Systematic Placement of the Enigmatic Southeast Asian Genus *Paralamium* and an Updated Phylogeny of Tribe Pogostemoneae (Lamiaceae Subfamily Lamioideae)

Fei Zhao†, Yi-Wen Wu‡, Bryan T. Drew§, Gang Yao¶, Ya-Ping Chen†, Jie Cai†, En-De Liu†, Bo Li* and Chun-Lei Xiang*

1 CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China, 2 College of Life Sciences, Shaanxi Normal University, Xi’an, China, 3 Department of Biology, University of Nebraska at Kearney, Kearney, NE, United States, 4 South China Limestone Plants Center, College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou, China, 5 Germplasm Bank of Wild Species in Southwest China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China, 6 Research Centre of Ecological Sciences, College of Agronomy, Jiangxi Agricultural University, Nanchang, China

*Correspondence: Chun-Lei Xiang
xiangchunlei@mail.kib.ac.cn
Bo Li
hanbolijx@163.com
†These authors have contributed equally to this work

**Specialty section:**
This article was submitted to Plant Systematics and Evolution, a section of the journal Frontiers in Plant Science

**Received:** 25 December 2020
**Accepted:** 22 March 2021
**Published:** 16 April 2021

**Citation:** Zhao F, Wu Y-W, Drew BT, Yao G, Chen Y-P, Cai J, Liu E-D, Li B and Xiang C-L (2021) Systematic Placement of the Enigmatic Southeast Asian Genus *Paralamium* and an Updated Phylogeny of Tribe Pogostemoneae (Lamiaceae Subfamily Lamioideae). Front. Plant Sci. 12:646133. doi: 10.3389/fpls.2021.646133

**Keywords:** Lamioideae, molecular phylogenetics, nutlet morphology, plastome phylogenomics, *Paralamium*, Pogostemoneae

**INTRODUCTION**

Lamiaceae, as currently defined, contains about 7000 species and is subdivided into 12 subfamilies (Li et al., 2016; Li and Olmstead, 2017; Zhao et al., 2021). Lamioideae, containing at least 1260 species and about 61 genera, is the second-largest subfamily (after Nepetoideae) within Lamiaceae in terms of both the number of species and genera (Harley et al., 2004). Although the subfamily has a subcosmopolitan distribution, it is most common in southwest Asia and the Mediterranean region, China, and sub-Saharan Africa. During the past two decades, relationships and circumscription of constituent genera of Lamioideae have largely been clarified through both morphological (Abu-Asab and Cantino, 1992, 1994; Cantino, 1992a,b; Cantino et al., 1992; Ryding, 1994a,b,c, 1995, 1998, 2003, 2008; Salmaki et al., 2008; Xiang et al., 2013a; Seyedi and Salmaki, 2015) and molecular
phylgenetic studies at various taxonomic levels (Wink and Kaufmann, 1996; Lindqvist and Albert, 2002; Scheen and Albert, 2007, 2009; Scheen et al., 2008, 2010; Bendiksby et al., 2011, 2014; Salmaki et al., 2012, 2013; Xiang et al., 2013b; Chen et al., 2014; Roy and Lindqvist, 2015; Li et al., 2016; Yao et al., 2016; Siadati et al., 2018). In particular, the molecular phylogenetics analyses of Scheen et al. (2010), Bendiksby et al. (2011), and Zhao et al. (2021) have dramatically improved our understanding of both tribal classification and character evolution within Lamioideae. Systematic positions of several enigmatic genera which were previously unplaced within Lamioideae have been recently elucidated (Scheen et al., 2010; Bendiksby et al., 2011; Chen et al., 2014; Roy and Lindqvist, 2015; Olmstead, 2016; Zhao et al., 2021), while a few genera, namely the rare and monotypic Paralamium Dunn. and Metastachydium Airy Shaw ex C.Y. Wu & H.W. Li, and Roylea Wall. ex Benth., remain unclassified at the tribal level because of insufficient molecular data available to date. The aforementioned Paralamium and Metastachydium have not been included in any published molecular phylogenetic study.

The genus Paralamium was originally described by Dunn (1913) and reported to be endemic to southeast Asia with a sporadic distribution in humid regions of southwestern China (subtropical Yunnan), northern Vietnam, northern Burma, and eastern India (Assam) (Li and Hedge, 1994; Harley et al., 2004; Suddee and Paton, 2004). The genus is distinguished from other Lamioideae genera mostly based on calyx morphology. Paralamium has unequal calyx-lobes, with the posterior calyx tooth being the largest and having a truncate apex flanked by smaller triangular lateral lobes, and lanceolate-triangular anterior lobes (Figure 1). Harley et al. (2004) called this unique calyx morphology a 1/2/2 split, while Li and Hedge (1994) recognized this shape as a 1/4 split. In addition, this genus is characterized by possessing very small pollen grains with the polar length and/or equatorial width less than < 18 µm (Harley et al., 2004), which is an uncommon feature within Lamioideae.

Paralamium is monotypic, with the sole species, P. gracile Dunn (1913) described on the basis of a specimen collected from Yunnan, China (Henry 10636). However, before the description of this species, Hooker (1885) described Plectranthus griffithii Hook.f. based on a collection from eastern Assam, India (Herb Griffith 4056). After careful examination of the type materials, Suddee and Paton (2004) suggested that Plectranthus griffithii Hook.f. and Paralamium gracile Dunn. were conspecific. Thus, they formerly transferred the former species to Paralamium and a new combination, Paralamium griffithii (Hook.f.) S. Suddee & A.J. Paton, was created, making the latter species (Paralamium gracile) a synonym.

The systematic position of Paralamium has been enigmatic ever since its original description. When establishing the genus, Dunn (1913) noted that the calyx is the “most striking” character of Paralamium and similar to Orthosiphon Benth. (Nepetoideae), Coleus Lour. (Nepetoideae) and Teucrium L. (Ajugoideae) by virtue of the following calyx characters: a broad upper calyx tooth with recurved decurrent margins and a conspicuously veined calyx tube. However, in the protologue for Paralamium (Dunn, 1913), the genus was also considered to be closely related to Lamium L. (Lamiodeae) based on nutlet and corolla characters, hence the name “Paralamium” which can be translated to mean “resembling Lamium.” Studies on the genus after its original description have been scarce. Li (1977) placed Paralamium within subtribe Lamini of tribe Lamiae in subfamily Lamioideae sensu Briquet (1895–1897) based on its morphology provided in the protologue (Dunn, 1913). Later, Cantino and Sanders (1986) considered Paralamium as an anomalous genus within Lamioideae because of its morphology similar to various genera in different subfamilies, but discreetly suggested that it could probably be related to Lamium based on their similar tricolpate and two-celled pollens observed by Abu-Asab and Cantino (1994). Harley et al. (2004) also placed Paralamium within Lamioideae in their comprehensive classification of Lamioideae. In the most recent classifications of Lamioideae based on molecular data (Scheen et al., 2010; Bendiksby et al., 2011), Paralamium was provisionally treated as incertae sedis within Lamioideae but additionally suggested to be a member of tribe Pogostemoneae based on nutlet morphology (e.g., small glossy nutlets) (Bendiksby et al., 2011). While in the updated online synoptical classification of Lamiales, Olmstead (2016) placed Paralamium within tribe Stachydeae of Lamioideae. However, Paralamium has never been included in a published molecular phylogenetic analysis, making the above empirical placement of Paralamium within Lamioideae untested.

The main reason that Paralamium has not been included in any molecular phylogenetic studies is a lack of suitable leaf tissue for DNA extraction. However, during collecting expeditions in the Yunnan province of China in 2018 and 2019, we discovered two populations of P. griffithii. These collections allowed us to investigate the phylogenetic position of this monotypic and enigmatic genus based on molecular data. Here, using both plastid and nuclear ribosomal DNA markers, we present molecular phylogenetic analyses using different sampling strategies to finally establish the tribal affinities of Paralamium within Lamioideae and provide an updated phylogeny of the tribe Pogostemoneae. Furthermore, we provide a dichotomous key for genera within Pogostemoneae.

MATERIALS AND METHODS

Field Collections
Specimens from two populations of Paralamium griffithii were collected from Malipo County (Liu et al. 7859) and Jinping County (Z.Y. Cai and X.E. Ye 22-36) within the Yunnan Province of China. Fresh leaves were collected and dried with silica gel. Voucher specimens were deposited in the Herbarium of Kunming Institute of Botany (KUN), Chinese Academy of Sciences.

Taxon Sampling and Genetic Markers Selected
In order to better evaluate the systematic position of Paralamium and assess the phylogenetic relationships of this enigmatic genus and related genera, we experimented with three datasets. The first dataset included 79 plastid protein-coding genes within Lamioideae (dataset CP79) aiming to confirm the subfamilial
position of *Paralamium*. In total, 84 accessions from 84 species and 63 genera of Lamiaceae were included for this initial analysis, covering 11 of the 12 subfamilies recognized by Li et al. (2016) and Li and Olmstead (2017). The plastome of *P. griffithii* (Z.Y. Cai and X.E. Ye czy-36) was newly sequenced for this dataset. Outgroups of the dataset CP79 were selected from Mazaceae [Mazus pumilus (Burm. f.) Steenis], Wightiaceae (Wightia speciosissima (D. Don) Merr.), Phrymaceae (Phryma leptostachya L. subsp. *asiatica* H. Hara), Paulowniaceae (Paulownia coraena Uyeki), and Orobanchaceae (*Castilleja paramensis* F. González et Pabón-Mora), according to recent Lamiales-wide phylogenies (Refulio-Rodriguez and Olmstead, 2014; Liu et al., 2020). GenBank accession numbers and the source publications for taxa in this dataset are provided in Supplementary Table 1. We used the phylogenetic results from this first set of analyses as a basis for a more focused second round of analyses.

Because the first set of analyses demonstrated that *Paralamium* has affinities with tribe Pogostemoneae of Lamioideae, we expanded the sampling of Pogostemoneae in a second round of analyses. These analyses focused on further exploring the placement of *Paralamium* within Pogostemoneae and explicating relationships among genera of the tribe. Chen et al. (2014) demonstrated that the monotypic genus *Holocheila* (Kudô) S. Chow is a member of Pogostemoneae, so we also included this
genus for analysis. In total, for the first time, all 12 genera (including *Paralamium*) of Pogostemoneae were included as part of our Pogostemoneae-wide analyses. This comprehensive generic sampling offers the opportunity to clarify generic relationships of Pogostemoneae using five plastid regions (*matK, rbcL, rps16, trnH-psbA, trnL-trnF*; dataset CP5) and the nuclear ribosomal internal transcribed spacer (dataset nrITS). In total, 56 sequences were newly sequenced for 13 species in 8 genera, while others were taken from previous studies (Chen et al., 2014; Yao et al., 2016) or downloaded from GenBank (Table 1). Outgroups for the dataset CP5 and the dataset nrITS were sampled from tribe Gomphostemmateae (*Chelonopsis souliei* (Bonati) Merr., *Gomphostemma lucidum* Wall. ex Benth., and *Gomphostemma* sp.) according to Yao et al. (2016).

**DNA Extraction, Amplification, and Sequencing**

Total genomic DNA was extracted from fresh or silica-gel-dried leaf fragments using the CTAB procedure of Doyle and Doyle (1987), then dissolved in double-distilled water and kept at −20°C for future polymerase chain reaction (PCR) amplification.

Primers and PCR thermal cycler settings for *matK* and *rbcL* followed Chen et al. (2014), and those for nrITS, *trnL-trnF*, *rps16*, and *trnH-psbA* were as described by Xiang et al. (2013b). Amplified PCR products were visualized on 1% TBE agarose gel, stained with ethidium bromide and then sequenced by an ABI-PRISM3730 sequencer after purification with a QIAquick PCR purification Kit (BioTek, Beijing, China). Voucher information for newly sequenced species and GenBank accession numbers for all sequences used in the current study are listed in the Table 1.

**Plastome Sequencing, Assembly, Annotation, and Gene Region Extraction**

The DNA concentration of *Paralamium griffithii* was at least 35 ng/µL as measured by a NanoDrop spectrophotometer 2000 (Thermo Scientific, Carlsbad, CA, United States). DNA integrity was detected and purified by 1% Agarose Gel Electrophoresis for 40 min at 150 V. Subsequently, the DNA samples were sheared into 300 bp fragments for paired-end library construction according to manufacturer's instructions (Illumina, San Diego, CA, United States), details are provided in Zhao et al. (2020a).

Prior to genome assembly, adapter sequences and low-quality reads were removed using the ea-utils package1. Quality control of raw sequence reads was carried out using FastQC 0.11.8 (Andrews, 2018) with the parameter set as Q ≥ 25. We used the GetOrganelle pipeline (Jin et al., 2020) for the *de novo* assembling. The software Bandage v. 0.8.1 (Wick et al., 2015) was employed for contig visualization and editing. Lastly, in order to validate the assembly error, the raw reads were mapped to the assembled plastid genome sequences by the Bowtie2 (Langmead and Salzberg, 2012) plugin in Geneious v. 11.0.3 (Kearse et al., 2012). In addition to the newly sequenced plastome of *Paralamium griffithii* and downloaded plastomes of 54 species from GenBank (Supplementary Table 1), 32 data from the Sequences Read Archive (SRA) were included for reassembling.

1https://code.google.com/p/ea-utils/

The Initial annotations were implemented in the Plastid Genome Annotator (PGA) (Qu et al., 2019), and the published plastome of *Phlomoides betonicoides* (Diels) Kamelin & Makhm (MN617020; Zhao et al., 2020b) was set as a reference, then Geneious v.11.0.3 (Kearse et al., 2012), and tRNAscan-SE service (Lowe and Chan, 2016) were used adjusting of the putative starts, stops, intron positions, and tRNA boundaries as described by Zhao et al. (2021) and Xiang et al. (2020). Finally, the circular physical map of the plastome of *Paralamium* (Supplementary Figure 1) was drawn by the Organellar Genome DRAW tool (Lohse et al., 2013). The coding regions (CR) were extracted from the annotated complete plastome sequences for phylogenetic analyses.

**Sequence Alignment and Phylogenetic Analyses**

Sequences were assembled and edited using Geneious v.11.0.3 (Kearse et al., 2012). Sequences were aligned using MAFFT v.7.221 (Katoh and Standley, 2013), and then adjusted manually in PhyDE v.0.9971 (Müller et al., 2010) for minor corrections. All datasets were submitted to TreeBASE (study ID: S27475).

Since topological incongruence between the combined cpDNA and nrITS data was reported in Yao et al. (2016), the nrITS and cpDNA datasets were not combined for analyses here. However, because plastome regions typically have a shared genetic history, the five plastid DNA regions were combined for phylogenetic analyses. All datasets were analyzed using Maximum Likelihood (ML) and Bayesian Inference (BI) algorithms on the CIPRES Science Gateway (*Miller* et al., 2010). The ML analyses were implemented with RAxML v.8.2.9 (Stamatakis, 2014), bootstrap probabilities were generated by conducting 1000 bootstrap iterations, and details for parameter settings are described by Xiang et al. (2020). Bayesian inference analyses were performed using MrBayes v.3.2.2 (Ronquist et al., 2012). The best-fit nucleotide substitution models were selected under the Akaike Information Criterion (AIC) using jModelTest v.3.7 (Posada, 2008). The models used were the GTR+I+G for dataset CP79, TVM+I+G for dataset CP5, and for the nrITS dataset. In addition, a partitioned strategy for the dataset CP5 also used for Bayesian inference analyses (GTR + G for *matK*, GTR + I for *rbcL*, TVM + G for *rps16*, TPM1uf + G for *trnH-psbA*, GTR + I for *trnL-trnF*). Specific steps for analyses are described in detail in Chen et al. (2016) and references provided therein. Finally, we used FigTree v.1.4.2 (Rambaut, 2014) to visualize and edit all resulting trees. We defined branches with posterior probabilities (PP) ≥ 0.95 and bootstrap values (BS) ≥ 80% as strongly supported, PP = 0.90–0.95 and BS = 70–80% as moderately supported, while PP < 0.90 and BS < 70% were defined as weakly supported.

**Nutlets Morphology**

Mature nutlets were collected from both wild-collected or herbarium plant specimens from the Germplasm Bank of Wild Species in Southwest China, Kunming Institute of Botany, for light microscope (LM) and scanning electron microscope (SEM) analysis.
| Taxa                                      | Voucher information | nrITS   | matK    | rbcL    | rps16   | trnH-psbA  | trnL-trnF  |
|-------------------------------------------|---------------------|---------|---------|---------|---------|------------|------------|
| Chelonopsis souliei (Bonati) Merr.        | Xiang et al. 1638 (KUN) |         |         |         |         |            |            |
| Gomphostemma lucidum Wall. ex Benth.     | Xiang et al. s.n. (KUN)   |         |         |         |         |            |            |
| Gomphostemma sp.                         | G. Yao 298 (IBSC)    |         |         |         |         |            |            |
| Cotlebrookea oppositifolia Sm. 1          | G. Yao 342 (IBSC)    |         |         |         |         |            |            |
| Cotlebrookea oppositifolia Sm. 2          | G. Yao 367 (IBSC)    |         |         |         |         |            |            |
| Cotlebrookea oppositifolia Sm. 3          | G. Yao 385 (IBSC)    |         |         |         |         |            |            |
| Paralamium griffithii (Hook.f.) Suddee & A.J. Paton 1 | Liu et al. 7859 (KUN) |         |         |         |         |            |            |
| Paralamium griffithii (Hook.f.) Suddee & A.J. Paton 2 | Z.Y. Cai and X.E. Ye czy-36 (IBSC) |         |         |         |         |            |            |
| Craniothe furcata (Link) Kuntze 1         | G. Yao 346 (IBSC)    |         |         |         |         |            |            |
| Craniothe furcata (Link) Kuntze 2         | G. Yao 361 (IBSC)    |         |         |         |         |            |            |
| Holochela longipedunculata S.Chow 1       | Xiang et al. 142 (KUN) |         |         |         |         |            |            |
| Holochela longipedunculata S.Chow 2       | Peng et al. PLJ00048 (KUN) |         |         |         |         |            |            |
| Achyrospermium wallichianum (Benth.) Benth. ex Hook.f. | Liu et al. 16cs11840 (KUN) |         |         |         |         |            |            |
| Euryssolen gracile Prain 1                | G. Yao 366 (IBSC)    |         |         |         |         |            |            |
| Euryssolen gracile Prain 2                | G. Yao 366 (IBSC)    |         |         |         |         |            |            |
| Leucosceptrum canum Sm. 1                 | G. Yao 349 (IBSC)    |         |         |         |         |            |            |
| Leucosceptrum canum Sm. 2                 | Peng et al. PLJ00049 (KUN) |         |         |         |         |            |            |
| Comanthosphace ningpoensis (Hems.) Hand.-Mazz. | Dong et al. HGNU-0864 |         |         |         |         |            |            |
| Comanthosphace japonica (Miq.) S. Moore   | NA                  |         |         |         |         |            |            |
| Rostrinucula dependens (Rehder) Kudô       | W. Fang hw11123 (KUN) |         |         |         |         |            |            |
| Rostrinucula sinensis (Hemsl.) C.Y.Wu      | C.L. Xiang 355 (KUN) |         |         |         |         |            |            |
| Microtnea sp.                             | G. Yao 377 (IBSC)    |         |         |         |         |            |            |
| Microtnea urticifolia Hemsli.             | Y.P. Chen and Q.R. Zhao EM065 (KUN) |         |         |         |         |            |            |
| Microtnea malvensis C.Y.Wu                | F. Zhao et al. LGH111 (KUN) |         |         |         |         |            |            |
| Microtnea delavayi Prain                 | Y.P. Chen EM599 (KUN) |         |         |         |         |            |            |
| Microtnea moospinepinsis (Franch.) Prain  | Y.P. Chen EM631 (KUN) |         |         |         |         |            |            |
| Microtnea robusta Hemsli.                 | Y.P. Chen EM605 (KUN) |         |         |         |         |            |            |
| Anisomeles indica (L.) Kuntze 1           | G. Yao 442 (IBSC)    |         |         |         |         |            |            |
| Anisomeles indica (L.) Kuntze 2           | G. Yao 448 (IBSC)    |         |         |         |         |            |            |
| Pogostemon barbatus Bhoti & Ingr. 1       | G. Yao 274 (IBSC)    |         |         |         |         |            |            |
| Pogostemon barbatus Bhoti & Ingr. 2       | G. Yao 446 (IBSC)    |         |         |         |         |            |            |
| Pogostemon auricularius (L.) Hass.ik.     | G. Yao 362 (IBSC)    |         |         |         |         |            |            |
| Pogostemon hispidocalyx C.Y.Wu & Y.C.Huang | Expedition to QTP 9446 (KUN) |         |         |         |         |            |            |
| Taxa | Voucher information | nrITS | matK | rbcL | rps16 | trnH-psbA | trnL-trnF |
|------|---------------------|-------|------|------|-------|------------|-----------|
| Pogostemon litigiosus Doan ex Suddee & A.J.Paton 1 | V. D. Nong 31712077 (IBSC) | KR608776 | KR608458 | KR608519 | KR608645 | KR608582 | KR608707 |
| Pogostemon litigiosus Doan ex Suddee & A.J.Paton 2 | V. D. Nong 6467 (IBSC) | KR608777 | KR608459 | KR608520 | KR608646 | KR608583 | KR608708 |
| Pogostemon brachystachyus Benth. 1 | G. Yao 358 (IBSC) | KR608775 | KR608455 | KR608517 | KR608642 | KR608579 | KR608704 |
| Pogostemon brachystachyus Benth. 2 | G. Yao 359 (IBSC) | KR608774 | KR608454 | KR608516 | KR608641 | KR608578 | KR608703 |
| Pogostemon fraternus Miq. | Syn. 7655 (KUN) | KR608781 | KR608461 | NA | KR608648 | KR608585 | KR608710 |
| Pogostemon rogersii N.E.Br. Phillips 3855 (K) | G. Yao 358 (IBSC) | KR608775 | KR608455 | KR608517 | KR608642 | KR608579 | KR608704 |
| Pogostemon aquaticus (C.H.Wright) Press Bidgood et al. 3387 (K) | T. P. Zhu 528 (KUN) | KR608771 | KR608466 | KR608525 | KR608653 | KR608590 | KR608715 |
| Pogostemon petelotii Doan ex Gang Yao, Y.F.Deng & X.J.Ge | T. Sorensen et al. 6313 (KUN) | KR608772 | KR608470 | KR608529 | KR608657 | KR608594 | KR608719 |
| Pogostemon stellatus (Lour.) Kuntze | B. Z. Xiao 4826 (K) | KR608768 | KR608464 | KR608523 | KR608651 | KR608588 | KR608713 |
| Pogostemon crassicaulis (Benth.) Press | J. T. Yin 594 (HTBC) | KR608770 | KR608469 | KR608528 | KR608656 | KR608593 | KR608718 |
| Pogostemon samsonii (F.Ince) Press | G. Yao 273 (IBSC) | KR608769 | KR608465 | KR608524 | KR608652 | KR608589 | KR608714 |
| Pogostemon heyneanus Benth. | G. Yao 297 (IBSC) | KR608751 | KR608427 | KR608492 | KR608616 | KR608551 | KR608679 |
| Pogostemon cablin (Blanco) Benth. 1 | G. Yao 292 (IBSC) | KR608752 | KR608499 | KR608504 | KR608628 | KR608563 | KR608691 |
| Pogostemon cablin (Blanco) Benth. 2 | G. Yao 296 (IBSC) | KR608756 | KR608434 | KR608508 | KR608632 | KR608567 | KR608695 |
| Pogostemon parviflorus Benth. 1 | G. Yao 365 (IBSC) | KR608749 | KR608436 | KR608501 | KR608625 | KR608560 | KR608688 |
| Pogostemon parviflorus Benth. 2 | G. Yao 365 (IBSC) | KR608750 | KR608437 | KR608502 | KR608626 | KR608561 | KR608689 |
| Pogostemon pleuranthoides Desf. | W. Koelz 4153 (US) | KR608760 | KR608446 | KR608509 | KR608634 | KR608570 | KR608696 |
| Pogostemon pleuranthoides Desf. | G. Yao 449 (IBSC) | KR608758 | KR608447 | KR608510 | KR608635 | KR608571 | KR608697 |
| Pogostemon xanthiiphyllus C. Y. Wu et Y. C. Huang | H. T. Tsai 59-10586 (KUN) | KR608746 | KR608428 | KR608493 | KR608617 | KR608552 | KR608680 |
| Pogostemon formosanus Oliv. 1 | C. H. Lin 370 (US) | KR608744 | KR608434 | KR608499 | KR608623 | KR608558 | KR608686 |
| Pogostemon formosanus Oliv. 2 | R.Q. Gao and S.H. Lai 710 (PE) | KR608779 | KR608435 | KR608500 | KR608624 | KR608559 | KR608677 |
| Pogostemon glaber Benth. 1 | G. Yao 364 (IBSC) | KR608739 | KR608429 | KR608494 | KR608618 | KR608553 | KR608681 |
| Pogostemon glaber Benth. 2 | G. Yao 386 (IBSC) | KR608741 | KR608430 | KR608495 | KR608619 | KR608554 | KR608682 |
| Pogostemon chinensis C.Y.Wu & Y.C.Huang 1 | J. Chen 656 (KUN) | KR608743 | KR608426 | KR608491 | KR608615 | KR608550 | KR608678 |
| Pogostemon chinensis C.Y.Wu & Y.C.Huang 2 | G. Yao 445 (IBSC) | KR608742 | KR608449 | KR608512 | KR608637 | KR608573 | KR608699 |
| Pogostemon septentrionalis C.Y.Wu & Y.C.Huang 1 | G. Yao 264 (IBSC) | KR608747 | KR608432 | KR608497 | KR608621 | KR608556 | KR608684 |
| Pogostemon septentrionalis C.Y.Wu & Y.C.Huang 2 | G. Yao 272 (IBSC) | KR608748 | KR608433 | KR608498 | KR608622 | KR608557 | KR608685 |
| Pogostemon amaranthoides Benth. | J. Chen 668 (KUN) | KR608745 | KR608425 | KR608490 | KR608614 | KR608549 | KR608677 |
aligned positions (ambiguously aligned characters. The nrITS matrix contained 656 trn(2021) with the exclusion of the coding genes for phylogenetic analyses based on Zhao et al. Supplementary Table 2 unique genes (80 protein-coding genes, 30 tRNAs, and 4 rRNAs; Supplementary Figure 1 large single copy (LSC; 83,788 bp) and small single copy (SSC; 152,664 bp and displayed the typical quadripartite structure coverage of 179 \times paired-end sequencing generated 20,321,882 clean reads, with database with the accession number MW201575. Illumina The newly sequenced and annotated plastome was submitted Content of terminology followed Moon et al. (2009).

**RESULTS**

**Genome Assembly, Features and Gene Content of *Paralamium griffithii***
The newly sequenced and annotated plastome was submitted to the National Center for Biotechnology Information (NCBI) database with the accession number MW201575. Illumina paired-end sequencing generated 20,321,882 clean reads, with coverage of 179 \times for *P. griffithii*. The plastome size was 152,664 bp and displayed the typical quadripartite structure consisting of a pair of IR regions (25,617 bp) separated by the large single copy (LSC; 83,788 bp) and small single copy (SSC; 17,642 bp) regions (Supplementary Figure 1). In total, 114 unique genes (80 protein-coding genes, 30 tRNAs, and 4 rRNAs; Supplementary Table 2) were identified (duplicated genes in IR regions were counted only once). We used 79 common protein-coding genes for phylogenetic analyses based on Zhao et al. (2021) with the exclusion of the ycf15 gene because it could not be extracted from most plastome reassembled from SRA database.

**Sequence Characterization**
Properties for different datasets are summarized in Table 2. The aligned length of the combined 79 protein coding regions (CP79) was 70,100 bp. Removal of ambiguous sites and single taxon insertions resulted in an aligned length of 69,276 bp, of which 47,566 sites were constant (68.66%). The aligned regions and the excluded ambiguous sites of the individual loci are listed in Supplementary Table 3.

In the second set of analyses, the combined cpDNA dataset was 3,439 bp (832 bp for matK, 574 bp for rbcL, 880 bp for trnL-trnF; 861 bp for rps16, and 292 bp for trnH-psbA) after excluding ambiguously aligned characters. The nrITS matrix contained 656 aligned positions (Table 2).

**Phylogenetic Analysis**
For each combined dataset (CP79, CP5, and nrITS), ML and BI analyses yielded identical topologies, respectively (Figures 2–4; Supplementary Figures 2–8). Therefore, only the trees resulting from maximum likelihood analysis of each dataset are presented, with posterior probability values from BI analyses indicated.

In our phylogenetic analyses based on 79 coding plastome sequences (CP79), Lamioideae are supported as monophyletic (Figure 2; ML-BS = 100%/BI-PP = 1.00; all support values follow this order hereafter) and subfamilial relationships are identical to those recovered by Zhao et al. (2021), and 11 tribes were recovered within Lamioideae (Figure 2). In all analyses, the focal species *Paralamium griffithii* was sister to *Craniotome furcata* (Link) Kunze (100%, 1.00) within tribe Pogostemoneae of subfamily Lamioideae.

This recognition guided the second set of analyses, which aimed to further clarify the position of *Paralamium*, reassess generic relationships within Pogostemoneae, and update the phylogeny of Pogostemoneae by including as comprehensive taxon sampling as possible using both nrITS and cpDNA data. In all analyses, Pogostemoneae is robustly supported as monophyletic (Figures 3, 4), but the topologies differed between the nrITS and cpDNA phylogenetic trees. In the nrITS phylogeny, Pogostemoneae was found to have two major clades (labelled A and B in Figure 3). Clade A, or the Pogostemon group, includes *Pogostemon Desf.*, *Anisomeles R. Br.*, and *Microtoena Prain*, in which the former two genera formed a clade (100%, 1.00) sister to *Microtoena* (98%, 1.00). Clade B is poorly supported (59%, -) and includes nine genera. Clade B in turn is comprised of two subclades: one containing *Colebrookea Sm.*, *Paralamium* + *Craniotome*Rchb., weakly supported (57%, -); and another subclade composed of *Holocheila* and the “*Achyropermum* group” (i.e., *Achyropermum* Blume, *EurysoLEN Prain, Leucoceptrum Sm.*, *Comanthophase S. Moore*, and *Rorinucula Kudô*), also poorly supported (63%, -).

All analyses based on the combined cpDNA dataset (CP5) also strongly supported the monophyly of Pogostemoneae (Figure 4; 100%, 1.00). At this point in the Pogostemoneae topology, the two samples of *Holocheila* formed a well-supported clade (100%, 1.00) and were recovered as sister to the remaining Pogostemoneae, which formed a weakly supported clade (59%, -). This “remaining Pogostemoneae” clade included *Colebrookea* (100%, 1.00), the *Achyropermum* group (92%, 1.00), *Paralamium* + *Craniotome* (100%, 1.00), and Clade A (i.e., the *Pogostemon* group, 89%, 1.00).

**Nutlets Morphology**
The nutlets of the genera in clade A (Figure 3) are glossy and smooth (Figures 5, 6A–P) compared with those of genera in clade B (Figure 3). As reported previously (Ryding, 1994a; Bongcheewin et al., 2017), the nutlets of *Pogostemon* (Figures 5A–P) and *Anisomeles indica* (L.) Kunze (Figures 5Q–T) are orbicular to subglobose, dark-brown to black, and the surface is very smooth (*P. chinensis* C.Y. Wu et Y.C. Huang, Figures 5A–D; *P. glaber* Benth., Figures 5E–H) or finely striato-reticulate (*P. brachystachyus* Benth., Figures 5I–L; *P. amaranthoides* Benth.; Figures 5M–P). In *Microtoena* (Figures 6A–P), nutlets are ovoid or subglobose, brown to black, glossy, and the surface is relatively smooth (*M. delavayi* Prain, Figures 6A–D; *M. prainiana* Diels, Figures 6E–H; *M. stenocalyx*
TABLE 2 | The statistics of all datasets for phylogenetic analysis.

| Datasets | No. Taxa | Nucleotides (with ambiguous sites excluded) [bp] | GC content (%) | No. constant sites [bp] | No. variable sites [bp] | No. parsimony-informative sites [bp] |
|----------|---------|---------------------------------------------|----------------|----------------------|----------------------|-----------------------------------|
| CP79     | 65      | 69,276                                      | 38.30%         | 47,566 (68.66%)      | 21,710 (31.34%)       | 13,285 (19.18%)                   |
| matK     | 66      | 832                                         | 33.90%         | 683 (82.09%)         | 149 (17.91%)          | 113 (13.58%)                      |
| psbA-trnH| 65      | 292                                         | 32.60%         | 202 (69.18%)         | 90 (30.82%)           | 71 (24.32%)                       |
| rbcL     | 65      | 574                                         | 44.10%         | 535 (93.21%)         | 39 (6.79%)            | 35 (6.1%)                         |
| rps16    | 66      | 861                                         | 34.70%         | 693 (80.49%)         | 168 (19.51%)          | 123 (14.29%)                      |
| trnL-trnF| 66      | 880                                         | 35.90%         | 774 (87.96%)         | 106 (12.04%)          | 68 (7.73%)                        |
| CP5      | 66      | 3,439                                       | 36.20%         | 2,887 (83.95%)       | 552 (16.05%)          | 410 (11.92%)                      |
| nrITS    | 65      | 656                                         | 62.80%         | 305 (46.49%)         | 251 (52.51%)          | 279 (42.53%)                      |

C.Y. Wu et S.J. Hsuan, Figures 6M–P), or finely granulated (M. esquirolii H. Lév.; Figures 6I–L).

In Rostrinucula, the nutlets are narrowly ellipsoid with curved hook-like apices, brown, pubescent outside with glands and eglandular trichomes (R. sinensis (Hemsl.) C.Y. Wu, Figures 6Q–T). Nutlets of Comanthosphae are obovate, light brown, and the surface is rough and has subsessile and eglandular trichomes (C. ningpoensis (Hemsl.) Hand.-Mazz., Figures 6U–X). In Leucosceptrum canum Sm., the nutlets are obovate, brown, with sharp edges or ribs apically, and a surface more or less smooth but with sparse subsessile glands (Figures 7A–D). Nutlets of Eurysole can be obovate, dark brown, dull, and densely glandular along the ventral side (Figures 7E–H). Only one species of Achyrospermum, A. wallichianum (Benth. ex Hook. f., was included for this study. Achyrospermum wallichianum has somewhat elliptic light brown nutlets that are hairy at apex and reticulate on the surface (Figures 7I–L). Nutlets of Craniotome (Figures 7M–P) and Paralamium (Figures 7Q–T) are subobovate, brown and black respectively, and slightly reticulate outside. Nutlets of Colebrookea (Figures 7U–X) are obovate to oblong, light brown, with apices and fruit navel densely covered with glands, and a surface that is smooth and sometimes with subsessile glands.

**DISCUSSION**

*Paralamium* as a Member of Pogostemoneae in Subfamily Lamioideae

The resulting topologies of Lamioideae from the dataset CP79 are consistent with that of previous studies (Li et al., 2016) based on five cpDNA regions and relationships among these subfamilies are well resolved. Moreover, all tribes of Lamioideae are strongly supported as monophyletic (Figure 2), which is in concordance with previous studies (Scheen et al., 2010; Bendiksby et al., 2011; Roy and Lindqvist, 2015; Zhao et al., 2021).

Cantino and Sanders (1986) considered *Paralamium* as an anomalous genus within Lamioideae because of its morphological similarities to genera from various subfamilies (i.e. Orthosiphon and Coleus of Nepetoideae, Ajuga of Ajuoidae, and Lamium of Lamioideae). However, the presence of tricolpate and two-celled pollens in *Paralamium* suggested its placement within Lamioideae (Cantino and Sanders, 1986). The genus was suggested to be closely related to Pogostemoneae by Bendiksby et al. (2011) based on nutlet morphology, but they explicitly treated it as *incertae sedis* within Lamioideae due to the lack of molecular phylogenetic data. Here, both the plastid and nuclear DNA data (Figures 2–4) support that *Paralamium* is a member of tribe Pogostemoneae, and is sister to the monotypic genus Craniotome.

Previous studies based on cpDNA sequences showed that Craniotome grouped with Microtoena, Anisomeles, and Pogostemon (Scheen et al., 2010; Bendiksby et al., 2011; Chen et al., 2014). Using low-copy nuclear pentatricopeptide repeat (PPR) data, Roy and Lindqvist (2015) also recovered a close relationship among Craniotome, Anisomeles, and Pogostemon (Microtoena not sampled). In our analyses, however, Craniotome consistently grouped with Paralamium with high support values (Figures 2–4). Some morphological characters support the close relationship between Paralamium and Craniotome. For example, the size of pollen grains is very similar in *Paralamium* (16.3 × 15.0 µm) and Craniotome (16.6 × 14.9 µm), and are smaller than other lamiod genera (Abu-Asab and Cantino, 1994). Additionally, nutlet morphology supports the sister relationship between Paralamium and Craniotome. Nutlets in both genera are obovate and glossy (Figures 7O, S) and have reticulate ornamentation on the surface (Figure 7P, × 750; Figure 7T, × 1200), while in other related genera in clade B (Figure 3), nutlets are oblong (Leucosceptrum, Figures 7A–C), hooked (Rostrinucula, Figures 6Q–S) or hairy (Achyrospermum, Figures 7J–I) at apex, or has eglandular (Comanthosphae, Figure 6X) or glandular (Eurysole, Figure 7G; Colebrookea, Figure 7X) trichomes. At the same time, Paralamium has some unique morphological characters, especially its unequal calyx lobes (i.e., 1/2/2 split), can differentiate it from other genera (calyx lobs 3/2 or 1/4 split, or (sub)equal) within Pogostemoneae.

Circumscription and Relationships Within Pogostemoneae

The monophyly of Pogostemoneae was supported by most studies (Scheen et al., 2010; Bendiksby et al., 2011; Chen et al., 2014) based on cpDNA sequences, but not by Roy and Lindqvist (2015) using PPR data, who revealed that genera of Pogostemoneae were included in two separate clades. The first
clade was referred as the *Achyrospermum* group (i.e., subclade A in Figure 2 sensu Roy and Lindqvist, 2015), forming the first-diverging clade within Lamioideae. The second clade consist of *Pogostemon*, *Anisomeles*, and *Craniotome* (i.e., subclade B in Figure 2 sensu Roy and Lindqvist, 2015), forming the second diverging clade sister to remainder of Lamioideae.

In our analyses, the cpDNA datasets strongly support the monophyly of Pogostemonae (Figure 2), and the monophyly of...
this tribe was recovered based on nrITS dataset, although only two genera were selected as outgroup (Figure 3). Based on the results from present as well as previous studies (Scheen et al., 2010; Bendiksby et al., 2011; Chen et al., 2014; Roy and Lindqvist, 2015), Pogostemonae comprises 12 genera: Pogostemon, Anisomeles, Microtoena, Rostrinucula,
Comanthophace, Leucosceptrum, Eurysolem, Achyropermum, Holocheila, Craniote, Paralamium, and Colebrookea. Most genera are monotypic or oligotypic, excepting Pogostemon (80 spp.), Achyropermum (ca. 25 spp.), and Microtoena (19 spp.). Morphologically, Pogostemonceae is a very heterogeneous group, and synapomorphies for the tribe are still unclear. However, some morphological and anatomical characters can be used to distinguish Pogostemonceae from other lamioid members. Most genera of Pogostemonceae possess small and relatively glossy nutlets with pericarps often lacking a
sclerenchyma region (Ryding, 1994a, 1995), generally long-exserted stamens with bearded filaments, weakly 2-lipped corollas, and broad bracts (Scheen et al., 2010). Additionally, pollen grains of Pogostemoneae are typically smaller (less than $28 \times 27 \mu m$) than that of most genera of Lamioideae (Abu-Asab and Cantino, 1994).

In addition to the confirmation of the systematic position of Paralamium and sister relationship between Paralamium and Craniotome, some other well-supported groups within Pogostemoneae are also recovered in this study, which enables us to further discuss the relationships within the tribe. Based on nrITS phylogeny (Figure 3), two subclades (i.e., clade A and clade B) can be recognized. Clade A is strongly supported and composed of three genera (Pogostemon, Anisomeles, and Microtoena), while clade B is composed of the remaining genera of Pogostemoneae. Although clade B is weakly supported (0.59, -), this split is supported by nutlet morphology. In the present study, nutlets of 17 species representing 11 out of 12 genera (except Holocheila) of Pogostemoneae were included for analyses. Based on our LM and SEM observations, we found that nutlets of genera in clade A (Figure 3; Pogostemon, Anisomeles, and Microtoena) are glossy and relatively glabrous (Figures 5, 6A–P), and the sclerenchyma region is very distinctive (Bendiksby et al., 2011), while genera in clade B (Rostrinucula, Comanthosphace, Leucosceptrum, Eurysolen, Achyrospermum, Paralamium, Craniotome) have dull and glandular nutlets (Figures 7Q–X), and the sclerenchyma region is often absent or indistinct (Ryding, 1994a, 1995).

Within clade A, Anisomeles is sister to Pogostemon, with Microtoena sister to the Anisomeles-Pogostemon clade (Figures 3, 4). The three genera form a clade referred as clade A, which was supported by previous molecular phylogenetic studies (Scheen et al., 2010; Bendiksby et al., 2011). Cantino (1990, 1992a,b) suggested a close relationship between Anisomeles and Pogostemon based on their bearded staminal filaments and lustrous pericarps, as well as the presence of minute glands with unicellular caps on the leaf epidermis. Later, Abu-Asab and Cantino (1994) found that the two genera have very
similar pollen grains with regular polygonal lumina and large perforations (see also Bean, 2015).

The close relationship between Microtoena and the Pogostemon-Anisomeles clade has been reported in previous studies (Scheen et al., 2010; Bendiksby et al., 2011; Chen et al., 2014; Roy and Lindqvist, 2015). The three genera are similar in terms of calyx morphology, with the calyx splitting the upper two and bottom three lobes up to ca. 1/2 of its length. Furthermore, linear bracts are present in Anisomeles and most species of Microtoena, while lanceolate or ovate bracts can be found in some species of Microtoena and Pogostemon (Wang, 2018). Geographically, most of the species of clade A are distributed in tropical East Asia (Scheen et al., 2010), although some species occur on islands within the Pacific and West Indian Oceans (Anisomeles), Africa (Pogostemon), and the Himalayas (Craniotome and Pogostemon glaber).

Microtoena was shown to be polyphyletic in some studies (Bendiksby et al., 2011; Roy and Lindqvist, 2015) based on cpDNA regions, but our results recover it as monophyletic with convincing support (Figure 3). A possible reason for this discrepancy may be that only two species and three cpDNA markers (matK, trnL-trnF, rps16 intron) were used in previous studies. Wang (2018) included 11 species for the phylogenetic reconstruction of Microtoena. Though his study was based only on two cpDNA regions (matK, trnL-trnF), the monophyly of Microtoena was well supported, as in our
present study using nrITS (Figure 3, 100%, 1.00) and additional cpDNA markers (Figure 4, 74%, 0.98). *Microtoena* is a poorly understood genus and was previously placed within Stachydeae (Prain, 1889; Briquet (1895–1897)). Although recent molecular phylogenetic studies (Bendiksby et al., 2011; Yao et al., 2016; Zhao et al., 2021) confirmed its placement within Pogostemoneae, corroborating the taxonomic treatment of Harley et al. (2004), species relationships within *Microtoena* remain unresolved.

Another subclade (i.e., *Achyrosperrnum* group) composed of *Achyrosperrnum*, *Eurysolen*, *Leucosceptrum*, *Rostrinucula*, and *Comanthosphace* is also strongly supported in both the nrITS (Figure 3) and cpDNA trees (Figure 4), among which *Rostrinucula* and *Comanthosphace* are consistently resolved as sister genera (Figures 3, 4). The *Achyrosperrnum* group was first reported by Bendiksby et al. (2011) using cpDNA markers and subsequently recovered by Roy and Lindqvist (2015) based on the PPR region, but neither of them sampled *Leucosceptrum*. Species of the *Achyrosperrnum* group are distributed mainly in tropical East Asia and share several morphological characters. For example, the sclerenchyma region in the fruit pericarp is present in most lamioid members (Ryding, 1995; Bendiksby et al., 2011), but is obsolete, indistinct, or absent in the *Achyrosperrnum* group (Ryding, 1994b, 1995). Moreover, genera in this subclade have dull and glandular nutlets (Figures 6Q–X, 7A–L), while other genera within Pogostemoneae have glossy and glabrous nutlets (Bendiksby et al., 2011). Stamens long-exserted
from the corolla are rare in Lamioideae, and are restricted to Comanthosphace, Rostrinucula, and Leucoceptrum in the Achyrospermum group, as well as a few species of Pogostemon in clade A. As suggested by Scheen et al. (2010), this character may be a synapomorphy for the small clade consisting of Comanthosphace, Rostrinucula, and Leucoceptrum. Molecular phylogenetic and morphological studies based on a broader sampling and more DNA sequences may further help to elucidate relationships within Pogostemoneae and identify morphological synapomorphies for the tribe.

**Incongruence Between Nuclear and Plastid Phylogenies**

In this study we provide the first comprehensive molecular phylogenetic study of Pogostemoneae. Though the intergeneric relationships within this tribe are generally well resolved, the placement of four monotypic genera (Colebrookea, Holocheila, Paralamium and Craniotome) is still uncertain due to incongruent topologies between nrITS and cpDNA trees. In the nrITS phylogeny (Figure 3), the Paralamium-Craniotome clade is sister to Colebrookea but weakly supported (57%, -). The Paralamium-Craniotome-Colebrookea clade is then sister to a clade including Holocheila and the Achyrospermum group, which is also weakly supported (-, 0.90) again. In the cpDNA tree (Figure 4), however, Holocheila is the first diverging clade, followed by Colebrookea, the Achyrospermum group, and then Paralamium-Craniotome + clade A, which is largely consistent with the topology of Chen et al. (2014). Most genera in clade B (excepting Achyrospermum, 25 spp.), all other genera are monotypic (Colebrookea, Craniotome, Eurysolen, Holocheila, Leucoceptrum, Paralamium, Rostrinucula) or oligotypic (Comanthosphace, 4 spp.) and mainly distributed in East Asia.

Incongruence between genomes have been noted within several genera in Lamioideae, and ancient hybridization and chloroplast capture have often been posited to have contributed to the discordance (e.g., Albaladejo et al., 2005; Drew and Sytsma, 2013; Drew et al., 2014; Deng et al., 2015; Walker et al., 2015; Hu et al., 2018). Roy and Lindqvist (2015) suggested ancient reticulation events are likely to be responsible for the discordance between the plastid and PPR topologies of Pogostemoneae. They also demonstrated that ancestors of Pogostemoneae may have undergone rapid diversification during the middle Miocene in East Asia, which may have been triggered by climatic changes resulting from the uplift of the Qinghai-Tibetan Plateau (QTP) (Roy and Lindqvist, 2015). Considering that incomplete lineage sorting (ILS) among taxa is often associated with rapid radiations (Enard and Paabo, 2004; Pollard et al., 2006), ILS may also be a cause of the incongruences between the nuclear and plastid trees of Pogostemoneae. In the present study, two clades (clade A and clade B) are recognized based on nrITS phylogeny, but clade B is weakly supported by nrITS data and not recovered using cpDNA data. Although nutlet morphology supported the division of these two clades, futures studies involving next-generation sequencing and increased taxon sampling are need to provide insights into the complex evolutionary history of this group.

**Key to All Genera of Pogostemoneae**
The following circumscription of Pogostemoneae is based on this as well as previous studies (Scheen et al., 2010; Bendikovsky et al., 2011). We provide a key to the 12 genera of Pogostemoneae below.

| Key | Description | Genera |
|-----|-------------|--------|
| 1   | Creeping herb; corolla with two entire lips (1/1) | Holocheila |
| 2   | Shrub, subshrubs or erect herb; corolla 2-lipped, 4-lobed (1/3, 1/3 or 2/3) | Microtoena |
| 3   | Calyx 5-lobed, lobes unequal (1/2/2), posterior lip very broad | Paralamium |
| 4   | Nutlet narrowly ellipsoid, hooked at apex | Rostrinucula |
| 5   | Nutlet not narrowly ellipsoid, unhooked at apex | Pogostemon |
| 6   | Filaments usually bearded along center with moniliform hairs | Craniotome |
| 7   | Flowers dioecious with dimorphic male and female flowers | Colebrookea |
| 8   | Shrub or small tree; nutlets cylindrical-oblong | Leucoceptrum |
| 9   | Rhizomatous perennial herbs; nutlets obovate | Comanthosphace |
| 10  | Nutlets scaly at apex | Achyrospermum |
| 11  | Nutlets never scaly at apex | Anisomeles |
| 12  | Anthers 1-celled; corolla tube saccate in front | Eurysolen |
| 13  | Anthers 2-celled; corolla tube not saccate in front | Pogostemon |
| 14  | Stamens long-exserted from corolla | Holocheila |
| 15  | Stamens not or shortly exserted from corolla | Paralamium |
| 16  | Flowers monoecious | Leucoceptrum |
| 17  | Corolla-tube less than 1 cm long | Microtoena |
| 18  | Corolla-tube less than 1 cm long | Pogostemon |
| 19  | Filaments usually bearded along center with moniliform hairs | Comanthosphace |
| 20  | Flowers dioecious with dimorphic male and female flowers | Colebrookea |

**CONCLUSION**

This study confirms the systematic placement of Paralamium for the first time inferred from chloroplast and nuclear DNA data. Paralamium is a member of the tribe Pogostemoneae within Lamioideae and is sister to Craniotome. As currently defined, the tribe Pogostemoneae is composed of 12 genera, and the monophyly of Pogostemoneae is supported in all analyses. Phylogenetically, Pogostemoneae are the first diverging tribe and sister to the remaining Lamioideae. Morphologically, Pogostemoneae are a remarkably diverse group and lack clear synapomorphies. Although some well-supported groups were identified within Pogostemoneae, relationships of some monotypic genera (e.g., Holocheila,
Colebrookea, Paralamium and Craniotome) remain unclear. Thus, studies using broad sampling of low-copy and/or single-copy intrageneric phylogenies and detailed comparative morphological investigation are needed.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

AUTHOR CONTRIBUTIONS

FZ, C-LX, BD, and BL conceived the idea and designed the research. Y-WW, FZ, and Y-PC conducted experiments. E-DL and JC conducted specimen and seed collection. FZ, Y-WW, Y-PC, GY, BD, JC, E-DL, BL, and C-LX wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was jointly supported by the “Ten Thousand Talents Program of Yunnan” (Grant No. YNWR-QNBJ-2018-279), Yunnan Fundamental Research Projects (Grant No. 2019Fl009), the CAS “Light of West China” program, and the Large-scale Scientific Facilities of the Chinese Academy of Sciences (2017-LSFGBOWS-02) granted to C-LX, and NSF-DEB grant DEB-1655611 granted to BD.

ACKNOWLEDGMENTS

We would like to thank Dr. Xing-Er Ye of South China Botanical Garden, Chinese Academy of Sciences, Dr. Hong-Jin Dong of Huanggang Normal University, Hubei, for their help in field collection, and Dr. Xin-Xin Zhu of Xinyang Normal University for providing photos. We gratefully thank Mr. Lian-Yi Li and Mr. Zhi-Jia Gu for their technical assistance in LM and SEM. Thanks are also extended to the staff Germplasm Bank of Wild Species in Southwest China for their valuable help in research facilities and providing seed materials.

REFERENCES

Abu-Asab, M. S., and Cantino, P. D. (1992). “Pollen morphology in subfamily Lamioideae (Labiatae) and its phylogenetic implications,” in Advances in Labiatae Science, eds R. M. Hartley and T. Reynolds (London: Royal Botanic Gardens, Kew), 361–379.
Abu-Asab, M. S., and Cantino, P. D. (1994). Systematic implications of pollen morphology in subfamilies Lamioideae and Pogostemonoideae (Labiatae). Ann. Mo. Bot. Gard. 81, 653–686. doi: 10.2307/2399915

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.646133/full#supplementary-material

Supplementary Figure 1 | Gene map of the complete chloroplast genome of Paralamium griffithii. 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.

Supplementary Figure 2 | Maximum likelihood phylogeny of Lamiaceae based on 79 chloroplast DNA regions (dataset CP79) of coding regions dataset, ambiguous sites were excluded for analyses. Maximum likelihood bootstrap support (MLBS) are near the branches. A “–“ indicates MLBS values < 50%.

Supplementary Figure 3 | Phylograms from Bayesian Inference (BI) analyses of Lamiaceae based on concatenated nucleotide sequences of 79 protein-coding genes (dataset CP79), ambiguous sites were excluded for analyses. Bayesian inference posterior probability (BIPP) are near the branches. A “–“ indicates BIPP values < 0.8.

Supplementary Figure 4 | Maximum likelihood phylogeny of Pogostemonoideae based on the nrITS region, ambiguous sites were excluded for analyses. Maximum likelihood bootstrap support (MLBS) are near the branches. A “–“ indicates MLBS values < 50%.

Supplementary Figure 5 | Phylograms from Bayesian Inference (BI) analyses of Pogostemonoideae based on the nrITS region, ambiguous sites were excluded for analyses. Bayesian inference posterior probability (BIPP) are near the branches. A “–“ indicates BIPP values < 0.8.

Supplementary Figure 6 | Maximum likelihood phylogeny of Pogostemonoideae based on the combined dataset of five cpDNA regions (matK, rbcL, rps16, trnH-psbA, and trnL-trnF), ambiguously aligned sites were excluded from analyses. Maximum likelihood bootstrap support (MLBS) are near the branches. A “–“ indicates MLBS values < 50%.

Supplementary Figure 7 | Phylograms from Bayesian Inference (BI) analyses of Pogostemonoideae based on the combined dataset of five cpDNA regions (matK, rbcL, rps16, trnH-psbA, and trnL-trnF), ambiguously aligned sites were excluded from analyses. Bayesian inference posterior probability (BIPP) are near the branches. A “–“ indicates BIPP values < 0.8.

Supplementary Figure 8 | Phylograms from Bayesian Inference (BI) analyses of Pogostemonoideae based on the combined dataset of five cpDNA regions (matK, rbcL, rps16, trnH-psbA, and trnL-trnF), under a partitioned strategy model, ambiguously aligned sites were excluded from analyses. Bayesian inference posterior probability (BIPP) are near the branches. A “–“ indicates BIPP values < 0.8.

Supplementary Table 1 | List of taxa sampled with information related to taxonomy, GenBank accession numbers and references.

Supplementary Table 2 | Features of newly sequenced plastome of Paralamium griffithii.

Supplementary Table 3 | Excluded ambiguous sites for each dataset.

Albaladejo, R. G., Aguilar, J. F., Aparicio, A., and Feliner, G. N. (2005). Contrasting nuclear-plastidial phylogenetic patterns in the recently diverged Iberian Philonis crinita and P. lychnitis lineages (Lamiaceae). Taxon 54, 987–998. doi: 10.2307/25065483
Andrews, S. (2018). FastQC: A Quality Control Tool for High Throughput Sequence Data. Available online at: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ (accessed October 18, 2020)
Bean, A. R. (2015). A taxonomic revision of Anisomeles R.Br. (Lamiaceae). Australbaileya 9, 321–381.
Bendiksby, M., Salmaki, Y., Brauchler, C., and Ryding, O. (2014). The generic position of Stachys tibetica Vatke and amalgamation of the genera Eriophyton and Stachyopis (Lamiaceae subfam. Lamioideae). Plant. Syst. Evol. 300, 961–971. doi: 10.1007/s00606-013-0935-2

Bendiksby, M., Thorbek, L., Scheen, A. C., Lindqvist, C., and Ryding, O. (2011). An updated phylogeny and classification of Lamiaceae subfamily Lamioideae. Taxon 60, 471–484. doi: 10.1205/tax.60.2010

Bongcheewin, B., Pramali, K., Traiperm, P., Chantaranothai, P., and Paton, A. (2017). Pogostemon nudus sp. nov. (Lamiaceae) from Thailand. Nord. J. Bot. 35, 289–299. doi: 10.1111/nj.10143

Briquet, J. (1895–1897). “Labiatate,” in Die natürlichen Pflanzenfamilien, ed. A. Engler and K. Prantl (Leipzig: Wilhelm Engelmann), 183–357.

Cantino, P. D. (2004). The phylogenetic significance of stomata and Trichomes in the Labiatae and Verbenaceae. J. Linn. Soc. Bot. 122, 649–668. doi: 10.1111/j.1756-1051.1994.tb00572.x

Cantino, P. D., and Sanders, R. W. (1986). Subfamilial classification of labiatae. Syst. Bot. 11, 163–185. doi: 10.2307/2418955

Chen, Y. P., Li, B., Olmstead, R. G., Cantino, P. D., Liu, E. D., and Xiang, C. L. (2017). Two new subfamilies in Lamiaceae. Phytotaxa 313, 222–226. doi: 10.11646/phytotaxa.313.2.9

Hooker, J. D. (1885). “Labiatae,” in The Families and Genera of Vascular Plants, eds A. Engler and K. Prantl (Leipzig: Wilhelm Engelmann), 183–357.

Lowe, T. M., and Chan, P. P. (2016). tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res. 44, W54–W57. doi: 10.1093/nar/gkw090

Li, B., and Olmstead, R. G. (2017). Two new subfamilies in Lamiaceae. Phytotaxa 313, 222–226. doi: 10.11646/phytotaxa.313.2.9

Li, H. W. (1977). “Paralamium Hand.-Mazz.” in Flora Reipublicae Popularis Sinicae, Vol. 65, ed. C. Y. Wu (Beijing: Science Press), 544–545.

Lohse, M., Drechsel, O., Kahlau, S., and Bock, R. (2013). OrganellarGenomeDRAW—a suite of tools for generating physical maps of plastid and mitochondrial genomes and visualizing expression data sets. Nucleic Acids Res. 41, W575–W581. doi: 10.1093/nar/gkt1289

Linn. Soc. (2002). Origin of the Hawaiian endemic mints within North American Stachys (Lamiaceae). Am. J. Bot. 89, 1709–1724. doi: 10.3732/ajb.89.10.1709

Miller, M. A., Pfeiffer, W., and Schwartz, T. (2010). “Creating the CIPRES Science Gateway for inference of large phylogenetic trees,” in Proceedings of the 2010 Gateway Computing Environments Workshop (GCE), (New York, NY: IEEE), 1–8.

Moon, H. K., Hong, S. P., Smets, E., and Huysmans, S. (2009). Micromorphology and character evolution of nutlets in tribe Mentheae (Nepetoideae, Lamiaceae). J. Syst. Evol. 48, 51–62. doi: 10.1007/s12304-010-0017-9

Müller, K., Müller, J., and Quandt, D. (2010). PhyloDE: Phylogenetic Data Editor, Version 0.9971. Available online at: http://www.phyde.de/index.html (accessed 14 November 2014)

Olmstead, R. (2016). A Synoptical Classification of the Lamiales, Version 2.6.2. Available online at: http://dept5.washington.edu/phylo/Classification.pdf (accessed 25 Feb 2020)

P_frontend/figtree/ (accessed October 13, 2018)

Qu, X. J., Moore, M. J., Li, D. Z., and Yi, T. S. (2019). PGA: a software package for rapid, accurate, and flexible batch annotation of plastomes. Plant Methods 15:50. doi: 10.1186/s13007-019-0435-7

Rambaut, A. (2014). FigTree, v.1.4.2. Available online at: http://tree.bio.ed.ac.uk/software/figtree/ (accessed October 13, 2018)

Refulio-Rodriguez, N. F., and Olmstead, R. G. (2014). Phylogeny of Lamioideae. Am. J. Bot. 101, 287–299. doi: 10.3732/ajb.1300394

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Hohna, S., et al. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542. doi: 10.1093/sysbio/sys029

Roy, T., and Lindqvist, C. (2015). New insights into evolutionary relationships within the subfamily Lamioideae (Lamiaceae) based on pentatricopeptide repeat (PPR) nuclear DNA sequences. Am. J. Bot. 102, 1721–1735. doi: 10.3732/ajb.1500233

Ryding, O. (1994a). Pericarp structure and phylogeny of Lamiaceae subfamily Pogostemonoideae. Nord. J. Bot. 14, 59–63. doi: 10.1111/j.1756-1051.1994. th00572.x

Ryding, O. (1994b). The pericarp structure in subtribe Melittidinae (Lamiaceae—Lamioideae) and its systematic implications. Bot. Jahrb. Syst. 115, 547–555.

Ryding, O. (1994c). Pericarp structure in the tribe Prasieae (Lamiaceae—Lamioideae) and its systematic implications. Bot. Jahrb. Syst. 116, 391–399.
Scheen, A. C., and Albert, V. A. (2007). Nomenclatural and taxonomic changes in generic boundaries within the Leucas clade (Lamiaceae). Bot. Jahrb. Syst. 127, 299–316. doi: 10.1127/0006-8152/2008/0127-0002

Scheen, A. C., and Albert, V. A. (2009). Molecular phylogenetics of the genus Chelonopsis (Lamiaceae subfamily Scutellarioideae) as inferred from nuclear and plastid DNA and potential pollination ecology. Plant Biosyst. 143, 670–677. doi: 10.3111/11263504.2012.748099

Scheen, A. C., Zhang, Q., Scheen, A. C., Cantino, P. D., Funamoto, T., and Peng, H. (2013b). Molecular phylogenetics of Cheloneopsis (Lamiaceae: Lamioideae) and allied genera, with reference to taxonomic implications and potential pollination ecology. Plant Biosyst. 147, 620–628. doi: 10.1080/11263504.2012.748099

Scheen, A. C., Zhang, Q., Scheen, A. C., Cantino, P. D., Funamoto, T., and Peng, H. (2013a). Pollen morphology of the East Asiatic genus Chelonopsis (Lamiaceae: Lamioideae) and allied genera. Cladistics 29, 205–227. doi: 10.1111/j.1095-8677.2012.00557.x

Schein, A. C., and Albert, V. A. (2007). Nomenclatural and taxonomic changes within the Leucas clade (Lamiaceae: Lamioideae). Syst. Geogr. Fl. 77, 229–238. Schein, A. C., and Albert, V. A. (2009). Molecular phylogenetics of the leucas group (Lamiaceae: Lamioideae). Syst. Bot. 34, 173–181. doi: 10.1600/036364409787602366

Schein, A. C., Bendiksbv, M., Ryding, O., Mathiesen, C., Albert, V. A., and Lindqvist, C. (2010). Molecular phylogenetics, character evolution, and suprageneric classification of Lamioideae (Lamiaceae). Ann. Mo. Bot. Gard. 97, 191–217. doi: 10.3417/2007174

Schein, A. C., Lindqvist, C., Fossdal, G., and Albert, V. A. (2008). Molecular phylogenetics of tribe Synandreae, a North American lineage of lamioiids (Lamiaceae). Cladistics 24, 299–314. doi: 10.1111/j.1096-0031.2007.00180.x

Seyedi, Z., and Salmaki, Y. (2015). Trichome morphology and its significance in the systematic studies of Phlomoidae (Lamiaceae: Lamioideae; Phlomidae). Flora 213, 40–48. doi: 10.1016/j.flora.2015.04.003

Siadati, S., Salmaki, Y., Mehrvarz, S. S., Heubl, G., and Weigend, M. (2018). Untangling the generic boundaries in tribe Marrubiaceae (Lamiaceae: Lamioideae) using nuclear and plastid DNA sequences. Taxon 67, 770–783. doi: 10.12705/674.6

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. doi: 10.1093/bioinformatics/btu033

Suddoe, S., and Paton, A. (2004). Some nomenclatural changes in South East Asian Lamiaceae. Kew Bull. 59, 315–318. doi: 10.2307/4115869

Walker, J. B., Drew, B. T., and Sytsma, K. J. (2015). Unravelling species relationships and diversification within the iconic California Floristic Province Sages (Salvia subgenus Audibertia, Lamiaceae). Syst. Bot. 40, 826–844. doi: 10.1600/036364415x689285

Wang, Q. (2018). A Monograph of the Genus Microtoena (Lamiaceae). Beijing: Science Press.