the greatest challenges due to limited availability of genetic data.

Results: We performed phylogenetic analyses of Lamiaceae to infer relationships at the tribal level using 79 protein-coding plastid genes from 175 accessions representing 170 taxa, 79 genera, and all 12 subfamilies. Both maximum likelihood and Bayesian analyses yielded a more robust phylogenetic hypothesis relative to previous studies and supported the monophyly of all 12 subfamilies, and a classification for 22 tribes, three of which are newly recognized in this study. As a consequence, we propose an updated phylogenetically informed tribal classification for Lamiaceae that is supplemented with a detailed summary of taxonomic history, generic and species diversity, morphology, synapomorphies, and distribution for each subfamily and tribe.

Conclusions: Increased taxon sampling conjoined with phylogenetic analyses based on plastome sequences has provided robust support at both deep and shallow nodes and offers new insights into the phylogenetic relationships among tribes and subfamilies of Lamiaceae. This robust phylogenetic backbone of Lamiaceae will serve as a framework for future studies on mint classification, biogeography, character evolution, and diversification.

Keywords: Lamiaceae, Lamioideae, Mints, Phylogenomics, Tribal relationships
Background
Lamiaceae, generally known as the mint family, have long been known for their aromatic oils, which have played an undeniably significant role within culinary, medicinal, and horticultural aspects of human history. Species of Lamiaceae are of wide economic importance as sources of wood (e.g., *Tectona grandis* L. f.), landscape ornamentals (e.g., scarlet sage [*Salvia splendens* Sellow ex Wied-Neuw.]), cosmetics (e.g., lavender [*Lavandula angustifolia* Mill.]), culinary herbs (e.g., basil [*Ocimum basilicum* L.], oregano [*Origanum vulgare* L.], thyme [*Thymus vulgaris* L.]), and medicinal herbs (e.g., Korean mint [*Agastache rugosa* (Fisch. & C.A. Mey.) Kuntze], peppermint [*Mentha × piperita* L.]). Despite the recognition of this family (Lamiaceae s.s.) from advances in systematics and taxonomy of the late twentieth century, the family has historically been considered a “natural” group based on a combination of readily recognizable features such as an herbaceous habit, quadrangular stems, opposite phyllotaxy, bilabiate flowers, a gynobasic style, and four nutlets. However, morphological and molecular phylogenetic studies in the past three decades have significantly changed the concept of the family, and an expanded Lamiaceae (Lamiaceae s.l.) is now widely accepted. As currently circumscribed, Lamiaceae comprise more than 230 genera and over 7000 species, making it the sixth largest angiosperm family and the largest according to molecular phylogenetic studies [1–3]. Although unequivocally shown to be members of the family, inclusion of some disparate groups such as *Vitex* L. (originally placed in Verbenaceae because they were trees with fleshy fruits) has challenged the earlier concepts of the family.

Early infrafamilial classifications within Lamiaceae were predominately based on the treatment of Bentham [4], who divided the family into eight tribes. Briquet [5], for example, followed the division of Bentham [4], but raised several of the tribes to subfamilial rank and merged four tribes into the single large subfamily Lamioideae. Erdtman [6], however, recognized only two subfamilies based on palynological distinctions, viz., Lamioideae (with tricolporate pollen shed at the two-celled stage) and Nepetoideae (with hexacolpate pollen shed at the three-celled stage). Combining the classifications of Briquet [5] and Erdtman [6], Wunderlich [7] recognized six subfamilies within Lamiaceae, rejecting Lamioideae as circumscribed by Briquet [5] and accepting a subfamily Nepetoideae close to that of Erdtman [6]. Cantino and Sanders [8] revealed that Nepetoideae sensu Erdtman [6] is monophyletic with several synapomorphies, whereas no synapomorphy was found for Lamioideae sensu Erdtman [6].

The mint family has long been thought to have evolved from Verbenaceae-like ancestors, and these two families were considered separate largely based upon gynoecial structure. Although a deeply four-lobed ovary with a gynobasic style is typical for most traditionally recognized Lamiaceae (i.e. Lamiaceae s.s.), and an unlobed ovary with a terminal style is typical of most Verbenaceae, there exists in both families a continuum in extent of lobing and separation of fruits into single seeded units [9]. Noting this, Cantino [9, 10] carried out a cladistic analysis of the Lamiaceae s.s. and the Verbenaceae s.l. based on 85 morphological and anatomical characters, which provided support to reject that the Lamiaceae s.s. was monophyletic, demonstrating several clades of the Verbenaceae s.l. recovered among clades of the Lamiaceae s.s. Based on these results, Cantino et al. [11] published a list of subfamilies and genera of the Lamiaceae s.l. that were treated as incertae sedis [12]. This incorporated the transfer of the cymose subfamilies Caryopteridoideae, Chloanthoideae, Viticoideae, Symphoremtatoideae, and tribe Monochlateae to the expanded Lamiaceae, rendering the Verbenaceae s.s. as only the subfamily Verbenoideae. Verbenaceae s.s. can be recognized by having racemose inflorescences, tricolporate pollen, and ovules attached to the carpel margins, while the Lamiaceae s.l. generally possess thyrsoid inflorescences, colpate pollen, and ovules attached to the sides of the false sepa of ovary [13]. Moreover, the Verbenaceae s.s. have thickened stigma lobes with conspicuous stigmatic tissue, hypocrateriform corollas with included stamens, and usually terete stems, whereas in the Lamiaceae s.l., stigma lobes are slender with inconspicuous stigmatic tissue, corollas that are rarely hypocrateriform, and stems are typically quadrangular. Since Cantino et al. [11], the expanded concept of the Lamiaceae s.l. has been consistently supported as monophyletic by molecular phylogenetic studies [14–20] and is widely accepted in various classifications [1, 3]. We acknowledge these results and use the names Lamiaceae and Verbenaceae in their contemporary circumscription. Though today both Lamiaceae and Verbenaceae are placed within “core Lamiales” of the asterids, they have unexpectedly not been recovered as sister taxa despite their morphological similarities: Lamiaceae belong to a clade that includes Mazaceae, Phrymaceae, Wightiaceae, Paulowniaceae, and Orobancheaceae, whereas Verbenaceae are recovered as sister to Thromandersiaceae [18, 21, 22].

Following Cantino et al. [11], Harley et al. [1] published a global, genus-level taxonomic conspectus of Lamiaceae. Except for the ten genera *Acrymia* Prain, *Callicarpa* L., *Cymaria* Benth., *Garrettia* H.R. Fletch., *Holocheila* (Kudô) S. Chow, *Hymenopyramis* Wall. ex Griff., *Ombrocharis* Hand.-Mazz., *Peronema* Jack, *Petraeovitex* Oliv., and *Tectona* L. that were treated as incertae sedis, the remaining 226 genera were assigned to seven subfamilies: Ajugoidae, Lamioideae, Nepetoideae, Prostantheroideae, Scutellarioideae, Symphorematoidae, and Viticoideae [1]. Since...
the publication of this classification [1], numerous molecular phylogenetic studies have been carried out to explore the relationships at the subfamilial [19], tribal [23–33], or generic [34–50] level. However, relationships among four subfamilies (Nepetoideae, Tectonoideae, Premnoideae, and Ajugoideae) remain unresolved and those among some tribes were also unclear in those studies.

In terms of taxon number, the most comprehensively sampled phylogenetic study of Lamiaceae was conducted by Li et al. [19] using an ingroup sampling of 288 species from 191 genera and employing five plastid DNA regions (matK, ndhF, rbcL, rps16, and trnL-trnF). The backbone of this phylogeny was comprised of 12 clades, all provided with high branch support, and seven of which corresponded to a portion of the Viticoideae and six of the previously recognized subfamilies of Harley et al. [1]. The other five clades consisted of previously incertae sedis genera and were each provided subfamilial rank as the Cymarioideae (including Acrymia and Cymaria), Peronematoidae (including Hymenopyramis, Petraevitex, Peronema, and Garrettia), Premnoideae (including Premna L., Gmelina L., and Cornutia L.), Callicarpoidae (including Callicarpa), and Tectonoideae (including Tectona) [19, 51].

Despite the improved resolution in our understanding of Lamiaceae and its subfamilies, the work by Li et al. [19] was not able to clarify relationships among Nepetoideae, Tectonoideae, Premnoideae, and Ajugoideae, nor were they able to provide resolution to understand the tribal classification within some subfamilies (viz. Lamioideae). While recent phylogenetic analyses have greatly improved our understanding of the major lineages and classifications of Lamioideae [52, 53], the tribal membership of Betonica L., Colquhounia Wall., Galeopsis L., Metastachydium Airy Shaw ex C.Y. Wu & H.W. Li, Paralamium Dunn., and Roylea Wall. ex Benth. remains unclear [2, 53]. Furthermore, Xiang et al. [54] identified four major clades within the Ajugoideae, but did not propose a formal tribal classification. The uncertain relationships among and within these subfamilies have hindered the further study of character evolution and diversification patterns within Lamiaceae.

Next-generation sequencing (NGS) provides a significantly larger amount of DNA sequence data than has been previously available for phylogenetic studies within angiosperms [55]. While the use of complete plastome sequences is not a panacea [56], it has successfully resolved previously intractable phylogenetic problems within flowering plants at multiple taxonomic levels [57–65]. Concordantly, recent phylogenomic studies based on plastome sequences have provided new insight into both generic and species-level relationships within Scutellarioideae [66] and Salvia [67], respectively. In order to resolve the remaining ambiguities at the tribal and subfamilial level, we sequenced and analyzed the complete plastome for 175 representative taxa from all currently recognized tribes in the 12 subfamilies of Lamiaceae. The focus of this study was to (1) improve the resolution of the phylogenetic backbone of Lamiaceae, (2) modify the tribal classification of Lamiaceae based on our results, and (3) provide a summary of the recent phylogenetic and taxonomic progress achieved for each subfamily and tribe.

Results

Characteristic of plastome features and datasets

Our sequencing generated between 13,829,468 (Siphocranion flavidum Y.P. Chen & C.L. Xiang) and 81,265,290 (Chloanthes coccinea Bartl.) clean reads from the 50 newly sequenced species, with the mean base coverage ranging from 110× (Congea tomentosa Roxb.) to 3104× (Lamium amplexicaule L.) estimated by the GetOrganelle pipeline [68]. Since we failed to assemble the complete plastome of Callicarpa americana L., the average base coverage for this species is unavailable (noted as “NA” in Table 1). Statistics about the assemblies for each newly sequenced species are provided in Table 1.

All plastomes exhibit a typical quadripartite structure of the large single-copy (LSC, 81,341–85,891 bp) and small single-copy (SSC, 9969–20,681 bp) regions, separated by a pair of inverted repeats (IR regions, 23,085–31,573 bp). The chloroplast genome maps are provided in Additional file 1 (Fig. S1). The GC content was evenly distributed, and the average GC content was 38.10% (Additional file 2: Table S1). All the newly sequenced and annotated plastomes in the present study were submitted to the National Center for Biotechnology Information (NCBI) database with accession numbers MT473738–MT473786 (Table 1).

The aligned length of the combined 79 protein-coding regions (CR) is 72,082 bp. Removal of ambiguous sites and single-taxon insertions results in an aligned length of 69,822 bp (CRM), of which 41,459 sites are constant (59.38%). The aligned regions and the excluded ambiguous sites of the individual loci are listed in Additional file 3 (Table S2), and properties of the five datasets are summarized in Table 2.

Phylogenetic analyses

All analyses yielded an identical topology for the ingroup at the tribal level (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5), although the support is variable among different datasets. All 12 subfamilies were recovered and well-supported in all analyses (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5). The topology recovered by the combined dataset with the ambiguously aligned positions excluded (CRM) is presented as the primary tree
Table 1 Newly sampled species in this study (NA data unavailable)

| Systematic assignment | Species | Locality | Clean reads | Mean coverage of base (x) | GenBank accession numbers |
|-----------------------|---------|----------|-------------|---------------------------|--------------------------|
| Phrymaceae (outgroup) | Mimulus sp. | The United States Botanic Garden (USBG), United States | 19,584,540 | 478 | MT473772 |
| Ajugoideae Ajugeae | Caryopteris forrestii Diels | Lijiang, Yunnan, China | 67,295,160 | 485 | MT473742 |
| Ajugoideae Teucrieae | Schnabelia oligophylla Hand.-Mazz. | Kunming, Yunnan, China | 67,359,376 | 726 | MT473777 |
| Ajugoideae Clerodendreae | Clerodendrum japonicum (Thunb.) Sweet | Kunming, Yunnan, China | 69,357,954 | 854 | MT473745 |
| Ajugoideae Clerodendreae | Clerodendrum trichotomum Thunb. | Huairou, Beijing, China | 69,621,568 | 536 | MT473746 |
| Ajugoideae Rotheceae | Rotheca serrata (L.) Steane & Mabb. | Kunming, Yunnan, China | 69,698,896 | 328 | MT473776 |
| Callicarpioideae – | Callicarpa americana L. | Gainesville, Florida, United States | 69,222,992 | NA | – |
| Callicarpioideae – | Callicarpa arborea Roxb. | Kunming, Yunnan, China | 70,066,596 | 341 | MT473738 |
| Callicarpioideae – | Callicarpa brevipes (Benth.) Hance | Guangzhou, Guangdong, China | 68,119,222 | 383 | MT473739 |
| Callicarpioideae – | Callicarpa macrophylla Vahl | Kunming, Yunnan, China | 69,104,110 | 499 | MT473740 |
| Callicarpioideae – | Callicarpa peichieniana Chun & S.L. Chen ex H. Ma & W.B. Yu | Guangzhou, Guangdong, China | 68,759,068 | 215 | MT473741 |
| Cymarioideae – | Cymaria dichotoma Benth. | Changjiang, Hainan, China | 68,070,464 | 1189 | MT473753 |
| Lamioideae Paraphlomideae | Paraphlomis javanica (Blume) Prain | Kunming, Yunnan, China | 66,797,022 | 239 | MT473773 |
| Lamioideae Gomphostemmateae | Gomphostemma lucidum Wall. ex Benth. | Changjiang, Hainan, China | 66,842,836 | 171 | MT473749 |
| Lamioideae Gomphostemmateae | Chelonopsis souliei (Bonati) Merr. | Litang, Sichuan, China | 67,646,436 | 572 | MT473743 |
| Lamioideae Colquhounieae | Colquhounia coccinea Wall. | Kunming, Yunnan, China | 66,842,836 | 171 | MT473730 |
| Lamioideae Colquhounieae | Colquhounia sequinii Vaniot | Kunming, Yunnan, China | 66,760,344 | 337 | MT473750 |
| Lamioideae Colquhounieae | Colquhounia vestita Wall. | Cuona, Xizang, China | 67,753,130 | 192 | MT473751 |
| Lamioideae Larnieae | Lamium amplexicaule L. | Zuoqong, Xizang, China | 67,339,814 | 3104 | MT473770 |
| Lamioideae Synandreae | Macbridea alba Chapm. | The United States Botanic Garden (USBG), United States | 20,514,794 | 474 | MT473771 |
| Lamioideae Stachydeae | Galeopsis bifida Boenn. | Deqin, Yunnan, China | 67,442,714 | 500 | MT473759 |
| Nepetoideae Elsholtzieae | Elsholtzia densa Benth. | Shangri-La, Yunnan, China | 18,273,016 | 888 | MT473757 |
| Nepetoideae Elsholtzieae | Elsholtzia rugulosa Hemsl. | Kunming, Yunnan, China | 67,318,028 | 553 | MT473758 |
| Nepetoideae Ocimeae | Siphocranion flavidum Y.P. Chen & C.L. Xiang | Malipo, Yunnan, China | 13,829,468 | 436 | MT473778 |
| Nepetoideae Ocimeae | Siphocranion macranthum (Hook. f.) C.Y. Wu | Nanchuan, Congqing, China | 13,860,798 | 241 | MT473779 |
| Nepetoideae Ocimeae | Hanuca exserta Y.Z. Sun ex C.Y. Wu | Hezhou, Guangxi, China | 67,557,758 | 203 | MT473765 |
| Nepetoideae Ocimeae | Isodon amethystoides (Benth.) H. Hara | Lin'an, Zhejiang, China | 25,146,824 | 696 | MT473767 |
| Nepetoideae Ocimeae | Isodon lophanthoides (Buch.-Ham. ex D. Don) H. Hara | Kunming, Yunnan, China | 40,730,966 | 316 | MT473768 |
| Nepetoideae Ocimeae | Isodon ternifolius (D. Don) Kudô | Longling, Yunnan, China | 32,984,960 | 542 | MT473769 |
Within Lamiaceae, two primary clades were recovered and subdivided as 12 clades corresponding to the 12 subfamilies (Fig. 1), with each subfamily being monophyletic (excepting Cymarioideae, which was represented by only one species). The first clade comprised the Prostantheroideae and Callicarpoideae (i.e., Calliprostantherina sensu Li et al. [19]), both with strong support (MLBS = 100%, BIPP = 1.00). The two tribes of Prostantheroideae, Chloantheae and Westringieae, were each recovered as monophyletic and sister taxa with strong support (100%, 1.00). The second clade of Lamiaceae consisted of Nepetoideae, Symphorematoideae, Viticoideae, Tectonoideae, Premnoideae, Ajugoideae, Peronematoideae, Scutellarioideae, Cymarioideae, and Lamioideae (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5).

Within Nepetoideae (100%, 1.00), the monophyly of Elsholtzieae, Ocimeae, and Mentheae was robustly supported in all analyses (100%, 1.00). However, relationships among the three tribes varied among different datasets. Most of the datasets (CRM, CR, CR3, dePCS) supported Elsholtzieae as sister to Ocimeae (Fig. 1, 86%, 1.00; Additional files 4, 5: Figs. S2, S3; Additional file 7: Fig. S5), while in the phylogeny based on dataset CR12, Elsholtzieae were weakly supported as sister to Mentheae (Additional file 6: Fig. S4, 45%, 0.66).

In tribe Elsholtzieae, the genus Elsholtzia Willd. was recovered as sister to Perilla L., and the sister relationships received maximal support in all analyses (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5). Representatives of all seven subtribes of Ocimeae formed a well-resolved clade, with subtribe Siphocranioninae (Siphocranion spp.) diverging first, followed by subsequent bifurcations for subtribes Lavandulinae (Lavandula spp.), Hanceoliniae (Hanceola exserta Y.Z. (Fig. 1) for the following discussion of phylogenetic relationships.

Within Lamiaceae, two primary clades were recovered and subdivided as 12 clades corresponding to the 12 subfamilies (Fig. 1), with each subfamily being monophyletic (excepting Cymarioideae, which was represented by only one species). The first clade comprised the Prostantheroideae and Callicarpoideae (i.e., Calliprostantherina sensu Li et al. [19]), both with strong support (MLBS = 100%, BIPP = 1.00; Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5, and all support values follow this order hereafter). The two tribes of Prostantheroideae, Chloantheae and Westringieae, were each recovered as monophyletic and sister taxa with strong support (100%, 1.00). The second clade of Lamiaceae consisted of Nepetoideae, Symphorematoideae, Viticoideae, Tectonoideae, Premnoideae, Ajugoideae, Peronematoideae, Scutellarioideae, Cymarioideae, and Lamioideae (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5).

### Table 1 Newly sampled species in this study (NA data unavailable) (Continued)

| Systematic assignment | Species | Locality | Clean reads | Mean coverage of base (x) | GenBank accession numbers |
|-----------------------|---------|----------|-------------|--------------------------|--------------------------|
| Nepetoideae Ocimeae   | Coleus xanthanthus C.Y. Wu & Y.C. Huang | Mengla, Yunnan, China | 25,669,120 | 821 | MT473748 |
| Nepetoideae Menheae   | Dracocephalum taliense Forrest | Heqing, Yunnan, China | 68,863,176 | 446 | MT473756 |
| Nepetoideae Menheae   | Clinopodium abyssinicum (Benth.) Kuntze | Kabarnet, Baringo, Kenya | 48,657,815 | 833 | MT473747 |
| Peronematoideae –     | Garretia siamensis H.R. Fletcher | Mengla, Yunnan, China | 69,566,486 | 1905 | MT473760 |
| Peronematoideae –     | Hymenopyramis cana Craib | Changjiang, Hainan, China | 66,946,216 | 298 | MT473766 |
| Premnoideae –         | Premna szemaoensis C. P‘ei | Kunming, Yunnan, China | 69,409,616 | 477 | MT473775 |
| Premnoideae –         | Premna vietnamensis Bo Li | K’Bang, Gia Lai, Vietnam | 80,675,070 | 460 | MT473774 |
| Premnoideae –         | Gmelina arborea Roxb. ex Sm. | Mengla, Yunnan, China | 67,974,942 | 493 | MT473761 |
| Premnoideae –         | Gmelina hainanensis Oliv. | Kunming, Yunnan, China | 67,354,640 | 1527 | MT473762 |
| Premnoideae –         | Gmelina philippensis Cham. | Mengla, Yunnan, China | 69,953,046 | 479 | MT473763 |
| Prostantheroideae Chloantheae | Chloanthes coccineae Bartl. | Australian National Botanic Gardens (ANBG), Australia | 81,265,290 | 598 | MT473744 |
| Prostantheroideae Chloantheae | Dasymalia teckiana (F. Muell.) B.J. Conn & Henwood | Australian National Botanic Gardens (ANBG), Australia | 41,308,508 | 519 | MT473754 |
| Prostantheroideae Chloantheae | Dicra stylos parvifolia F. Muell. | Australian National Botanic Gardens (ANBG), Australia | 81,081,410 | 577 | MT473755 |
| Symphorematoideae –   | Congea tormentosa Roxb. | Mengla, Yunnan, China | 40,494,132 | 110 | MT473752 |
| Symphorematoideae –   | Sphenodesme mollis Craib | Mengla, Yunnan, China | 81,008,454 | 529 | MT473780 |
| Tectonoideae –        | Tectona grandis L. f. | Mengla, Yunnan, China | 40,169,710 | 514 | MT473781 |
| Viticoideae –         | Vitex glabrata R. Br. | Kunming, Yunnan, China | 70,126,282 | 722 | MT473782 |
| Viticoideae –         | Vitex negundo var. cannabifolia (Siebold & Zucc.) Hand.-Mazz. | Kunming, Yunnan, China | 67,083,468 | 1387 | MT473783 |
| Viticoideae –         | Vitex quinata (Lour.) F.N. Williams | Mengla, Yunnan, China | 69,282,366 | 828 | MT473784 |
| Viticoideae –         | Vitex tripinnata (Lour.) Merr. | Guangzhou, Guangdong, China | 67,065,514 | 1404 | MT473785 |
| Viticoideae –         | Vitex yunnanensis W.W. Sm. | Luquan, Yunnan, China | 70,217,642 | 395 | MT473786 |
Sun ex C.Y. Wu), Isodoninae (Isodon spp.), Hypidinae (Mesorphaerum suaveolens (L.) Kuntze), Ociminae (Ocimum spp.), and Plectranthinae (Coleus spp.). Relationships within tribe Mentheae were also well resolved (100%, 1.00), with subtribe Salviinae recovered as sister to the remaining four subtribes, Prunellinae, Lycopinae, Menthinae, and Nepetinae.

Along the backbone of the tree, subsequent to the branching of the Nepetoideae, Symphorematoideae (100%, 1.00) and Viticoideae (100%, 1.00) formed a clade (i.e., Viticisymphorina sensu Li et al. [19]), which was followed by subsequent bifurcation supporting clades of the Tectonoideae (100%, 1.00), Premnoideae (100%, 1.00), and then Ajugoideae, respectively (Fig. 1, 100%, 1.00). Ajugoideae (100%, 1.00) were divided into four subclades that corresponded with the structure of tribal classification: each tribe was recovered as monophyletic and provided with high branch support (100%, 1.00). Within the Ajugoideae, Rotheceae were recovered as sister to the Teucrieae, Clerodendeae, and Ajugeae.

The sister clade of Ajugoideae was comprised of Peronematoideae, Scutellarioideae, Cymarioideae, and Lamioideae (i.e., the phylogenetically defined Perolamiina in Li et al. [19]). Monophyly of Ajugoideae plus Perolamiina was supported in all analyses with moderate support values (Fig. 1, 71%, 0.98; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5), and Peronematoideae were recovered as monophyletic and provided with high branch support (100%, 1.00). Within the Ajugoideae, Rotheceae were recovered as sister to the Teucrieae, Clerodendeae, and Ajugeae.

Discussion

It has been more than 20 years since the first attempt was made to employ molecular data as evidence to infer a phylogenetic tree for Lamiaceae, which made use of the rbcL region of the chloroplast genome [15]. Subsequently, various phylogenetic analyses have greatly contributed to our understanding of the circumscription, classification, and phylogeny of this family, progressively improving the resolution of relationships [15, 19, 25, 27–31, 44, 46, 52–54, 69]. This study, based on coding plastome sequences, provides the most comprehensive phylogeny of Lamiaceae at the tribal level to date. With increased taxon sampling and a vastly expanded DNA dataset, the results of our plastid phylogeny significantly clarify the remaining ambiguities for all relationships among subfamilies and provide better support for all nodes in the phylogenetic tree at the subfamilial level.

In our phylogenetic analyses, 12 subfamilies are recovered and well-supported as monophyletic (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5). Our results correspond with the most recent phylogenetic study using five cpDNA regions [19] and have resolved the placement of the Nepetoideae, Premnoideae, and Ajugoideae which were previously unknown. Nepetoideae, the largest subfamily of Lamiaceae, is sister to a grade of lineages comprising the Symphorematoideae, Viticoideae, Tectonoideae, Premnoideae, Ajugoideae, Peronematoideae, Scutellarioideae, Cymarioideae, and Lamioideae (Fig. 1). However, our results differ somewhat from those of the Mint Evolutionary Genomics Consortium [20], which used 520 single-copy nuclear genes from 48 Lamiaceae species representing 11 of 12 subfamilies. Their results of the first-diverging lineages were consistent with ours and only differ within the clade of Premnoideae, Ajugoideae, Peronematoideae, Scutellarioideae, Cymarioideae, and Lamioideae, where most of the relationships in their tree were weakly supported. Furthermore, taxon sampling was sparse in their study, and it is possible that additional taxon sampling

Table 2 Data characteristics with models selected for each dataset used for phylogenetic study in the present study

| Dataset    | CRM          | CR           | CR12         | CR3          | dePCS        |
|------------|--------------|--------------|--------------|--------------|--------------|
| GC content | 38.3%        | 38.3%        | 40.2%        | 34.5%        | 30.8%        |
| Alignment sites (bp) | 69,822       | 72,082       | 48,069       | 24,013       | 72,082       |
| Constant sites (bp) | 41,459       | 43,415       | 31,083       | 12,331       | 50,977       |
| Parsimony-informative sites (bp) | 29,945       | 20,185       | 11,561       | 8,624        | 14,473       |
| Variable sites (bp) | 28,363       | 28,667       | 16,986       | 11,682       | 21,105       |
| Missing data | 4.31%        | 4.31%        | 4.31%        | 4.31%        | 4.31%        |
| Best-fit model | GTR+I+G      | GTR+I+G      | GTR+I+G      | GTR+I+G      | GTR+I+G      |
could alter the subfamilial relationships that their analyses recovered.

Relationships within Lamioideae are also relatively similar with previous broad-scale studies [52, 53], but internal support values from our study are generally higher. Within Lamioideae, five genera (Betonica, Colquhounia, Galeopsis, Metastachydium, and Roylea) have not previously been assigned tribal status [2, 52, 53]. In addition, the phylogenetic position of Paralamium remains unclear [2, 53], since the genus has not been included in any published molecular phylogenetic study. We included three of these genera (Betonica, Colquhounia, and Galeopsis) in our study.

Colquhounia is recovered as sister (Fig. 1, 100%, 1.00) to the clade of Synandreae, Betoniceae, Galeopseae, Stachydeae, Paraphlomideae, Phlomideae, Leonureae, Marrubieae, Leucadeae, and Lamieae. The morphological distinctiveness and well-supported phylogenetic position of Colquhounia substantiates tribal recognition within Lamioideae as tribe Colquhounieae (see “Taxonomic treatment”).

Corroborating previous phylogenetic studies [52, 53], our chloroplast phylogeny demonstrates that Galeopsis and Betonica form a clade (Fig. 1, 64%, 0.98) that is sister to the Stachydeae (100%, 1.00). This clade in turn is recovered as sister to a clade of Paraphlomideae, Phlomideae, Leonureae, Marrubieae, Leucadeae, and Lamieae. Using cpDNA markers, Scheen et al. [52] and Bendiksby et al. [53] found this same structure, and our unpublished data based on chloroplast DNA markers (M. Bendiksby and Y. Salmaki, in prep.) also suggests these two genera occupy different positions within Lamioideae. In contrast, analyses using the low-copy nuclear pentatricopeptide repeat (PPR) region recovered Galeopsis as sister to tribe Synandreae rather than sister to Betonica, albeit this was provided with low support [69]. With the available evidence (see “Discussion”), the phylogeny supports that Betonica and Galeopsis are distinct from other tribes. As suggested by Li and Olmstead [51], “for the benefit of those who need a complete, rank-based classification of Lamiaceae to arrange genera and species in checklists”, a new monotypic tribe (i.e., Betoniceae) is established here and the tribe Galeopseae (also monotypic) is resurrected, to accommodate the
systematic positions of these two genera within Lamioideae. The tribal placement of the remaining three genera, Paralamium, Roylea, and Metastachydium, is still uncertain.

Within Ajugoideae, we recover the same relationships as reported by Xiang et al. [54], who sampled 51 taxa representing 22 of the 23 genera of the subfamily and identified four main clades. All clades are recovered as monophyletic and receive better resolution (Fig. 1). Although Xiang et al. [54] improved our understanding of relationships within Ajugoideae, a tribal classification scheme for the subfamily has been needed. Corroborating relationships within Ajugoideae, a tribal classification although Xiang et al. [54] improved our understanding of monophyletic and receive better resolution (Fig. 1). Al-

Representing 22 of the 23 genera of the subfamily and as reported by Xiang et al. [54], who sampled 51 taxa of complex dendritic trichomes (typically tomentose) covering branches, leaves, and flowers (except four species in the Westringieae).

Many taxonomic changes have been made for Chloantheae and its constituents. Since the description of Chloanthes and Pityrodia R. Br. [75], most genera were shuffled between different tribes of Verbenaceae [76, 77]. Most were allocated within the tribe Chloantheae (Verbenaceae) by Bentham [4]. This treatment was followed later by Hutchinson’s recognition as family Chloantheae [78], which was accepted by some authors [74, 79–83], but not all [84, 85].

Phylogenetic analysis of morphological [9] and molecular data [71] indicated that Chloantheae is sister to Westringieae within Lamiaceae, which is supported here (Fig. 1). The contemporary understanding of generic relationships within the tribe was informed by the comprehensively sampled molecular phylogeny of Conn et al. [24], which found that Pityrodia was not monophyletic, precipitating the description of Muniria N. Streiber & B.J. Conn and restoration of Dasymalla and Quoya Gaudich. [73]. Another new genus, Apatelantha, was recently described to accommodate a clade identified by Conn et al. [24] composed of individuals formerly assigned to Lachnostachys Hook., Newcastelia, and Physopsis Turcz. [86]. Although our study only samples three taxa in Chloantheae, as in previous studies [73], it supports the close relationship between Dasymalla and Chloanthes relative to Dicrastylis (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5).

**Tribe Westringieae Bartl.**

Westringieae consist of five genera and over ca. 210 species of subshrubs, shrubs, and small trees distributed across Australia [1]. Frequently found restricted to exposed and rocky or well-drained places, members of the tribe are distributed throughout habitats within which these places occur, from rainforests to ranges of the Australian arid inland.

Flowers are 5-merous and weakly to strongly zygomorphic, similar to bird or insect pollination syndromes typically found in other Lamiaceae [87–89]. The tribe
can be distinguished from Chloantheae by a four-lobed ovary, which develops into four nutlets [1]. The variation in anther morphology (e.g., outgrowth of the antherial connective of Prostanthera Labill.) combined with reductions in fertility (reduction of abaxial stamens to staminalodes in Westringia Sm.) in this tribe distinguishes it from Chloantheae (which typically has four bisericate anthers) and assists with informing the contemporary generic delimitation in the tribe [1, 90].

Tribal recognition of Westringiaceae and its generic constituency was first described by Bentham [91]. The monophyly of this tribe, in addition to its sister relationship to Chloantheae, has been substantiated by numerous phylogenetic analyses [9, 19, 71] including our own (Fig. 1). Further investigation into generic relationships has shown that Hemiandra R. Br., Hemigenia R. Br., Microcorys R. Br., and Westringia are closely related to each other with respect to Prostanthera [87, 90, 92], although the relationship between them still needs to be resolved by more comprehensively sampled phylogenetic studies.

Subfamily Callicarpoideae Bo Li & R.G. Olmstead
This recently described subfamily consists only of the genus Callicarpa which contains ca. 170 species of small trees or shrubs primarily distributed in tropical to temperate Asia, tropical and subtropical America, Australia, and some Pacific Islands [19, 51]. Callicarpoideae differs from other subfamilies by having a peltate or capitate stigma and a drupaceous fruit with four stony pyrenes [51]. Furthermore, Callicarpoideae possess actinomorphic flowers which are unusual within Lamiaceae (generally zygomorphic). The group is remarkably morphologically homogeneous given its broad geographical distribution, although there is variation in the number of flower parts and stamen structure among different species within Callicarpoideae.

Callicarpa was historically placed in Verbenaceae and treated as a member of tribe Callicarpeae in subfamily Viticoideae [5]. It was first transferred to Lamiaceae based on a cladistic analysis of morphological, anatomical, and palynological characters [9, 10] and later confirmed by molecular study [19]. Because only one or few representatives of the genus were included, different phylogenetic analyses resolved Callicarpa in different positions within Lamiaceae [19, 31, 52, 53, 71].

The sister relationship between Callicarpa and Prostantheroideae was first discovered by Olmstead et al. [71] and confirmed by subsequent studies [18–20, 31, 72]. In our analyses, they form a well-supported clade, which is sister to the remaining Lamiaceae (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5).

Subfamily Nepetoideae (Dumort.) Luerss.
Nepetoideae are the most species-rich subfamily within Lamiaceae, with about 3400 species divided into three tribes, Elsholtzieae, Mentheae, and Ocimeae [1]. Nepetoideae are native to every continent except Antarctica and are found in each of the seven global regions of high Lamiaceae diversity [1, 93]. Although only clarified when comparative pollen analyses were established [6, 8], Nepetoideae are now considered among the most clearly defined subfamilies of Lamiaceae and have consistently been supported as monophyletic in molecular analyses [15, 19, 31, 44, 94, 95]. Nepetoideae contain nearly all the aromatic species within Lamiaceae and are characterized by hexacolpate, trinucleate pollen [6, 8], an investing embryo [96], and the presence of rosmarinic acid [1]. Additionally, mucilaginous nutlets are only known to occur in the Nepetoideae within Lamiaceae and occur in all three tribes [97]. Thus, mucilaginous nutlets may also represent a synapomorphy within Nepetoideae.

The tribal assignment for groups now in Nepetoideae has been controversial [4, 5, 7] and was summarized by Cantino [10]. Results from morphological and molecular studies [9, 10, 95] led to a fundamentally new tribal classification for Nepetoideae proposed by Cantino et al. [11]. They recognized the four tribes Elsholtzieae, Occimeae, Lavanduleae, and Mentheae, with the latter containing the largest number of changes in circumscription. Harley et al. [1] basically adopted this treatment of Cantino et al. [11], with the exception of subsuming Lavanduleae within Occimeae. Although the three tribes of Harley et al. [1] are well-supported in both previous studies [16, 23, 27, 31, 98] and our analyses (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5), relationships among the three tribes remain murky. Previous studies have either found (1) Ocimeae to be sister to the Mentheae-Elsholtzieae clade [95], or (2) Mentheae to be sister to the Ocimeae-Elsholtzieae clade [16, 23, 27, 98], or (3) Elsholtzieae to be sister to the Mentheae-Ocimeae clade [31]. Our results reveal that Elsholtzieae is sister to Ocimeae in most of the analyses (CRM, CR, CR3, dePCS) (Fig. 1; Additional files 4, 5, 7: Figs. S2, S3, S5), but is weakly supported as sister to Mentheae by the dataset CR12 (Additional file 6: Fig. S4). Since none of the abovementioned relationships are strongly supported, nor a broad sampling within all three tribes are included in these studies, further studies are still needed to resolve the relationships among the three tribes.

Tribe Elsholtzieae (Burnett) R.W. Sanders & P.D. Cantino
Elsholtzieae are the smallest tribe of Nepetoideae, comprising eight genera and ca. 70 species mostly distributed across East and Southeast Asia. Collinsonia, which is
restricted to eastern North America, is the sole New World member of this tribe [1, 98]. Species of Elsholtzieae share divergent stamens, a weakly 2-lipped corolla, and an asymmetric disc with an elongate anterior lobe, but it is unclear whether these features are apomorphic [1, 31].

The tribe was formally validated by Sanders and Cantino [99] and consisted of six genera in the classification of Cantino et al. [11]: Collinsonia, Elsholtzia, Keiskea Miq., Mosla (Benth.) Buch.-Ham. ex Maxim., Perilla, and Perillula Maxim. In the molecular phylogenetic study of Nepetoideae by Wagstaff et al. [95], Elsholtzieae was represented by Elsholtzia, Collinsonia, and Perilla and formed a well-supported clade. Based on a sampling of all genera of Elsholtzieae using two nrDNA and four cpDNA markers, the results by Chen et al. [31] confirmed that the previously incertae sedis genus Ombrocharis is a member of the tribe and sister to Perillula. Contemporaneously, based on results from molecular phylogenetic analyses [31] and karyological studies [100], Mayta-Anco et al. [101] established a new genus, Vuhuangia Solomon Raju, Molinari & Mayta, to accommodate Elsholtzia flava (Benth.) Benth. and E. penduliflora W.W. Sm. However, Li et al. [98], apparently unaware of Vuhuangia, demonstrated that Elsholtzia was not monophyletic and outlined incertae sedis within Ocimeae, while Harley et al. [105] later established subtribe Hanceolineae to accommodate them.

Paton et al. [23] carried out the first molecular phylogenetic analyses of Ocimeae and revealed that the genus Lavandula L. was sister to the remaining Ocimeae and thus subtribe Lavandulinae was recognized within Ocimeae [23]. However, the two genera Hanceola and Siphocranion were not included in their analysis. The phylogenetic relationships within Ocimeae were further elucidated based on more comprehensive sampling by Zhong et al. [106], who demonstrated that Siphocranion, Hanceola, and Isodon each formed a distinct lineage within Ocimeae. The subtribes Siphocranioninae and Isodoninae were thus described to accommodate Siphocranion and Isodon, respectively, while subtribe Hanceolineae only includes Hanceola [106].

Recently, Chen et al. [107] reported a new species of Siphocranion, and in their molecular phylogenetic analyses based on six cpDNA markers, Siphocranioninae is shown to be sister to the remaining subtribes, with Lavandulinae further supported as the sister group of the clade including Hanceolineae, the Isodoninae-Hyptidinae clade, and the Plectranthinae-Ociminae clade. Our phylogenomic analyses largely confirm the results of Chen et al. [107], with the exception that Isodoninae is resolved as sister to the Hyptidinae-Ociminae-Plectranthinae clade (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5).

**Tribe Ocimeae Dumort.**

Ocimeae are characterized by stamens divergent or ascending (not declinate), a distinctly 2-lipped corolla (rarely weakly so), symmetric disc (if asymmetric and anterior lobe elongate, then corolla distinctly 2-lipped), and nutlets with an areolate abscission scar. Some of the most widely known medicinal and culinary plants are found within this group: mint, oregano, sage, savory, and thyme. Mentheae comprise both the largest number of genera and species of any tribe within Nepetoideae and Lamiaceae. Many of the plants in this group are of economic and ecological importance and thus have commonly attracted the attention of scientists. This has resulted in fundamentally differing taxonomic approaches at all taxonomic ranks, making it difficult to provide accurate numbers for genera (about 60) or species (at least 2000).

Due to the abovementioned fluidity regarding circumscription within Mentheae, the classification of Harley et al. [1] is regarded as the starting point for a modern subtribal classification. There, three subtribes were recognized, Menthinae, Nepetinae, and Salviinae, along with two genera of uncertain placement (Heterolamium C.Y. Wu and Melissa L.). Since the treatment of Harley et al. [1],
relationships within Mentheinae have been greatly clarified based on molecular phylogenetic studies [25, 27, 108–110]. Drew and Sytsma [27] accommodated Cleonia L., Hormium L., and Prunella L. in Prunellinae and erected a new subtribe, Lycopinae, for the enigmatic genus Lycopus L. (a tribe Lycopaeae was previously proposed [111]). Neoeplngia Ramamoorthy, Hiriart & Medrano along with Melissa were transferred to Salviniaceae [27] while Hyssopus L. and the previously unplaced Heterostamium were included in Nepetinae based on morphological [112] and molecular results [27, 113]. The currently accepted number of subtribes is thus five. This is also well-supported by our analyses, where Salviniaceae is sister to the other four subtribes; among the remaining subtribes, Nepetinae and Mentheinae are sister groups, with Prunellinae and Lycopinae as successive sister groups to Nepetinae and Mentheinae (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5).

Subfamily Symphorematoideae Briq.
Symphorematoideae contain about 21 species in three genera of woody climbers, Congea Roxb., Sphenodesme Jack, and Symphorema Roxb., and occur mainly in tropical regions of Asia. Symphorematoideae are characterized by having capitulate cymes surrounded by bracteoles which are often conspicuous, colorful, and accrescent, and incompletely 2-locular ovaries [19].

Historically, Symphorematoideae has been treated as a separate family with the same circumscription [114, 115] or (more commonly) as part of Verbenaceae [5, 116]. It was first found to be related to Lamiaeceae in the molecular era [15, 16], and then transferred to Lamiaceae and treated as a subfamily [1, 117]. Li et al. [19] were the first to include all three genera of Symphorematoideae in a comprehensive phylogenetic analysis of Lamiaeceae based on chloroplast sequences, and Symphorematoideae was found to be monophyletic and sister to Viticoideae. Such a sister relationship was further recovered in phylogenetic analyses based on nuclear genes [20] and confirmed in our phylogenomic analyses using plastome sequences (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5).

Subfamily Viticoideae Briq.
Viticoideae currently include ca. 280 species in three genera: Vitex (250 spp.), Teijsmanniodendron Koord. (23 spp.), and Pseudocarpodium Millspa. (9 spp.). These genera are distributed predominantly in the Tropics with a few species of Vitex occurring in temperate regions of the Northern Hemisphere [19].

Viticoideae as defined by Briquet [5] were a heterogeneous group whose circumscription has shrunk dramatically. Segregated from traditional Viticoideae are three subfamilies, Callicarpoideae, Premnoideae, and Tectonoideae in the present classification, and part of Ajugoideae and Scutellarioideae. Furthermore, the type genus of Viticoideae, Vitex, has expanded to include Paravitex H.R. Fletcher, Petitia Jacq., Tsongia Merr., and Viticipremna H.J. Lam based on molecular studies [19, 42]. Even though only three genera remain in Viticoideae as currently circumscribed, the intergeneric relationships are still questionable, with the positions of Teijsmanniodendron and Pseudocarpodium poorly resolved [19]. As mentioned above, the sister relationship between Viticoideae and Symphorematoideae is firmly supported, and the two subfamilies share several anatomical traits [19]. Morphologically, species of Viticoideae can be easily recognized by the palmately compound leaves and dry or fleshy drupes or schizocarps.

Subfamily Tectonoideae Bo Li & R.G. Olmstead
Tectonoideae comprise only the three species of Tectona. They are large trees native to tropical Asia from India to Southeast Asia, but are widely cultivated and naturalized in Africa, Central and South America, and the Caribbean [51].

Tectona was originally placed in tribe Tectoneae of Viticoideae [5], but was revealed to be sister to a large clade comprising Lamioideae, Cymarioideae, Scutellarioideae, Peronomatoideae, Ajugoideae, and Premnoideae [19]. The relationship is also confirmed by our analyses (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5). However, Tectona was recovered as sister to a larger clade including the aforementioned subfamilies (Cymarioideae not sampled) as well as Symphorematoideae and Viticoideae in an analysis using low-copy nuclear markers [20]. Regardless of phylogenetic position, Tectonoideae represents a genetically isolated clade in Lamiaeceae and has a series of distinct morphological traits [19, 51].

Subfamily Premnoideae Bo Li, R.G. Olmstead & P.D. Cantino
Premnoideae were recently established to include three former vitoid genera (Sensu Harley et al. [1]): Cornutia, Gmelina, and Premna [19], with the total species number estimated at about 150 (B. Li, pers. comm.). Nearly all species of this subfamily are woody shrubs, trees, or climbers, occurring mainly in Old World tropical to subtropical regions (Gmelina and Premna) and the New World Tropics (Cornutia) [19].

With the current circumscription, Premnoideae are well-supported in our phylogenomic trees (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5). However, in a phylogeny of Lamiaeceae based on nuclear genes, Cornutia was not recovered in Premnoideae but was sister to the Lamioideae-Ajugoideae-Peronomatoideae-Scutellarioideae clade [20, 72]. In the analyses of Li et al. [19], the relationships among Premnoideae, Ajugoideae,
and Lamioideae-Cymarioideae-Scutellarioideae-Peronematoideae were not well resolved, but in our phylogenomic analyses, Premnoideae are strongly supported to be sister to the clade comprising Lamioideae, Cymarioideae, Scutellarioideae, Peronematoideae, and Ajugoideae (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5).

**Subfamily Ajugoideae Kostel.**

Ajugoideae are the third-largest subfamily within Lamioideae and contain about 770 species in 23 genera [19, 48, 54, 118, 119] distributed worldwide but most common in tropical regions [1]. A possible synapomorphy of Ajugoideae may be pollen with branched to granular colu-
mellae [9].

Briquet [5] first elevated tribe Ajugeae sensu Bentham [4] to subfamilial rank, which was followed by most sub-
sequent treatments [1, 7, 116, 120]. Circumscription of Ajugoideae, however, has changed considerably. The recognition of some subfamilies (i.e., Teucrioideae and Caryopteridoideae) that include many traditionally ver-
benaceous genera (e.g., Caryopteris Bunge, Clerodendrum L., Schnabelia Hand.-Mazz., and Teucrium L.) was untenable. These genera were later transferred to Ajugoideae based on molecular phylogenetic [15, 16] and morphological evidence [121].

A recent phylogenetic study that sampled 22 out of the 23 genera of Ajugoideae and used four cpDNA markers (matK, rbcl, trnL-trnF, and rps16) strongly sup-
ported the monophyly of Ajugoideae and identified four major clades [54]. Relationships among these clades are consistent with the results in our study.

Currently, no tribal classification has been assigned for Ajugoideae. Although some old tribal names have been proposed [5, 91, 122], the circumscription of Lamioideae at that time was much narrower compared to our current understanding, and many genera now placed within Ajugoideae (e.g., Caryopteris, Clerodendrum, Rothecea, Schnabelia, Volkameria L.) were previously treated as members of Verbenaceae. Based on re-
results from both the present and previous studies [19, 54], we suggest that the four clades be recognized as tribes Ajugeae, Clerodendreae, Teucrieae, and Rothe-
ceae, with the last proposed here as a new tribe (see “Taxonomic treatment” below).

**Tribe Rothceae**

Rothceae are established as a new tribe (see “Tax-
onomic treatment” below) comprising four genera: Rotheca (60 spp.), Glossocarya Wall. ex Griff. (13 spp.), Discretitheca P.D. Cantino (1 sp.), and Karomia Dop. (9 spp.). The tribe is disjunctly distributed from Australia (Queensland) and tropical southern Asia to southern Af-
rica. No non-molecular synapomorphy has been found for this tribe.

Rotheca, the largest genus in this tribe, was resurrected by Steane and Mabberley [123] to maintain the mono-
phyly of the genus Clerodendrum [35]. In the present study, we demonstrate Rotheca to be sister to all other members of the subfamily, as reported by Yuan et al. [124]. Although only Rotheca was sampled here, a close relationship to the other three genera has been demon-
strated previously [54]. Steane et al. [36] found Karomia to be sister to Rotheca based on ndhF sequences, and this relationship was corroborated by Li et al. [19] based on five cpDNA markers. Xiang et al. [54] found that Karomia, Discretitheca, Glossocarya, and Rotheca formed a clade, but with moderate support. Discretitheca and Glossocarya were only first included in molecular phylogenetic analyses [54], and detailed morphological studies as well as molecular phylogenetic studies for these two genera are scarce and more studies are needed. As with Discretitheca and Glossocarya, only one species of Karomia (K. speciosa (Hutch. & Corbishley) R. Fern.) has been included in previous molecular phylo-
genetic analyses [36, 54], although DNA sequences of two species have been reported (the additional species is K. tettensis (Klotzsch) R. Fern. which was used mainly for ecological analyses [125]). Overall, the systematic re-
relationships within this tribe await to be fully clarified.

**Tribe Teucrieae Dumort.**

Teucrieae consist of ca. 260 species in three genera, Teu-
crium (ca. 250 spp.), Schnabelia (5 spp.), and Rubiteucris Kudô (2 spp.). The latter two genera are endemic to East Asia, while Teucrium has a subcosmopolitan distribu-
tion. A possible synapomorphy of the tribe is the conflu-
ce of anther thecae at anthesis, a feature that also characterizes Ajugeae, where it may have arisen indepen-
dently.

Teucrium is the largest genus in this tribe. A previ-
ous phylogenetic study [48] suggested the inclusion of Oncinocalyx F. Muell., Spartothamnella Briq., and Teucrium Hook.f. in Teucrium, and this treatment was confirmed by Xiang et al. [54]. Although both Rubiteucris and Schnabelia are small genera, the tax-
onomy and systematic relationships of Rubiteucris and Schnabelia were not sufficiently resolved until recent molecular phylogenetic studies based on a broad sampling [48, 54]. Here, the monophyly of Teucrieae is strongly supported (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5).

**Tribe Ajugeae Benth.**

Ajugeae contain 79 species in six genera: Ajuga L. (ca. 50 spp.), Amethystea L. (1 sp.), Caryopteris (7 spp.), Pseudocaryopteris (Briq.) P.D. Cantino (3 spp.), Trichos-
tema Gronov. (17 spp.), and Tripora P.D. Cantino (1 sp.). Ajuga is distributed primarily in Eurasia,
Amethystea is widespread in temperate Asia [1]. Trichostema is restricted to North America [126], and the remaining three genera are endemic to East Asia. A possible synapomorphy is the confluence of the anther thecae at anthesis (with a reversal in Caryopteris), a feature that also characterizes Teucriae and may have arisen independently in the two tribes. In most other species of Ajugoideae and in most of the closest outgroups, the thecae remain separate at anthesis. However, it is equally parsimonious to hypothesize that confluent anther thecae are a synapomorphy of the clade comprising Ajugoideae, Clerodendraceae, and Teucriae, with a subsequent reversal at the base of Clerodendraceae.

The traditionally delimited genus Caryopteris [5, 54, 127] is polyphyletic [9, 128] and species previously included in Caryopteris have been distributed in six genera: Caryopteris, Discretitheca, Pseudocaryopteris, Rubiteucris, Schnabelia, and Tripora, of which three were placed in tribe Ajugoideae, two belong to tribe Teucriae, and one belongs to tribe Rothoeae. A sister-group relationship between Tripora and Pseudocaryopteris was inferred in previous studies [54, 129, 130], but support values varied in different studies. The sister relationship between the North American genus Trichostema and the East Asian genus Caryopteris was also reported in many studies [15, 16, 35, 36, 130, 131]. Although Ajuga is the largest genus in this tribe, no phylogenetic study has been carried out for the genus to date, and infrageneric relationships within this genus still need further investigation.

Tribe Clerodendraceae Briq.
Clerodendraceae consist of ca. 350 species in ten genera: Clerodendrum (ca. 150 spp.), Volkameria (30 spp.), Kalaharia Baill. (1 sp.), Amasonia L.f. (8 spp.), Tetraclea A. Gray (2 spp.), Aegiphila Jacq. (120 spp.), Ovieda L. (21 spp.), Oxera Labill. (21 spp.), Hosea Ridl. (1 sp.), and probably Monochilus Fisch. & C.A. Mey. (2 spp.). Monochilus has not been included in any published molecular analysis, but based on a cladistic analysis of morphological data, Cantino [9] suggested a close relationship between Monochilus and Amasonia. Both genera usually have alternate to subopposite leaves, a rare feature in Lamiaceae. Monochilus was not included in the molecular results presented here but the presence of alternate to subopposite leaves suggests that Monochilus should be treated within tribe Clerodendraceae. However, this relationship needs to be tested using molecular evidence.

Clerodendraceae are pan-tropical/subtropical in distribution, predominantly distributed in the Americas, Africa, Asia, and Pacific Oceania. A probable synapomorphy for the tribe is a drupaceous fruit with four one-seeded pyrenes. In some species, the fruits split into four fleshy schizocarps. A similar fruit type is found in Rotheca (Tribe Rothoeae), where it apparently evolved independently. The character polarity is not entirely clear because Premnoideae also have drupaceous fruits. However, the fruits of Premnoideae contain a single four-seeded pyrene instead of four one-seeded ones. The other closely related groups (subfamilies Peronematoideae, Scutellarioideae, Cymarioideae, and Lamioideae) have dry fruits [19].

In terms of the number of genera, this is the largest tribe within subfamily Ajugoideae. Previous molecular phylogenetic studies concentrated mainly on two genera, Clerodendrum [34–36, 124] and Oxera [118, 119]. As a result of the disintegration of the traditionally defined Clerodendrum, some genera (i.e., Volkameria, Ovieda, Rotheca) were resurrected [34–36, 123, 124]. Species relationships within those genera, however, remain uncertain. In addition, relationships within the clade including Ovieda, Aegiphila, Clerodendrum, Tetraclea, Amasonia, Kalaharia, and Volkameria require further study.

Subfamily Peronematoideae Bo Li, R.G. Olmstead & P.D. Cantino
Peronematoideae were recently established to accommodate a well-supported clade comprising four small, mostly tropical Asian genera, Garrettiia (1 sp.), Hymenopyramis (7 spp.), Peronema (1 sp.), and Petraceovitex (8 spp.), which are sister to a larger clade formed by subfamilies Scutellarioideae, Cymarioideae, and Lamioideae [19]. These four genera were previously placed in the subfamily Caryopteridoideae of Verbenaceae [5, 132, 133] and were all transferred to Lamiaceae by Cantino et al. [11], with Hymenopyramis placed in Vitoioideae, Peronema and Petraceovitex in Teucrioidae, and Garrettiia in Ajugoideae. However, all the four genera were treated as incertae sedis in Harley et al.’s classification of Lamiaceae [1].

In recent molecular phylogenetic studies, Garrettiia was first inferred to be sister to a clade comprising Scutellarioideae, Acrynia, Cymaria, and Lamioideae [53], while the same sister relationship to an equivalent clade of the Scutellarioideae-Cymaria-Lamioideae clade (Acrynia was not sampled) was later found for a small well-supported clade comprised of Hymenopyramis, Petraceovitex, and Peronema [31, 44], as confirmed in our phylogenetic trees (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5). When Garrettiia, Hymenopyramis, Petraceovitex, and Peronema were included in the same analysis, they grouped together in a highly supported clade that is sister to the Scutellarioideae-Cymarioideae-Lamioideae clade [19]. Morphologically, the four genera are very heterogeneous but do share some common traits as noted by Chen et al. [44] and Li et al. [19].
**Subfamily Scutellarioideae (Dumort.) Caruel**

Scutellarioideae consist of ca. 390 species in five genera: Holmskioldia Retz. (1 sp.), Wenchengia (1 sp.), Renschia Vatke (1 sp.), Tinnea Kotschy ex Hook. f. (19 sp.), and Scutellaria L. (ca. 360 spp.) [1, 9, 121]. Species numbers and distribution of these genera are extremely uneven. Scutellaria is the largest and most widely distributed genus, having a cosmopolitan distribution [1, 134, 135]. Tinnea is much smaller and is distributed in tropical and southern Africa. The monotypic genera Renschia, Wenchengia, and Holmskioldia are endemic to Somalia, Southeast Asia (Hainan Island of China, Vietnam), and subtropical Himalayan regions, respectively. Scutellarioideae is diagnosed by the following synapomorphic characters: pericarps with tuberculate or elongate processes deae [15, 16, 19, 31, 44, 66, 136]. However, relationships within Scutellarioideae remain unresolved because Renschia has never been included in a molecular phylogenetic study. To date, four phylogenetic studies have focused on Scutellaria [66, 138–140], but none included a comprehensive taxon sampling of the genus or of Scutellarioideae as a whole. Thus, relationships within Scutellaria still need to be addressed in future studies.

**Subfamily Cymarioideae Bo Li, R.G. Olmstead & P.D. Cantino**

Cymarioideae were recently established to include two small genera that have previously been considered incertae sedis [1], Acrymia (1 sp.) and Cymaria (2 spp.), which are endemic to Southeast Asia.

Bendiksby et al. [53] found that Acrymia and Cymaria were the closest relatives of Lamioideae, which was supported by a subsequent study [44] but only with moderate support. Li et al. [19] further confirmed this relationship with high support values and consequently established a new subfamily, Cymarioideae, to accommodate the systematic position of the Acrymia-Cymaria clade. In the present study, Cymaria dichotoma Benth. is sister to Lamioideae in all analyses (Fig. 1; Additional files 4, 5, 6, 7: Figs. S2, S3, S4, S5).

Regarding the systematic placement of the Acrymia-Cymaria clade, two different treatments are feasible [19]. The Acrymia-Cymaria clade could be treated as a separate subfamily or as a distinct tribe within Lamioideae; both options are acceptable based on the principle of monophyly. However, as suggested by Bendiksby et al. [53] and Chen et al. [44], the inclusion of Acrymia-Cymaria within Lamioideae would make the subfamily morphologically heterogeneous and difficult to diagnose. The apomorphy of axial monochasial cymes which defines Cymarioideae is especially distinct and is not found within Lamioideae. Thus, we concur with the approach of Li et al. [19] and recognize Cymarioideae as a subfamily here.

**Subfamily Lamioideae Harley**

Lamioideae are the second largest subfamily within Lamiaceae, containing about 1260 species in 62 genera, with a near-cosmopolitan distribution, though concentrated in Eurasia and northern to tropical Africa [52, 53, 69].

Considerable progress has been made in our understanding of subfamily Lamioideae in recent years. Since Harley et al. [1], one genus has been established (Rydinaea Scheen & V.A. Albert [141]), four genera have been resurrected (Acanthoprasium (Benth.) Spenn. [53]; Betonica [52]; Plhnomoides Moench [142]; Pseudodictamnus Fabr. [33]), eight genera have been reduced to synonyms (Alajja Ikonn. and Sulaimania Hedge & Rech. f. [53]; Lamiophilomis Kudô, Notochaete Benth., and Pseuderecomostachys Popov [142]; Eremostachys Bunge [28]; Bostyranthera Benth. [43]; Stachyopsis Popov & Vved. [143]), and Holocheila, which was formerly treated as incertae sedis [1], has been shown to belong in Lamioideae [44]. Molecular phylogenies have also established that subfamily Cymarioideae is sister to Lamioideae [19].

A tribal classification of Lamioideae was the result of a molecular phylogeny based on cpDNA [52, 53]. The ten tribes have been corroborated as monophyletic groups using nuclear [143] and low-copy nuclear markers [67]. Four genera remained unplaced in the tribal classification because they formed monogenic clades [53, 67]; however, two new tribes, i.e., Colquhounieae and Betoniceae, are proposed here to accommodate the genera Colquhounia and Betonica, respectively. The monotypic Roylea has still not been attributed to a tribe. Roylea groups within tribe Marrubieae in some nuclear-based phylogenies, but not in all and not in phylogenies based on cpDNA data [33, 53, 67, 143]. To date, only two genera, Metastachydium and Paralumum, have still not been included in molecular phylogenetic studies of Lamioideae, and their relationship with the other genera remains enigmatic.

**Tribe Pogostemoneae Briq.**

Pogostemoneae consist of 11 genera as currently circumscribed [44, 52, 53], including Achyropermum...
Blume (25 spp.), Anisomeles R. Br. (26 spp. [144]), Cranioptome Rchb. (1 sp.), Colebrookea Sm. (1 sp.), Comanthosphace S. Moore (4 spp.), Eurysolesn Prain (1 sp.), Holocheila (1 sp.), Leucosceptrum Sm. (1 sp.), Microtoena Prain (19 spp.) [145], Pogostemon Desf. (80 spp.) [146, 147], and Rostrinucula Kudô. (2 sp.), and all genera are monophyletic [44, 52, 53, 145, 148]. Most genera of the tribe are distributed in East Asia to Southeast Asia, with three genera having a disjunct distribution between Asia and tropical Africa (Pogostemon, Achnypropermum, and Anisomeles). In addition, the monotypic genus Paralamium Dunn. is probably a member of Pogostemoneae based on the presence of small glossy nutlets [53].

Pogostemoneae were established by Briquet [5] and originally included seven genera (Elsholtzia, Comanthosphace, Keikkea, Pogostemon, Dysphylla Blume, Tetradenia Benth., and Colebrookea). Later, Kudô [149] and Press [150] circumscribed Pogostemoneae in a broad sense, adding 11 genera to the tribe [11, 52, 150]. A number of taxonomic and molecular phylogenetic studies [11, 19, 52, 53, 148, 150, 151] have indicated that six genera should be excluded from this tribe and that Dysphylla should be merged with Pogostemon, as suggested by Hasskarl [152] and Press [150], leaving the present total of 11 genera.

Cantino [10] and Cantino et al. [11] proposed a subfamily named Pogostemonoideae to include Colebrookea, Comanthosphace, Leucosceptrum, Pogostemon, Rostrinucula, Anisomeles, and Eurysolesn, but with hesitation regarding the two latter genera. Recent molecular phylogenetic studies have shown that Pogostemonoideae are sister to Lamioideae and have been included in that subfamily [52, 53]. Our results recover tribe Pogostemoneae as sister to the clade contain all other members of Lamioideae (Fig. 1). Previous studies based on plastid DNA regions [52, 53] identified two well-supported clades within Pogostemoneae. One clade includes Eurysolesn, Leucosceptrum, Rostrinucula, Comanthosphace, and Achnypropermum and is characterized by having dull and glandular nutlets, and the sclerenchyma region in the pericarp obsolete, indistinct, or absent. The second clade is composed of Colebrookea, Cranioptome, Microtoena, Anisomeles, and Pogostemon. Within this clade, two subclades were recognized [53]. Colebrookea is the only genus within the first subclade. This subclade is distinctive by possessing nutlets that are hairy and with eglandular hairs at the apex, while the remaining genera formed a second subclade united by having glossy and glabrous nutlets. Morphological studies focusing on traditionally defined Pogostemoneae (i.e., Pogostemonoideae; [153, 154]) identified some useful taxonomic characters. Subsequently, Scheen et al. [52], while not identifying any morphological synapomorphies, suggested that small and relatively glossy nutlets, pericarps (typically) lacking a sclerenchyma region [153, 154], generally long-exserted stamens with (usually) bearded filaments, a (generally) weakly 2-lipped corolla, and (generally) broad bracts are potentially useful morphological characters in defining the tribe. Further comparative morphological studies combined with well-supported phylogenetic trees based on extensive sampling and additional nuclear loci will be necessary to determine synapomorphies for this tribe.

**Tribe Gomphostemmateae Scheen & Lindqvist**

Gomphostemmateae were established by Scheen et al. [52] to include three genera, Gomphostemma Wall. ex Benth. (ca. 36 spp.), Chelonopsis Miq. (ca. 16 spp.), and Bostrychanthera (2 spp.), that are distributed in temperate to tropical East Asia [1, 155]. Since then, the genus Bostrychanthera was subsumed within Chelonopsis by Xiang et al. [43] based on morphological [156, 157] and molecular data [43] (see also Bongcheewin et al. [158]), thus leading to only two genera (Gomphostemma and Chelonopsis) currently retained in this tribe.

Gomphostemmateae were shown to be sister to a large group of Lamioideae in previous studies [52, 53], but these results were equivocal due to suboptimal support values. Here, we find the same relationship but with higher support values (Fig. 1). Possible synapomorphies for the tribe include pollen with branched columellae [159] and fibers in the mesocarp [160, 161]. However, pericarp structure has only been reported in a few species, and it is unclear whether unexamined species share these characters. Thus, future detailed morphological studies are needed.

**Tribe Colquhouniaceae**

Colquhouniaceae are newly established here to accommodate the enigmatic Colquhounia. The genus comprises approximately five species endemic to the Himalayan massif from Nepal and north India to southwest China and Vietnam. Morphologically, the genus is characterized by having nutlets winged at the apex, which is rare within subfamily Lamioideae [52]; besides Colquhounia, only some species of Chelonopsis have this character.

Based on trichome morphology, Hu et al. [162] classified the genus into two sections, Colquhounia sect. Simplicipilli C.Y. Wu & H.W. Li (including C. seguinii Vaniot) and C. sect. Colquhounia (all remaining species), but this classification was not supported by molecular phylogenetic results [43]. Although Scheen et al. [52] and Bendiksby et al. [53] found that Colquhounia occupied a phylogenetically distinct position within Lamioideae, they kept the genus unclassified at the tribal level, in part because only two species (C. coccinea Wall. and C. elegans Wall. ex Benth.) and only three markers...
(trnL-trnF, rps16, and matK) were used for phylogenetic reconstruction. In this study, five taxa were included for analyses and they form a distinct clade within Lamioideae. Thus, we describe this clade as a new tribe (see “Taxonomic treatment” below).

**Tribe Synandreae Raf.**

Synandreae were recircumscribed by Scheen et al. [163] to include the following five genera: *Brazoria* Engl. & A. Gray (3 spp.), *Macbridea* Elliott ex Nutt. (2 spp.), *Physostegia* Benth. (12 spp.), *Synandra* Nutt. (1 sp.), and *Warnockia* M.W. Turner (1 sp.). The only morphological synapomorphy for the tribe is a raceme-like inflorescence with sessile or very shortly pedicellate flowers [52, 163]. All five genera are characterized by having villous stamen filaments, but this is also found in some members of tribe Papogemoneae (e.g., *Pogostemon, Anisomeles*) and tribe Stachydeae [1] (*Chamae-sphacos* Schrenk ex Fisch. & C.A. Mey).

Previous studies involving cpDNA, nrDNA, and low-copy nuclear markers failed to adequately discern the position of Synandreae within Lamioideae [52, 53, 69, 163, 164]. Our results provide strong support for the placement of Synandreae, with the caveat that only one representative was included (Fig. 1). Only two lamiod tribe include species with a North American distribution, Synandreae and Stachydeae. The two tribes are not closely related [52, 53, 69] and therefore represent separate dispersals into North America [163].

**Tribe Betoniceae**

Betoniceae are newly established here to accommodate the phylogenetically and genetically isolated genus *Betonica* in Lamioideae. There are nine currently accepted *Betonica* species, three of which include 2–6 subspecific taxa distributed throughout Europe reaching Central Asia and Northwest Africa [165]. *Betonica* has repeatedly been included in, and excluded from, the genus *Stachys* L. Some authors have treated *Betonica* as a distinct genus [166–171], while Bentham [90] and Briquet [5] placed *Betonica* in its own section within *Stachys*. In the most recent morphological classification of *Stachys*, Bhattacharjee [172] recognized *Betonica* as a subgenus (S. subg. *Betonica* (L.) Bhattacharjee) within *Stachys*, defined by prominent sterile rosettes, usually unbranched flowering shoots arising from an axillary bud of the rootstock, and deeply crenate to serrate leaf margins; features that *Betonica* shares with the *Stachys* sections *Eriostomum* (Hoffmanns. & Link) Dumort. and *Ambleia* Benth. Yet, Bhattacharjee [172] mentions that *S. subg. Betonica* is divergent in the nature of the calyx (sessile calyx) and bracteoles (with a broad hardened base). Tomas-Báberán et al. [173] points to differences in phytochemistry between *Betonica* and *Stachys* species, as currently circumscribed. Recently, Giuliani and Bini [174] found that *Betonica* possesses only peltate trichomes, while *Stachys* has different types of large capitate hairs that are lacking in *Betonica*. In addition, Giuliani and Bini [174] also found that peltate trichomes of *Betonica* species have unusual secretion of flavonoids and essential oils and suggested that *Betonica* should be considered a genus of its own.

An early molecular phylogenetic analysis of *Stachys* s.l. [39] based on both plastid and nuclear DNA sequence data demonstrated that the type species of *Betonica*, *B. officinalis* L. (as *Stachys officinalis* (L.) Trevis.), fell outside of the clade that contained the remainder of *Stachys* including the type species, *S. sylvatica* L. Perhaps not being aware of this, Harley et al. [1] retained *Betonica* in synonymy under *Stachys*. Since then, further molecular phylogenetic evidence has corroborated the distinctness of *Betonica* [52, 53, 69]. Based on results from a comprehensive plastid phylogeny of Lamioideae that included five species of *Betonica*, Scheen et al. [52] suggested *Betonica* should be resurrected from synonymy under *Stachys*. The five species of *Betonica* formed a strongly supported clade sister to the remaining species under *Stachys*. Since *Betonica* so far has remained unclassified at the tribal level, and the genus seems to lack a clear affinity to any other lamiod taxon, we propose herein that the *Betonica* clade be recognized at the tribal level (see “Taxonomic treatment” below).

The intrageneric classification of *Betonica* into three sections [175, 176] (i.e., *B. sect. Betonica*, *B. sect. Foliosa* (Krestovsk. & Lazkov) Lazkov, and *B. sect. Macrosta-chya* (R. Bhattacharjee) Krestovsk.), has not received statistical support by any so far published molecular phylogenies [e.g., 52, 53]. *Betonica alopecuros* L., however, receives support as sister to the remaining species in these studies. The distinctness of *B. alopecuros* is also supported by morphology: yellow corollas with bifid upper lip and annulate corolla tubes [172, 177]. A more comprehensive study of this genus is needed.

**Tribe Galeopseae (Dumort.) Vis.**

In the present study, we propose resurrection of the tribe Galeopseae to accommodate the phylogenetically and genetically isolated genus *Galeopsis* in Lamioideae. Dumortier [167] established subtribe Galeopsinae (as “Galeopsisidae”) within the Stachydeae and included the two genera, *Galeopsis* and *Lamium* L. Later, Visiani [178] elevated subtribe Galeopsinae to the rank of tribe
as “Galeopsisideae” [98]) but included only *Galeopsis*. *Galeopsis* represents a morphologically highly distinct genus within subfamily Lamioideae, characterized by erect annual herbs with two conical protuberances near the base of the anterior lip of the corolla and anthers dehiscing by two valves, of which the upper is fimbriate [1, 179].

*Galeopsis* comprises 10 currently accepted species, two subspecies, and six documented hybrids [165]. The genus is distributed in temperate Eurasia with a center of species richness in Europe [180]. Strong support for the monophyly of *Galeopsis* was obtained in two recent molecular phylogenetic studies of Lamioideae that included three [52] and eight [53] species of *Galeopsis*, respectively.

Phylogenetically, *Galeopsis* holds a rather isolated, yet uncertain, position and has remained unclassified at the tribal level [52, 53, 69]. In both Scheen et al. [52] and Bendiksby et al. [53], *Galeopsis* was weakly supported as sister to *Betonica*, which in turn was sister to tribe Stachydeae, with even weaker support. Hence, a close relationship to *Lamiun* and *Lamastrum* Heist. ex Fabr., with which *Galeopsis* had been classified in most traditional classifications (e.g., subtribe Galeopsidinae Dumort.) based on the shared feature of a swollen corolla tube, was discarded. The sister relationship between *Betonica* and *Galeopsis* received some support from other sources of data. The two genera share the same base chromosome number [181] (x = 8), and flavonoid p-coumaryl glucosides are present in both *Betonica* and *G. subg. Galeopsis* [182]. The placement of *Galeopsis* in the nuclear PPR phylogeny by Roy and Lindqvist [69], however, does not support a sister relationship to *Betonica*, and *Galeopsis* falls out largely unresolved in their Lamioideae phylogeny. Although our current plastome-based phylogeny corroborates a sister relationship between *Galeopsis* and *Betonica*, support values remain low (Fig. 1). Based upon this phylogenetic uncertainty, the lack of support from nuclear data, and a goal of achieving taxonomic stability, we resurrect the tribe Galeopseae to encompass the single genus *Galeopsis*.

Reichenbach [169] divided *Galeopsis* into two subgenera, *G. subg. Galeopsis* and *G. subg. Ladanum* Rchb. Subgenus *Galeopsis*, is readily distinguished from *G. subg. Ladanum* by the presence of rigid hairs and swollen stem nodes in the former. The division of the genera into two equally sized subgenera is supported by phytogeography [182], crossing experiments [183], and molecular phylogenetics [53, 184]. *Galeopsis subg. Galeopsis* comprises the following five species: *G. bifida* Boern., *G. pubescens* Bess., *G. speciosa* Mill., *G. tetrahit* L., and *G. sulphurea* Jord. According to molecular analyses by Bendiksby et al. [184], the latter appears to represent a valid species, distinct from *G. speciosa*, and represents the most likely maternal parent to *G. tetrahit* (*G. pubescens* being the paternal parent). *Galeopsis subg. Ladanum* comprises the following five species: *G. ladanum* L., *G. nana* Otsch., *G. pyrenaica* Bartl., *G. reuteri* Rchb. f., *G. segetum* Neck. Species within *G. subg. Ladanum* have proven indistinguishable in DNA phylogenetic analyses involving nuclear (NRPAP2, 55-NTS) and chloroplast (*matK, psbA-trnH, rps16, trnL-trnF, and* trnS-trnG) DNA regions (M. Bendiksby, unpubl.). Morphologically, however, they appear highly distinct, and AFLP data (genomic fingerprint) group accessions according to species [M. Bendiksby, unpubl.]. Hence, the species of *G. subg. Ladanum* have probably diverged recently and the multilocus data suffers from incomplete lineage sorting.

**Tribe Stachydeae Dumort.**

Cosmopolitan Stachydeae are the largest and taxonomically most challenging alliance of all recognized tribes in subfamily Lamioideae [29, 30, 52, 53, 69]. Stachydeae have previously been the subject of several molecular phylogenetic investigations [30, 37–39, 52, 53, 185]. Lindqvist and Albert [39] revealed that three genera endemic to Hawaiian (dry fruited *Haplostaechys* (A. Gray) W.F. Hillebr., fleshly fruited *Phyllostegia* Benths., and *Ste-nogyne* Benths.) as well as the genera *Prasium* L., *Phlomidoschema* (Benth.) Vved., and *Sideritis* L. are nested within the large genus *Stachys*. Both *Prasium* with fleshy schizocarp and *Phlomidoschema*, which is characterized by a small corolla and branched hairs, are monotypic [1]. In contrast, *Stachys* comprises about 275 species and *Sideritis* comprises about 125 species [1]. This paraphyly of *Stachys* was corroborated by Scheen et al. [52] who showed that the Asian genera *Chamaephasacos*, *Suzukia* Kudô, and *Thuspeinanta* T. Durand also are embedded within *Stachys* and that the monotypic genus *Melittis* L. represents the sister to all other Stachydeae. In a later work, Bendiksby et al. [53] added *Hypogomphia* Bunge to the list of taxa nested within *Stachys*. Morphologically, the annuals *Chamaephasacos*, *Hypogomphia*, and *Thuspeinanta* are characterized by 1–3-flowered cymes and narrow nutlets, while *Suzukia* is recognized by a creeping habit and racemose inflorescences [1]. Thus, 12 genera and ca. 470 species are currently recognized in Stachydeae, but generic realignments are needed to reflect phylogenetic relationships. Scheen et al. [52] found no non-molecular synapomorphies for this diverse tribe, but listed the following characters as common among its members: calyx campanulate or weakly 2-lipped, calyx lobes often spiny, calyx throat often hairy, corolla strongly 2-lipped, anterior pair of stamens bending outwards after pollination, and nutlets usually apically rounded.
Besides some studies focusing on certain groups, such as Hawaiian [39, 185] and New World Stachys [30, 69], a comprehensive phylogenetic study of Stachydeae based on multiple loci analyses was performed by Salmaki et al. [29]. Analyses of nuclear ribosomal (nrITS) and plastid DNA data corroborated the monophyly of the tribe, with Melittis as sister to all remaining Stachydeae. Salmaki et al. [29] suggested the phylogenetic name “Eurystachys Y. Salmaki & M. Bendiksby” for the clade including all genera attributed to Stachydeae except Melittis. Although the plastid DNA markers provided well-supported backbone resolution in the Eurystachys clade, the nrITS phylogenetic tree recovered several groups with relatively poorly supported and short branches [29]. Therefore, detailed conclusions on the phylogenetic relationships in the Eurystachys clade needed using additional nuclear markers.

Recently, phylogenetic relationships in the Eurystachys clade utilizing two additional nuclear ribosomal DNA sequences (nrETS and 5S-NTS) provided high resolution allowing recognition of 12 well-supported clades within the Eurystachys clade, which also were recovered in the previous phylogenetic analyses using plastid DNA sequences [186]. The 12 clades were formally named in the Eurystachys clade following a PhyloCode nomenclature [187] and provided the basis for a future rank-based classification of Stachydeae with two options: (1) splitting the Eurystachys clade into 12 individual genera, each based on a pre-existing genus name and redefined to encompass additional taxa, but without clear morphological apomorphies; or (2) lumping of all these formal clades into a broadly defined Stachys, including widely recognized and morphologically well-defined segregates such as Prasium and Sideritis [186]. Clearly, more studies using various sources of evidence are needed to clarify the taxonomic borders in this tribe. A micro-morphological approach [153, 159–161, 188–192] at a global scale may provide a promising supplement to the more traditionally applied macro-morphological approaches.

**Tribe Paraphlomideae Bendiksby**

Paraphlomideae were established by Bendiksby et al. [53] to accommodate Matsumurella Makino (5 spp.), Ajugoides Makino (1 sp.), and Paraphlomis (Prain) Prain (ca. 25 spp.), together which have been found to form a distinct lineage within Lamioideae. Though the tribe has no clear synapomorphy, it can be distinguished from other tribes of Lamioideae by the following set of characters: herbs or subshrubs, indumentum of simple hairs, actinomorphic calyx, corolla (1/3) with hairy upper lip but scarcely bearded along the margin, included stamens, and an apically truncate ovary [1, 53, 193]. Most species of the tribe are restricted to East Asia (south China and Japan), with some species of Paraphlomis extending to Southeast Asia [1, 193].

**Tribe Phlomideae Mathiesen**

Based on the most recent molecular phylogenetic study of Phlomideae [28], the tribe now consist of only two genera: Phlomis L. (ca. 50–90 spp.) and Phlomoides (ca. 150–170 spp.).

Phlomideae were established by Mathiesen in Scheen et al. [52], in which six genera were recognized in the tribe: Eremostachys, Lamiophilomis, Notochaete, Phlomis, Phlomoides, and Pseudemostachys. Phlomideae are usually characterized by having calyx lobes abruptly narrowed to a narrow apex and expanded at the corolla margins that are bearded and densely pubescent outside and have branched hairs [52]. Mathiesen et al. [142] later reduced Pseudemostachys, Lamiophilomis, and one species of Notochaete (N. hamosa Benth.) to synonyms of Phlomoides. Combining multilocus molecular phylogenetic analyses and morphological evidence, Salmaki et al. [28] continued to show that Eremostachys, Notochaete, and Paraemostachys Adylov, Kamelin & Makhm should all be transferred to Phlomoides. Thus, the number of recognized genera in Phlomideae was reduced to two, i.e., Phlomis and Phlomoides. Species of Phlomis are shrubs or subshrubs with simple leaves, laterally compressed, flattened, sickle-shaped, but not fringed or incised upper corolla lips, and with nutlet pericarps possessing a sclerenchyma region (indistinct in a few species). In contrast, Phlomoides are herbaceous with simple or laciniate to pinnatisect leaves and with upper corolla lips that are arch-shaped, and always hairy or fringed-incised, but not laterally compressed or flattened, and have pericarps lacking a sclerenchyma region [194]. Phlomis have a mostly circum-Mediterranean distribution, while the centers of diversification of Phlomoides include Central Asia, the Iranian highlands, and China [28, 142, 195].

**Tribe Leonureae Dumort.**

Leonureae were recircumscribed by Scheen et al. [52] and Bendiksby et al. [53] based on phylogenetic and morphologic data. They are comprised of 80 species in six genera: Chaiturus Willd. (1 sp.), Lagochilus Bunge ex Benth. (45 spp.), Leonurus L. (24 spp.), Panzerina Sojak (2 spp.), Loxocalyx Hemsl. (3 spp.), and Lagopsis (Bunge ex Benth.) Bunge (5 spp.). The tribe is distributed primarily in Central Asia. Phylogenetic studies have shown that Lagopsis and Leonurus are poly- or parphyletic [53]. Possible morphological synapomorphies for the tribe are short stamens included in the corolla tube and more or less palmate venation and lobing of the leaves. The genus Loxocalyx lacks these characters but shares
zygomorphic calyces with longer abaxial lobes with many Leonureae.

**Tribe Marrubieae Vis.**

Marrubieae, with about 91 species, consist mostly of non-aromatic herbs or subshrubs, with thyroid inflorescences, few- to many-flowered cymes, widely campanulate to rotate calyces often with secondary calyx lobes, zygomorphic and 2-lipped corollas, and included or shortly exserted stamens [1]. The tribe is distributed from Europe to west and central Asia as well as North and South Africa with the highest number of species in southern Europe and North Africa [33].

The taxonomy and generic delimitations within Marrubieae have been controversial [33, 52, 53, 196–198]. Marrubieae contained three genera, *Ballota* L., *Marrubium* L., and *Moluccella* L. based on Scheen et al. [52]. Later, Bendiksby et al. [53] showed that the two species of *B. sect. Acanthoprasium* Benth. (*B. integrifolia* Benth., *B. frutescens* (L.) Woods) form a clade separate from the remaining species of *Ballota*. Therefore, Bendiksby et al. [53] resurrected the genus *Acanthoprasium* as proposed (but not formalized) by Scheen et al. [52]. The monotypic *Sulaimania* Hedge & Rech. f. was recovered as a member of the *Moluccella* clade and reduced to synonymy of *Moluccella* [53]. In a recent phylogenetic study of tribe Marrubieae using four plastid and one nuclear DNA locus (ITS), *B. sect. Beringeria* (Neck.) Benth. was raised to generic rank, as *Pseudodictamnus* Fabr. [33]. Therefore, the tribe now comprises five genera: *Acanthoprasium* (2 spp.), *Ballota* (3 spp.), *Marrubium* (ca. 50 spp.), *Moluccella* (8 spp.), and *Pseudodictamnus* (28 spp.) [33].

Members of the genus *Acanthoprasium* are shrubby and woody, have long spiny bracteoles, and occur in Europe, while species of *Pseudodictamnus* are herbaceous, have leafy bracteoles, and are predominantly Mediterranean-African in distribution [33, 91]. *Ballota* as now circumscribed includes herbaceous species covered by simple trichomes and are distributed from Europe to West Asia (including also the Mediterranean) [33]. *Marrubium* was also recircumscribed recently to include *B. deserti* (de Noé) Jury, Rejdali & A.J.K. Griffiths. There are around 50 species assigned to this genus, which are characterized by a bifid upper corolla lip and distributed from Macaronesia to temperate Eurasia.

**Tribe Leucadeae Scheen & Ryding**

Leucadeae were established by Scheen et al. [52] and include ca. 134 species in six genera: *Acrotome* Benth. ex Endl. (8 spp.), *Isoleucas* O. Schwarz (2 spp.), *Leonotis* (Pers.) R. Br. (9 spp.), *Leucas* R. Br. (ca. 100 spp.), *Otostegia* Benth. (ca. 8 spp.), and *Rydningia* (4 spp.). These genera are distributed from Africa through the Indian subcontinent to Queensland, Australia [199]. With a few exceptions, members of Leucadeae have a calyx that is distinctly zygomorphic with secondary lobes and a bearded margin of the upper lip of the corolla [52]. The latter character is also found in the genus *Phlomoides* [52]. The monophyly of Leucadeae has been corroborated using low-copy nuclear data [69], although only a small but representative selection of species was included in this study.

One molecular phylogeny has included a wide representation of species from all six genera, but only cpDNA markers were analyzed [199]. The large genus *Leucas*, with more than 100 species occurring on dry or disturbed ground in tropical to southern Africa and tropical and subtropical parts of Asia [1], was shown to be paraphyletic with respect to *Acrocone* and *Leonotis*, *Isoleucas*, and *Otostegia* [199]. Only a few of the Asian species of *Leucas* were included, but they formed a clade separate from the remaining *Leucas* [199]. More data are needed, including low-copy nuclear markers, before taxonomic changes can be proposed.

The genus *Otostegia*, as traditionally circumscribed, was clearly polyphyletic [199]. To make *Otostegia* monophyletic, the genus *Rydningia* was described to accommodate four Asian species, one species was transferred to *Isoleucas*, and one species was transferred to *Moluccella* [141]. Since then, an additional four species of *Otostegia* have also been transferred to *Moluccella* [53] (see also the discussion on tribe Marrubieae). Thus, the recircumscribed *Otostegia* is reduced to ca. eight species, most of which are endemic to Africa [53], with *O. fruticosa* (Forssk.) Schweinf. ex Penz. extending to the Arabian Peninsula [200].

Molecular phylogenies have resolved *Rydningia* as sister to the rest of Leucadeae, with this relationship recovered based on cpDNA [52, 53] and low-copy nuclear DNA [69]. However, more data are still needed to resolve the generic boundaries of the paraphyletic genus *Leucas* in relation to *Acrocone*, *Isoleucas*, *Leonotis*, and *Otostegia*.

**Tribe Lamieae Coss. & Germ.**

Lamieae are comprised of four genera: *Lamium* (including *Wiedemannia* Fisch. & C.A. Mey and *Lamiastrum*; ca. 25 spp.), *Eriophyton* Benth. (including *Alajja*; ca. 8 spp.), *Stachyopsis* (4 spp.), and possibly *Menitiska* (Krestovsk.) Krestovsk. (1 sp.). These genera are widely distributed in the temperate and subtropical regions of Europe, Asia, and Northern Africa. Five East Asian species of *Galeobdolon* and *Lamium chinense* Benth. were transferred into the genus *Matsumurella* in tribe Paraphlomideae by Bendiksby et al. [53]. Possible morphological synapomorphies for the tribe are hairy anthers (except for *Lamium galeobdolon* L., *L. flexuosum* Ten.,...
of Lamiaceae. Morphologically, however, Stachyopsis intermediates between Lamium and Wiedemannia, but did not have sufficient sampling to assess monophyly of Lamium, e.g., if Lamiastrum and Wiedemannia were excluded. Subsequent studies, with more complete sampling of Lamium, found Lamiastrum to be nested within Lamium [203, 204].

Bendiksby et al. [53] also determined that two other genera, Eriophyton (including Alajja and three species of Lamium) and Stachyopsis, should be included in tribe Lamiaeae. Bendiksby et al. [143] found that Stachys tibetica Vatke (= Mentiskia tibetica (Vatke) Krestovsk.) did not belong in Stachys (tribe Stachydeae), but was most closely related to Stachyopsis in Lamiaeae. Morphologically, however, S. tibetica has an intermediate position between Stachyopsis and Eriophyton. They expanded Eriophyton to include Stachyopsis and S. tibetica, in order to make Eriophyton monophyletic. Lazkov and Sennikov [176] stated that the genus Stachyopsis is similar to Eriophyton but differs in the habit, shape of leaves (oblong-ovate vs. broadly rhomboid-ovate), and shorter flower tube which is enclosed within the calyx; therefore, they suggested that the genus Stachyopsis should retain its generic status. At the same time, they resurrected Menitskia to accommodate S. tibetica as Menitska tibetica. The genus Menitska differs from Eriophyton and Stachyopsis by its narrower posterior corolla lip, stiffer bracteoles, and often deeply crenate to lobed leaves [143, 205].

**Taxonomic treatment**

**Colquhounieae**

C.L. Xiang, Bo Li & R.G. Olmstead, **trib. nov.** Type: Colquhounia Wall.

Shrubs erect or ascending. Stems and branches terete, with simple and/or branched hairs. Leaves toothed, petiolate; inflorescence thyrsoid, pedunculate to subsessile; cymes 1–5-flowered. Calyx tubular-campanulate, 10-veined, 5-lobed, lobes often equal. Corolla strongly 2-lipped, 4-lobed (1/3), often purple, sometimes spotted; posterior lip moderately long, hooded with upcurved margins, anterior lip slightly subequally 3-lobed, corolla tube strongly dilated distally; stamens 4, not exserted from corolla, thecae ± confluent; stigma lobes unequally 2-cleft. Nutlets narrowly obovoid-oblong, winged at apex.

Colquhounieae consist of one genus and approximately five species, occurring from Nepal, across north India to southwest and central China and Vietnam.

**Rothceae**

C.L. Xiang, Bo Li & R.G. Olmstead, **trib. nov.** Type: Rotheca Raf.

Shrubs, subshrubs, and perennial herbs. Leaves simple, opposite, or whorled with 3–4 leaves per node, often toothed. Flowers often in terminal and/or axillary cymes. Calyx actinomorphic, 5-lobed or truncate. Corolla ± zygomorphic, expanding abruptly on lower side only; 5 lobes ± unequal, anterior corolla lobe frequently much larger than the other four, limb in bud asymmetrical. Stamens 4, didynamous to subequal, long-exserted; anthers usually basifixed (occasionally approaching versatile). Ovary unlobed in flower but becoming imperfectly 4-lobed during fruit development. Style terminal, stigma lobes frequently unequal. Drupes (2–) 4-lobed, mesocarp ± fleshy, endocarp separated into 4 stones or 2 pairs of stones.

The tribe contain four genera, Rotheca (60 spp.), Glossocharya (9 spp.), Discretitheca (1 sp.), and Karomia (9 spp.), and are distributed in tropical southern Asia to southern Africa, and Australia (Queensland).

**Betonicae**

Bendiksby & Salmaki, **trib. nov.** Type: Betonica L.

Perennial herbs. Leaves deeply crenate-dentate. Flowering stems unbranched, lateral to rootstock, verticillasters condensed (rarely remote), 16–20-flowered. Bracteoles scarious or herbaceous, apex spineous, base broad and hardened. Flowers sessile, median lobe of lower corolla lip emarginate. Calyx sessile, ± regular. Another cells subparallel to parallel.

Betonicae are monotypic comprising the genus Betonica with about 10 species distributed in western Eurasia.

**Conclusions**

This is the first study to use plastome data to estimate family-wide relationships within Lamiaceae. We demonstrate that increased taxon sampling in concert with phylogenomic analyses based on plastome sequence data provides superior support and resolution at both deep and shallow nodes relative to previous studies and offers new insights into phylogenetic relationships among and between tribes and subfamilies of Lamiaceae. The monophyly of all 12 subfamilies is corroborated, and we recognize a total of 22 tribes within Lamiaceae, three of which are newly established here (i.e. Colquhounieae, Rothceae, and Betonicae). This study provides a detailed summary of the taxonomic history, generic and species diversity, morphology, synapomorphies, and
distribution for each tribe and subfamily, representing the most comprehensive overview of Lamiaceae since Harley et al. [1]. The classification presented herein is the most definitive tribal-level taxonomy of the mint family to date, and the robust phylogenetic backbone of Lamiaceae reconstructed here provides an extendable dataset for future studies on mint family classification, biogeography, character evolution, and diversification.

Materials and methods

Taxon sampling
In this study, plastomes of 50 taxa were newly sequenced and 61 taxa were reassembled from the sequence read archive (SRA) database; others were acquired from previous studies [66, 67, 206, 207] or downloaded from NCBI (https://www.ncbi.nlm.nih.gov; Additional file 8: Table S3). In total, the ingroup sampling included 170 taxa (175 accessions), 79 genera, and represented all 15 currently recognized tribes and all 12 subfamilies within Lamiaceae [19, 51]. Twenty-two species from five families of Lamiales (Mazaceae, Orobanchaceae, Phrymaceae, Paulowniaceae, and Wightiaceae) were selected as outgroups based on phylogenetic results of previous studies [18, 22, 208]. Voucher specimens of the newly sequenced taxa (Table 1) were deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN).

DNA isolation and sequencing

DNA was extracted from healthy and fresh leaves frozen in liquid nitrogen or dried in silica gel using the CTAB protocol of Doyle and Doyle [209] and sheared into ca. 300 bp fragments using a Covaris M220 Focused-ultrasonicator. Libraries for paired-end (PE) Illumina sequencing were constructed from fragmented genomic DNA following the standard protocol of manufacturer (NEBNext® Ultra II”DNA Library Prep Kit for Illumina”) and sequenced from both ends of 150 bp fragments on the Illumina HiSeq 2000 platform (Illumina, San Diego, CA, USA) at BGI Genomics (BGO-Shenzhen, China). Approximately 2–10 GB of raw data was generated with 150 bp paired-end read lengths.

Plastome assembly and annotation

Quality control of raw sequence reads was carried out using FastQC toolkit [210] (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) with the parameter set as Q≥25 to acquire high-quality clean reads. The de novo assembling of the plastome was implemented in the GetOrganelle pipeline [68], in which plastome reads were extracted from total genomic reads and then SPAdes v.3.10 [211] was used for assembly. For those plastomes we can acquire complete sequences, genome annotation was performed using Geneious v.11.0.3 [212], and the start and stop codons were manually adjusted by comparison with the plastome of Salvia miltiorrhiza Bunge [213] (HF586694). The online tRNAscan-SE web servers [214] were used to confirm the tRNA genes. Circular plastome maps were drawn using the Organellar-GenomeDRAW tool [215]. For 19 species, the plastomes were assembled from RNA-seq data and only contigs were obtained. Bowtie2 [216] was then used to map contigs to the reference sequences extracted from S. miltiorrhiza [213].

Sequence alignment and dataset generation

Since noncoding regions can be variable even among species and are often difficult to align across a family as large as Lamiaceae, only 79 protein-coding genes were used for phylogenetic analyses. Alignments of individual loci were performed using the MAFFT v.7.308 [217] plugin in Geneious v.11.0.3 [212] with G-INS-I algorithm, and the final alignments were manually adjusted in PhyDE v.0.9971 [218].

Since the plastome is uniparentally inherited in most angiosperms and generally does not undergo recombination, sequences of the 79 coding genes were concatenated in our study to generate a supermatrix of all coding regions (CR). Removal of problematic aligned regions may result in a better resolved phylogeny [219]; therefore, ambiguously aligned positions (e.g., characters of uncertain homology among taxa and single-taxon insertions; see [31, 46]) were removed manually in our analyses to construct the “Coding region manual” dataset (CRM, Additional file 3: Table S2).

Additional matrices for the 79 genes were constructed based on (1) the 1st and 2nd codon positions (CR12); (2) only the 3rd codon positions (CR3); and (3) the degenerated coded sequences (dePCS) generated using Degenerate v.1.4 (http://www.phylotools.com/). Thus, a total of five datasets (CR, CRM, CR12, CR3, dePCS) were used in subsequent analyses.

Phylogenetic analyses

Phylogenetic trees based on all datasets were built by two approaches including Bayesian inference (BI) analysis and maximum likelihood (ML) analysis. jModelTest v.2.1.4 [220] was used to determine the best-fit models for nucleotide sequences for BI analyses.

Bayesian analyses were executed using MrBayes v.3.2.2 [221]. Four iterations of 50,000,000 generations were run on four chains, sampling every 1000 generations on the Cyberinfrastructure for Phylogenetic Research Science (CIPRES) Gateway v.3.3 server [222] (http://www.phylo.org/). Default priors, unlinked parameter estimates, and best-fit models suggested by jModelTest v.2.1.4 [220] for each dataset were used for each iteration. Convergence of runs was accepted when the average standard
deviation of split frequencies (ASDSF) dropped below 0.01. Tracer v.1.6.0 [223] was used to inspect the convergence of model parameters and check whether the values of effective sample size (ESS) were ≥ 200. A majority-rule consensus tree was created from the runs, after a 25% burn-in. All resulting trees with nodal support values were visualized and edited in FigTree v.1.4.2 [224]. ML analyses were performed using RAxML v.8.2.9 [225] as implemented in the XSEDE interface of CIPRES [222]. The GTRCAT model was used for analyses and bootstrapping; bootstrap iterations (~#|–N) were set to 1000, and other parameters used the CIPRES default settings.

We defined branches with posterior probabilities (PP) < 0.90 and bootstrap values (BS) < 70% as weakly supported, PP = 0.90–0.95 and BS = 70%–80% as moderately supported, and PP > 0.95 and BS ≥ 80% as strongly supported [107]. The alignments and ML tree are deposited at TreeBase with study #S26639 (http://treebase.org/treebase-web/phylowas/study/TB2:S26639?x-access-code=bb02a4c5bc226f4604690ea0f21cccd41&format=html) [226].

Supplementary Information
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Additional file 1: Figure S1. Gene maps of the complete chloroplast genomes newly sequenced in this study. Genes inside and outside of the circle are transcribed in the clockwise and counterclockwise directions, respectively. Genes belonging to different functional categories are color-coded.

Additional file 2: Table S1. Features of newly sequenced plastomes.

Additional file 3: Table S2. Excluded ambiguous sites for 79 genes of coding regions (dataset CRM).

Additional file 4: Figure S2. Phylograms inferred from ML analysis of concatenated nucleotide sequences of 79 protein-coding genes (dataset CR). A, phylogram showing branch lengths, where tip names are absent follow the same order as shown in B. Scale bar represents the mean number of nucleotide substitutions per site. B, maximum likelihood bootstrap support values and Bayesian inference posterior probabilities are shown above and below the branches, respectively.

Additional file 5: Figure S3. Phylograms inferred from ML analysis of concatenated nucleotide sequences of the 3rd codon positions (dataset CRM). A, phylogram showing branch lengths, where tip names are absent follow the same order as shown in B. Scale bar represents the mean number of nucleotide substitutions per site. B, maximum likelihood bootstrap support values and Bayesian inference posterior probabilities are shown above and below the branches, respectively.

Additional file 6: Figure S4. Phylograms inferred from ML analysis of concatenated nucleotide sequences of the 1st and 2nd codon positions (dataset CR2). A, phylogram showing branch lengths, where tip names are absent follow the same order as shown in B. Scale bar represents the mean number of nucleotide substitutions per site. B, maximum likelihood bootstrap support values and Bayesian inference posterior probabilities are shown above and below the branches, respectively.

Additional file 7: Figure S5. Phylograms inferred from ML analysis of concatenated nucleotide sequences of the degeneracy nucleotide sequence (dataset dePCG). A, phylogram showing branch lengths, where tip names are absent follow the same order as shown in B. Scale bar represents the mean number of nucleotide substitutions per site. B, maximum likelihood bootstrap support values are shown above the branches.

Additional file 8: Table S3. List of taxa sampled with information related to taxonomy, GenBank accession numbers, references, and vouchers. Herbarium acronyms follow Index Herbariorum [227].

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Authors’ contributions
CLX, BL, FZ, YPC, HP, and RGO conceived this research. FZ, YPC, BTD, TCW, FC, and CLX collected materials. FZ performed the experiments. FZ, YPC, and XCL analyzed the data. All the authors wrote the manuscript. All authors read and approved the final version of manuscript.

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Availability of data and materials
All the newly sequenced and annotated plastomes in the present study were submitted to the National Center for Biotechnology Information (NCBI) database with accession numbers MT473738–MT473786 (Table 1). Other plastomes analyzed were acquired from previous studies [66, 67, 206, 207] or downloaded from NCBI (https://www.ncbi.nlm.nih.gov; Additional file 8; Table S3). The alignments and ML tree are deposited at TreeBase with study #S26639 (http://treebase.org/treebase-web/phylowas/study/TB2:S26639?x-access-code=bb02a4c5bc226f4604690ea0f21cccd41&format=html) [226].

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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