Molecular phylogeny of Lepidagathis sensu lato with notes on its biogeography and subgenus Lophostachys

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Abstract

Lepidagathis Willd., a genus belonging to the family Acanthaceae, is present pantropically and consists of about 151 species. Classification of *Lepidagathis* and *Lophostachys* has been under debate for a long period. Earlier, they were separated based on their distribution and morphology. However, according to Benoist 1911, the morphological differences were very slight; hence, both were considered under the genus *Lepidagathis*. Molecular analysis is the most effective method to clear up taxonomic issues, but more data is needed regarding *Lepidagathis* and *Lophostachys*. *Lepidagathis cristata*, the type species of this genus, has no molecular sequences available. Therefore, the purpose of this study was to sequence several plastid markers, which are frequently employed for the phylogeny of Acanthaceae and species DNA barcoding. Subsequently, phylogenetic trees using Bayesian and maximum likelihood bootstrap methods were built. The predominantly Asian species formed a clade which we characterize as *Lepidagathis* s.s was found monophyletic with strong Bayesian posterior probability support as well as good maximum-likelihood bootstrap support. *Lepidagathis* s.s was sister to another well-supported clade consisting of the genus *Lophostachys*. This phylogenetic study, along with the biogeographical distribution proves that *Lepidagathis* and *Lophostachys* are independently evolving clades, which can be treated as different subgenera.

Introduction

Acanthaceae is a family belonging to the dicotyledonous angiosperms. It consists of approximately 4900 species distributed throughout the tropical and temperate regions (Manzitto-Tripp et al. 2022). The plants in the family vary significantly in morphological and ecological characteristics (Manzitto-Tripp et al. 2022).

The genus *Lepidagathis* Willd., (Acanthaceae) is one of the largest genera in the tribe Barlerieae, with over 151 species (POWO 2023) found throughout the tropical and subtropical parts of the planet (GBIF.Org 2023). *Lepidagathis* was first described by Willdenow in 1800 based on the species *Lepidagathis cristata* Willd. from peninsular India (Willdenow 1800). *L. cristata* is a small shrub that is adapted to hot and dry tropical habitats. It has a trailing stem, narrow leaves, and dense clusters of flowers that are protected by pointed bracts. As described by (Nees 1832) *Lepidagathis* is distinguished by a five-partite calyx with the upper segment larger than the others and bract-like, a bilabiate corolla, didynamous stamens, bithecous anthers, a capsule with a membranous wall, and a hard septum. Additionally, short spikes with simple, lateral branches forming glomerules and bracts spirally arranged are present (Hirao et al. 2019). In more recent studies, *Lepidagathis* has been described to have prolate, tricolporate, coarsely reticulate pollen; frequently zygomorphic, 5-lobed or occasionally 4-lobed calyces; bilabiate corollas with a hooded upper lip; and an androecium with four bithecous stamens, or two bithecous with two monothecous stamens, or two bithecous stamens and two staminodes (Kameyama 2008; Champluvier and Darbyshire 2012; Darbyshire et al. 2019; Manzitto-Tripp et al. 2022).

Lepidagathis has been used as a traditional medicine (Ahirwar and Ahirwar 2021) in various cultures mostly in India. In Ayurvedic practice, *Lepidagathis cuspidata* is known as 'Kudajatri pacha' (Rao et al. 2019). *Lepidagathis cristata* is known as 'Bukhar Jadi' in Hindi (India Biodiversity Portal 2023). 'Pathar-phor buti' and 'safed rasna' are the hindi names of *Lepidagathis trinervis* (Flowers of India 2023). Pharmacological studies suggests that extracts from different species of *Lepidagathis* show antibacterial, antifungal, immunosuppressive, anti-inflammatory, analgesic, antipyretic, antiemetic, hypoglycaemic, and wound healing properties (Yadava 2001; Ravikanth et al. 2001); (Richard et al. 2011); (Yadava 2001; Abubacker and Devi 2014). Extract from *L. cristata* was discovered to be highly effective against a few human pathogenic fungi and plant pathogenic fungi (Abubacker and Devi 2014).

Lepidagathis was formerly kept in three separate genera and it has been difficult to correctly identify the genus. In 1831, Pohl described the genus *Lophostachys* based on three Brazilian species: *L. floribunda*, *L. villosa*, and *L. sessilifolia* (Pohl 1827). *Lophostachys* is characterized by the calyx with four unequal sepals, the two-lipped corolla, and the flowers that are arranged in a single row on the stem. (Kameyama 2008). Earlier, the genera *Lepidagathis*, *Lophostachys*, and *Teliostachya* were separated based on their distribution, inflorescence, and number of calyx (Nees 1847). However Bentham in 1876, believed *Teliostachya* and *Lepidagathis* to be synonymous, reasoning that there was no distinguishing trait between the two genera. He retained *Lepidagathis* and *Lophostachys* separately on the basis of differences in the sepals and stamens (Bentham 1876). (Benoist 1911) merged *Lophostachys* with *Lepidagathis* stating that there was no significant difference in the morphology of these genera, the change in the number of sepals was solely caused by how much they are fused, and the various inflorescence structures were only more simplified or enriched version of the same pattern (Benoist 1911). Again, due to the misconception of the insertion of anther theca and ornamentation of pollen grains, (Bremekamp 1938) separated *Teliostachya* and *Lepidagathis* and kept the latter in the tribe Lepidagathidae. (Daniel 1995) stated that the only difference between *Lepidagathis* and *Teliostachya* was the presence of radial symmetry in inflorescence. (McDade and Moody 1999) placed *Lepidagathis* as the sister taxon to *Barleria*. Later, in the year 2000, *Lepidagathis* and *Lophostachys* were placed in the subtribe Barleriinae along with the genus *Barleria* (Scotland and Vollesen 2000). (Kameyama 2008), merged *Lophostachys* and *Teliostachya* under *Lepidagathis* in accordance with Benoist's theories and stressed the need for additional research to confirm or disprove the new classification. Hirao et al. (2019) did further investigation on the floral development of three species and concluded that *Lophostachys* and *Teliostachya* should be included in *Lepidagathis.* Manzitto-Tripp et al. (2022) gave a revised classification of the family Acanthaceae, in which they followed the concept of including *Lophostachys* and *Teliostachya* in *Lepidagathis* following Kameyama (2008). They divided four morphologically separate subfamilies under the family Acanthaceae - Nelsonioidae, Avicennioideae, Thunbergioideae, and Acanthoideae. According to the study, there are eight recognised tribes under the subfamily Acanthoideae - Acantheae, Physacantheae, Barlerieae, Andrographideae, Whitfieldieae, Neuracantheae, Ruellieae, and Justicieae (Manzitto-Tripp et al. 2022). The tribe Barlerieae can be differentiated from other Acanthaceae by its quincuncial aestivation of corolla (Darbyshire et al. 2019). The tribe consists of 13 genera and 500 species, and *Lepidagathis* is also placed in this tribe (Manzitto-Tripp et al. 2022).

In a recent study, (Kadam et al. 2023) used the nuclear internal transcribed spacer (ITS) and the chloroplast *trn S-G* and *trn L-F* intergenic spacers to build the phylogeny of *Lepidagathis* including five Indian endemic species namely, *L. rigida* Dalzell, *L. cuspidata* Nees, *L. lutea* Dalzell, *L. sabui* Chandore, Borude, Madhav & S.R.Yadav, and *L. clavata* Dalzell. Based on their phylogenetic analysis by Bayesian and Maximum Likelihood methods *Lepidagathis* was divided into two clades, and they concluded that *Lophostachys, Teliostachya* and *Acanthura* can be included in the genus *Lepidagathis.*

Of about 151 species (POWO 2023) of *Lepidagathis* s.l., India is home to roughly 31 species, and ten varieties, of which 17 are endemic to the nation (Singh et al. 2015; BSI 2023; GBIF.Org 2023) (details are mentioned in Online Resource 1). Out of the 31, nine have been discovered after the 2000s.

In this study, two *Lepidagathis* samples were collected from two populations, in the University of Hyderabad (UoH). The voucher specimen (No. UH00097 and UH00117) is kept in the herbarium of the Department of Plant Sciences, University of Hyderabad. The specimens were putatively identified as *L. cristata* based on morphological characters (Fig. 1) as well as their distribution range. It is present in dry places and is a perennial herb with a central rootstock, and diffused stem branches arising from the rootstock. Stem is quadrangular in shape and glabrous. Leaves are lineolate, sessile, arranged in opposite phyllotaxy, with an acute tip, truncate base, and entire margin. At the base of the stem lies the globose inflorescence. Flowers are zygomorphic, bracteate, bracteolate, sessile, and pale pink or pale blue in colour. 5 lobed persistent calyx: 1 largest upper lobe, 2 opposite, and 2 inner small lobes, hairy, and spiny at the tip. Corolla is bi-lipped with 5 lobes: bifid upper lip and 3 lobed lower lip, brown and purple colour spots are present towards the base of the petal. Didynamous androecium with dithecous anthers, bilocular ovary with 2 ovules is present. Style is slender and stigma is capitellate. Fruit is capsule oblong and 2 seeded.

Molecular sequences for *L. cristata*, the type species of this genus are not available. Therefore in this study, we aimed to sequence several plastid markers, widely used for the phylogeny of Acanthaceae (Tripp and McDade 2014) and nuclear ITS (nrITS) markers for barcoding the species. Further, we aimed to build a phylogeny of the genus using the sequences available in the GenBank database. This work will act as the basis of future phylogenetic work on this under-studied genus in India and the globe.

Material and methods

Taxon sampling and DNA extraction

Two specimens of *Lepidagathis* were collected from the campus of the University of Hyderabad in December, 2022. Voucher specimens were deposited in the University of Hyderabad Herbarium (UH). Both the specimens were used for DNA extraction followed by PCR (Polymerase Chain Reaction) amplification of various makers for molecular identification and phylogenetic analysis. The corresponding genes in *Barleria* and *Crabbea*, also from the tribe Barlerieae were downloaded from GenBank and included in the analysis. *Justicia* from the sister tribe Justicieae served as the outgroup. Some of the taxa were also

reported in the phylogeny of the family Acanthaceae (McDade et al. 2008; Tripp and McDade 2014; Kadam et al. 2023). Samples were collected and stored in silica gel. Once dried, they were used for extraction of the total genomic DNA using the CTAB (cetyltrimethylammonium bromide) method along with some modifications (Doyle and Doyle 1987). For the quantification and checking the purity of the extracted DNA, NanoDrop 2000/2000c spectrophotometer (Thermo Scientific, USA) was used.

PCR amplification and sequencing

For this work, we targeted the plastid intergenic spacers: *trnS-G*, *trnL-F*, *rbcL*, *trnH-psbA*, *rps16*, *trnG-R* and nuclear ITS (for primer details, refer Online Resource 2). PCR amplification of the targetted regions was carried out. Each PCR was carried out in a 25 µl volume containing the 2X PCR mix (APS Labs, Pune, India) 10 pmol each of the forward and reverse primers and 20 ng of DNA. For amplification of the plastid markers and nuclear ITS, different programs were set. The PCR cycle for plastid genes were as follows: initial denaturation at 95℃ for 2 minutes followed by 40 cycles of (i) denaturation at 95℃ for 25 seconds, (ii) annealing at 58℃ for 30 seconds and (iii) extension at 72℃ for 50 seconds, followed by a final extension for 4 minutes at 72℃. The PCR products were analysed by running in 1% agarose gel (2 µL of each PCR product was loaded in the well) stained with GreenR dye (www.genetoprotein.com). The PCR product was then sent for purification and sequencing to Barcode Biosciences, Bangalore, India (www.barcodebiosciences.com). The accession number of all the samples are provided in Table 1.

Table 1
GenBank accession no. of the *Lepidagathis cristata*

Gene	UH:LS:0097	UH:LL:0117
trnS-trnG	OQ849586	OQ849585
trnL-trnF	OQ877054	OQ877053
trnG-trnR	OQ849588	OQ849587
trnH-psbA	-	OQ919473
rps16	-	OQ877055
rbcL	OQ877052	OQ877051

Sequence alignment and phylogenetic analysis

Chromatograms were viewed using Chromas Lite version 2.6.6 (technelysium.com.au). Forward and reverse chromatograms were checked for quality and aligned to a consensus using the Staden Package ver. 2.0.0.b11 (Staden et al. 2000). The FASTA sequences were aligned using the MUSCLE algorithm in AliView ver. 1.28 (Larsson 2014). Checking and refinements of the alignments were done using AliView. The preliminary phylogenetic tree from the aligned sequences were created using FastTree ver. 2.1.11 (Price et al. 2010), and the trees were viewed using FigTree.v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) in Aliview. Further, the best-fit model of sequence evolution

was selected by the Akaike Information Criterion using jModeltest ver. 2.1.10 (Darriba et al. 2012). Bayesian phylogenetic inference based on the best-fit model was performed in MrBayes ver. 3.2.7a (Ronquist et al. 2012). Two independent runs of two million Metropolis-coupled Markov chain Monte Carlo simulations were performed, and the consensus tree was obtained after a burnin of the initial 25% runs. Convergence of the runs was checked using Tracer ver. 1.7.1 (http://tree.bio.ed.ac.uk/software/tracer/). Maximum Likelihood bootstrap analysis was performed in MEGA ver. 11.0.13 (Tamura et al. 2021). One hundred bootstrap replicates were performed with the best-fit model under the Maximum Likelihood method. Trees were viewed using FigTree. Genetic distances between genera and within a genus were calculated using MEGA.

Results

The number of taxa for each dataset, the aligned characters, variable characters, parsimoniously informative characters, and the best model of sequence evolution are listed in Table 2. The nrITS could not be sequenced, and all the sequences obtained were fungal DNA contamination. The plastid *trnS-G* spacer had the highest number of taxa of *Lepidagathis* taxa which is 15 species. The *trnS-G* phylogenetic tree was well resolved (Fig. 2). In the *trnS-G* dataset *Lepidagathis* was found monophyletic with strong Bayesian posterior probability > 0.95 (BPP) support as well as good maximum-likelihood bootstrap support > 50% (MLBS). Within the *Lepidagathis* s.l. clade, the Asian species formed a well-supported clade sister to the African as well as South American distributed, *Lepidagathis alopecuroides* (Vahl) R. Br. ex Griseb. and the South American *Acanthura mattogrossensis* Lindau (synonymized with *Lepidagathis riedeliana* Nees). Together, these taxa were sister to another well supported clade consisting of the Central American species of the former genus *Lophostachys*: *Lophostachys uxpanapensis* Acosta (synonymized with *Lepidagathis uxpanapensis* (Acosta) Kameyama), *Lophostachys chiapensis* Acosta (synonymized with *Lepidagathis chiapensis* (Acosta) Kameyama) and *Lophostachys pubiflora* Lindau (synonymized with *Lepidagathis sessilifolia* (Pohl) Kameyama ex Wassh. & J.R.I.Wood). The Asian distributed *Lepidagathis falcata* Nees and African distributed *Lepidagathis scabra* C.B.Clarke were closest in relation to the *Lophostachys* clade. The *Lepidagathis* s.l. clade was sister to other genera of the tribe Barlerieae, *Barleria*, *Golaea*, *Acanthostelma*, *Crabbea*, and *Pseudodicliptera* which are all Old World in distribution, that too predominantly African.

Table 2
Dataset characteristics

Gene	No. of taxa	Length	Constant characters	Variable	Parsimony informative characters	Model of sequence evolution
trnSG	34	925	547	312	191	GTR + G
trnLF	36	961	678	237	135	GTR + G
trnGR	23	1096	704	309	165	GTR + I + G
psbA-trnH	22	677	305	285	166	GTR + G
rps16	23	964	566	271	133	GTR + G
rbcL	35	551	487	64	36	SYM + I + G
ITS	38	765	316	427	299	GTR + I + G
trnSG + *trnLF* + nrITS	31	2652	1567	925	557	GTR + I + G

The *trnL-trnF* phylogeny was highly congruent with the *trnS-G* phylogeny (Fig. 3). Asian *Lepidagathis* was monophyletic with strong branch support. This clade was in close relation to *Lepidagathis alopecuroides* and *Acanthura mattogrossensis* just as in the *trnS-G* dataset. This group was found sister to the well-supported clade containing Asian *L. falcata* and African *L. scabra* and Central American *Lophostachys*. *Lepidagathis* s.l. was sister to the other genera of tribe Barleriae which are predominantly Old World.

The *trnG-R* dataset phylogeny (Online Resource 3) was also similar to the *trnS-G* and *trnLF* intron phylogeny. The Asian species of *Lepidagathis* formed a well-supported clade, sister to the South American *Lepidagathis alopecuroidea*. This clade was in turn sister to the *Lophostachys* clade in which the African *Lepidagathis scabra* was nested in.

The *rps16* intron phylogeny (Fig. 4) was also congruent with the previous three dataset phylogenies. The Asian *L. cristata* and *L. formosana* were sister to *L. alopecuroides* and *Acanthura mattogressensis* with good BPP support but not MLBS support. This clade was in turn sister to the clade containing *Lophoystachys* and the African *L. scabra* with good MLBS support but not BPP support. The rest of the taxa of Barlerieae were sister to *Lepidagathis* s.l. but this clade was with moderate support.

The molecular sequences of *Lepidagathis* for the *psbA-trnH* region were very limited in the GenBank database. Only two Asian taxa were available. *L. cristata* and *L. incurva*, they were strongly supported and sister to the rest of the genera of the tribe Barlerieae (Online Resource 4).

The *rbcL* phylogeny was poorly resolved and was also depauperate in *Lepidagathis* species in representation. However, the available taxa of *Lepidagathis* were monophyletic with good MLBS support and the monophyly of Barleriae was also retained with only good MLBS support (Online Resource 5).

As the nrITS could not be sequenced for *B. cristata*, only the taxa whose ITS sequences were available in the GenBank were used for the analysis. The nrITS dataset was also congruent with the plastid datasets (Fig. 5). All Asian *Lepidagathis* samples were found in a strongly supported clade which was sister to *Lepidagathis alopecuroidea* and *Acanthura mattogrossensis* (syn. *Lepidagathis reidliana* Nees.), similar to the plastid datasets. The South American, *Lophostachys* group along with *L. scabra* and *L. falcata* formed a strongly supported clade but its relationship with the other *Lepidagathis* was not clear as it formed a polytomy with *Barleria* (Fig. 5). The relationship of *L. scabra* and *L. falcata* was also similar to that of the cpDNA datasets, i.e., closest to *Lophostachys*.

Since the topology of the plastid intron genes as well the nrITS dataset was congruent, we merged the three datasets to infer a combined phylogeny of the plastid *trnSG*, *trnL-F* and the nuclear ITS regions. The combined dataset, showed a very good resolution of the clades (Fig. 6). The Asian *Lepidagathis*, *Lepidagathis* s.s. formed a well-supported clade with one African species *L. villosa*. The closest in relation to this clade was the clade containing *Lepidagathis alopecuroides* and *Acanthura mattogrossensis*. The *Lophostachys* clade was sister to the above two clades which included *L. falcata* and *L. scabra*. The rest of the taxa of Barleriae was also well resolved and showed a sister relation to Whitfieldieae.

Discussion

We have sequenced six plastid DNA marker genes for *Lepidagathis cristata* for our study. However, the ITS sequence could not be amplified with multiple different primers and the bands amplified were fungal sequences. All markers used gave the same phylogenetic relationships and there was no incongruence between the nuclear and the plastid data.

Lepidagathis s.l. with about 151 species, according to POWO (2023) requires a thorough study. This initial molecular study provides evidence for multiple lineages which could be characterized primarily by biogeography and substantiated by morphological synapomorphies. In other words, *Lepidagathis* s.l. which is truly pantropical in distribution could be split into at least two different subgenera using both morphological, distribution and molecular data. In the very recent comprehensive classification of the family Acanthaceae, (Manzitto-Tripp et al. 2022) have considered *Lepidagathis* with the subsuming of *Lophostachys* following Kameyama (2008). But our study shows that the Asian *Lepidagathis* of which at least ten species have been sampled is a separate radiation which is related to the South American species *Acanthura mattogrossensis* (synonymized with *Lepidagathis riedeliana*) and *Lepidagathis alopecuroidea*, the latter also being African in distribution (the names used are as given in the original sequences from GenBank data). We propose this lineage as *Lepidagathis* sensu stricto. This lineage supports the idea that long distance dispersal from South American into Africa followed by long disperal into Asia and further eastward dispersal and diversification. The seasonal rainfall system, popularly known as Monsoon has played an important role in the rise in diversification rates of various plant as well as animal groups (Surveswaran et al. 2021). Alternately, *Lepidagathis* s.s. could be Asian in origin

followed by dispersal into Africa and South America and this hypothesis awaits testing. It would be important to date the diversification of *Lepidagathis* s.l using a comprehensive sampling.

Furthermore, based on the phylogeny, we propose raising *Lophostachys* to a subgenus level as it forms a separate clade predominantly containing South American species. Interestingly, this lineage also has Asian elements and African elements: *L. falcata* and *L. scabra*. The groupwise genetic distance between the samples of *Lepidostachys* and *Lophostachys* is quite high compared to the genetic distance between *Lophostachys* and *Barleria* (Table 3). The calyx and the anther characters (Table 5) are the two major distinguishing characters between *Lepidagathis* and *Lophostachys* (Benoist 1911) as used by (Bentham 1876). The calyx of *Lepidagathis* has five sepals, while the calyx of *Lophostachys* has four sepals. The androecium of *Lepidagathis* has all anthers with two thecae, while the androecium of *Lophostachys* has two anterior stamens with two thecae and the posterior ones with one or no thecae. *L. scabra* has 5 sepals whereas the anther character is similar to that of *Lophostachys* (Clarke 1899). We hypothesize that there were colonizations from the *Lophostachys* clade into Africa and Asia, however, this needs comprehensive sampling.

Table 3
Group-wise genetic differences between *Lophostachys*, *Lepidagathis*, and *Barleria* (Distance data between groups).

Gene	*Lophostachys-Lepidagathis*	*Lophostachys-Barleria*	*Lepidagathis-Barleria*
trnSG	0.0781	0.0601	0.0924
trnLF	0.0537	0.0327	0.0671
trnGR	0.0513	0.0376	0.0592

Majority of the workers have supported the merger of *Lophostachys* with *Lepidagathis* (Benoist 1911; Kameyama 2008; Hirao et al. 2019; Manzitto-Tripp et al. 2022; Kadam et al. 2023), however, based on biogeographical distribution, molecular phylogeny and morphological data, we propose retaining *Lophostachys* separate from *Lepidagathis*.

The branch lengths of *Lepidagathis* s.s. clade is considerably high corresponding to the high within-genus genetic distance across 4 genes (Table 4). Though it could be argued that the sampling for the other genera is less, even with the 10 species taken for this study, there is clearly a high genetic diversification rate among branches of *Lepidagathis* s.s. We speculate that this might be due to the open habitat in which the plant grows. In general, being a perennial with dried inflorescence facing the direct summer sunlight and the associated UV radiation might be a reason for the higher nucleotide diversification rate in the genus. Moreover, additional mutation rates might have played an important role

in the diversification of the species enabling it to conquer the harsh hot and dry habitat in which the plant survives. Engineered hypermutations in the model cyanobacterium *Synechocystis* have helped it survive high light and high temperature stress (Sun et al. 2023). Further studies on the plastome as well as the transcriptome is necessary to understand the survival ability of this genus in such harsh environments.

Table 4
Group-wise genetic differences within *Lophostachys*, *Lepidagathis*, and *Barleria*

Gene	*Lepidagathis*	*Lophostachys*	*Barleria*
trnSG	0.06940	0.01033	0.02268
trnLF	0.04446	0.01098	0.00590
trnGR	0.06136	0.02577	0.01110

Table 5
Differences between *Lepidagathis* and *Lophostachys*

Character	*Lepidagathis*	*Lophostachys*	References
Calyx	5-partite calyx, the upper one larger and the fused two lower segments longer.	4 segments, the upper and the lower wider	(Thomas F. 1993; Scotland 1994; Scotland and Vollesen 2000)
Corolla aestivation	Quincuncial left contort	Quincuncial with the abaxial corolla lobe wholly overlapped	(Scotland 1994)
Anthers	All anthers bithecous	Two anterior stamens, bithecous, posterior stamens monothecous or absent,	(Kameyama 2008)
Pollen	Size and shape vary from subprolate to prolate, 3-colporate, reticulate, heterobrochate, the lumina are verrucate, pilate or baculate, the lumina adjacent to the colpi, at the poles and the centre of mesolcopi are smaller.	Prolate, 3-colporate, reticulate subhomobrochate, lumina mostly pentagonal or hexagonal and baculaté, pilate, and/or gemmate	(Thomas F. 1993; Kameyama 2008)

Declarations

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Kuldeep Prasad Agarwala, Shriti Somani, Bhagyasree Raveendran V.R. The first draft of the manuscript was written by Siddharthan Surveswaran, Kuldeep Prasad Agarwala, Shriti Somani and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability

All the specimens are deposited in the Herbarium UH, University of Hyderabad, Hyderabad, India. Sequences have been deposited in the GenBank. The alignment file can be obtained from the corresponding author.

Conflict of interest

The authors declare no conflict of interest.

References

1. Abubacker MN, Devi PK (2014) In vitro antifungal potentials of bioactive compound oleic acid, 3-(octadecyloxy) propyl ester isolated from *Lepidagathis cristata* Willd. (Acanthaceae) inflorescence. Asian Pac J Trop Med 7S1:S190-3. https://doi.org/10.1016/S1995-7645(14)60230-3
2. Ahirwar B, Ahirwar D (2021) *Lepadigathis cristata* Willd. Traditional Plant of Chhattisgarh Tribes: An Alternative Cure. Rese Jour of Pharm and Technol 3430–3432. https://doi.org/10.52711/0974-360X.2021.00596
3. Benoist R (1911) Les genres *Lepidagathis* et *Lophostachys* sont-ils distincts? Notul Syst 2:139–144
4. Bentham G (1876) Acanthaceae. In: Genera plantarum. Vol. 2. Lovell Reeve, London, pp 1060–1122
5. Bremekamp (1938) Notes on the Acanthaceae of Surinam. Recueil Trav Bot Néerl 35:130–176
6. BSI (2023) Botanical Survey of India | Flora of India. https://efloraindia.bsi.gov.in/eFlora/speciesList_PCL.action?resultType=genusWise&parent_Id=23712&parent_Name=LEPIDAGATHIS. Accessed 17 Apr 2023
7. Champluvier D, Darbyshire I (2012) *Schaueriopsis*: a new genus of Acanthaceae (Acanthoideae: Barlerieae) from the Democratic Republic of Congo. Plant Ecol Evol 145:279–284. https://doi.org/10.5091/plecevo.2012.657
8. Clarke CB (1899) Acanthaceae. In: Dyer WTT (ed) Flora of Tropical Africa. Lovell Reeve and Co., London
9. Daniel TF (1995) New and reconsidered Mexican Acanthaceae VI. Chiapas. Proc Calif Acad Sci 48:253–282
10. Darbyshire I, Fisher AE, Kiel CA, McDade LA (2019) Phylogenetic relationships among species of *Barleria* (Acanthaceae, Lamiales): Molecular data reveal complex patterns of morphological evolution and support a revised classification. Taxon 68:92–111. https://doi.org/10.1002/tax.12029
11. Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772. https://doi.org/10.1038/nmeth.2109
12. Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

13. Flowers of India (2023) *Lepidagathis trinervis* - Frilly Lepidagathis. https://www.flowersofindia.net/catalog/slides/Frilly%20Lepidagathis.html. Accessed 18 Apr 2023
14. GBIF.Org (2023) Occurrence Download. The Global Biodiversity Information Facility. https://doi.org/10.15468/dl.gx742r
15. Hirao YV, Monteiro MM, Demarco D (2019) Structure and development of flowers in *Lepidagathis* and implications for systematics of the genus and floral evolution in Acanthaceae. Botan J Linn Soc 189:153–168. https://doi.org/10.1093/botlinnean/boy076
16. India Biodiversity Portal (2023) *Lepidagathis cristata* Willd. | Species. https://indiabiodiversity.org/species/show/266059. Accessed 18 Apr 2023
17. Kadam SK, Tamboli AS, Mane RN, et al (2023) Molecular phylogeny and genetic diversity of some *Lepidagathis* species (Acanthaceae) based on nuclear and chloroplast regions. Plant Biosyst 157:455–464. https://doi.org/10.1080/11263504.2023.2165557
18. Kameyama C (2008) New species, nomenclatural changes and lectotypifications in Neotropical *Lepidagathis* Willd. (Acanthaceae). Kew Bull 63:565–581. https://doi.org/10.1007/s12225-008-9066-2
19. Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics 30:3276–3278. https://doi.org/10.1093/bioinformatics/btu531
20. Manzitto-Tripp EA, Darbyshire I, Daniel TF, et al (2022) Revised classification of Acanthaceae and worldwide dichotomous keys. Taxon 71:103–153. https://doi.org/10.1002/tax.12600
21. McDade LA, Daniel TF, Kiel CA (2008) Toward a comprehensive understanding of phylogenetic relationships among lineages of Acanthaceae s.l. (Lamiales). Am J Bot 95:1136–1152. https://doi.org/10.3732/ajb.0800096
22. McDade LA, Moody ML (1999) Phylogenetic relationships among Acanthaceae: evidence from noncoding *trn L-trn F* chloroplast DNA sequences. Am J Bot 86:70–80. https://doi.org/10.2307/2656956
23. Nees CG (1832) Acanthaceae Indiae orientalis. Plantae asiaticae rariores 3:70–117
24. Nees CG (1847) Acanthaceae. In: Martius, von KFP (eds) Flora Brasiliensis. pp 65–74
25. Pohl JE (1827) Plantarum Brasiliae icones et descriptiones. Antonii Strauss, Vindobonae, Vienna
26. POWO (2023) *Lepidagathis* Willd. | Plants of the World Online | Kew Science. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:30029079-2#source-KB. Accessed 10 Apr 2023
27. Price MN, Dehal PS, Arkin AP (2010) FastTree 2 — approximately maximum-likelihood trees for large alignments. PLoS ONE 5:e9490. https://doi.org/10.1371/journal.pone.0009490
28. Rao S, Swamy RK, Kumar D, et al (2019) Flora of Peninsular India. http://flora-peninsula-indica.ces.iisc.ac.in/herbsheet.php?id=225&cat=7. Accessed 17 Apr 2023
29. Ravikanth V, Niranjan Reddy VL, Ramesh P, et al (2001) An immunosuppressive tryptophan-derived alkaloid from *Lepidagathis cristata*. Phytochemistry 58:1263–1266. https://doi.org/10.1016/s0031-

9422(01)00383-1

30. Richard SW, Marius L, Noya S, et al (2011) Anti-inflammatory, analgesic and antipyretic effects of *Lepidagathis anobrya* Nees (Acanthaceae). Afr J Tradit Complement Altern Med 8:420–424. https://doi.org/10.4314/ajtcam.v8i4.12
31. Ronquist F, Teslenko M, van der Mark P, et al (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. https://doi.org/10.1093/sysbio/sys029
32. Scotland RW, Vollesen K (2000) Classification of Acanthaceae. Kew Bull 55:513. https://doi.org/10.2307/4118776
33. Singh P, Karthigeyan K, Lakshminarasimhan P, Dash SS (2015) Endemic vascular plants of India. Botanical Survey of India, Kolkata
34. Staden R, Beal KF, Bonfield JK (2000) The Staden package, 1998. Methods Mol Biol 132:115–130. https://doi.org/10.1385/1-59259-192-2:115
35. Sun H, Luan G, Ma Y, et al (2023) Engineered hypermutation adapts cyanobacterial photosynthesis to combined high light and high temperature stress. Nat Commun 14:1238. https://doi.org/10.1038/s41467-023-36964-5
36. Surveswaran S, Kambale SS, Srivatsav M, et al (2021) Origin and diversification of Indian Ceropegieae (Apocynaceae) and possible relation to the Indian monsoon. J Syst Evol 59:93–112. https://doi.org/10.1111/jse.12578
37. Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 38:3022–3027. https://doi.org/10.1093/molbev/msab120
38. Tripp EA, McDade LA (2014) A rich fossil record yields calibrated phylogeny for Acanthaceae (Lamiales) and evidence for marked biases in timing and directionality of intercontinental disjunctions. Syst Biol 63:660–684. https://doi.org/10.1093/sysbio/syu029
39. Willdenow KL (1800) Species plantarum. Impensis G.C. Nauk, Berlin
40. Yadava RN (2001) A new biologically active triterpenoid saponin from the leaves of *Lepidagathis hyalina* Nees. Nat Prod Lett 15:315–322. https://doi.org/10.1080/10575630108041298

Figures

Figure 1

Lepidagathis samples collected from UoH. **a** Plant; **b**Inflorescence; **c** Stem

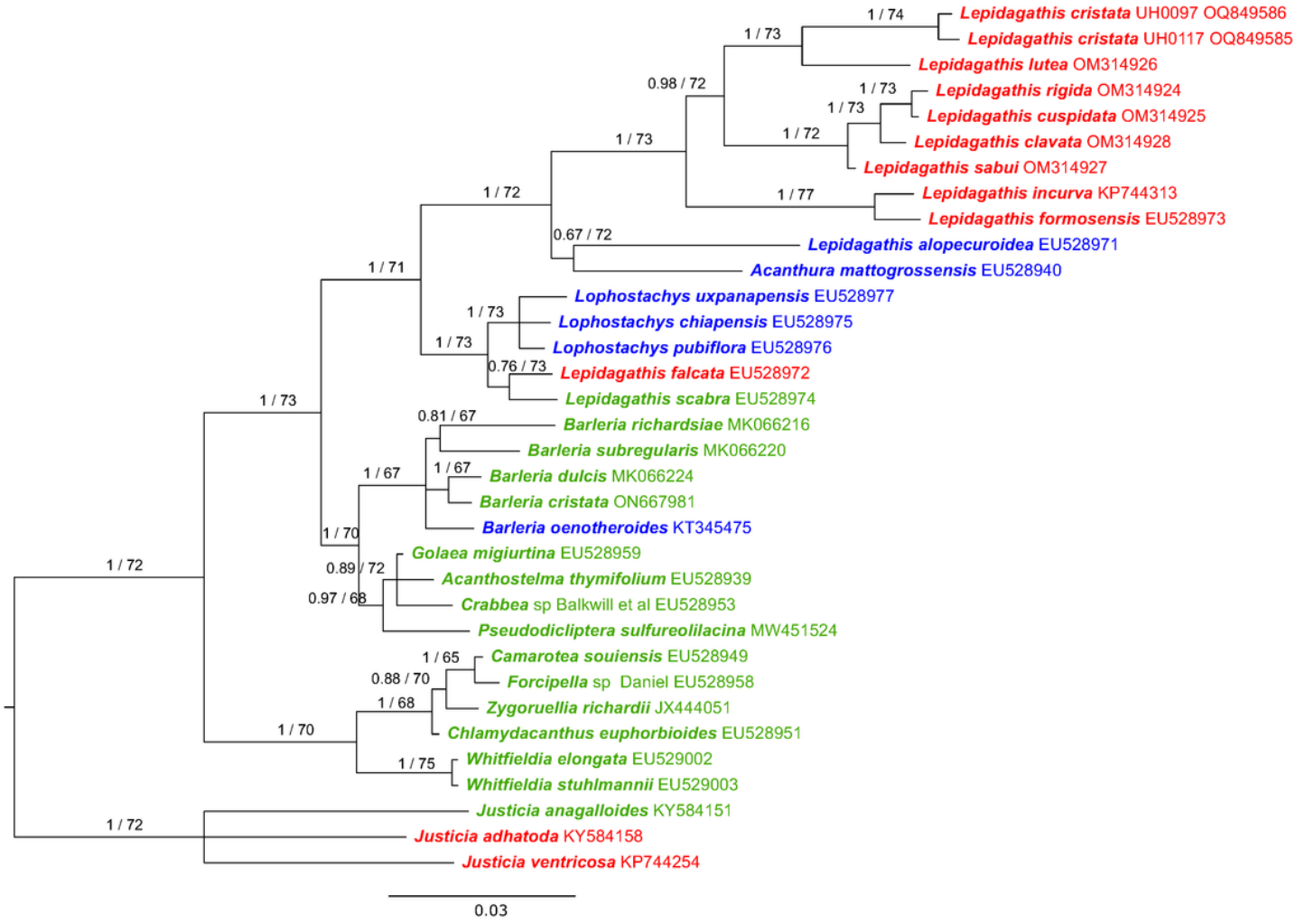

Figure 2

Bayesian phylogenetic tree of plastid *trnS-trnG* intron. The numbers above branches indicate BPP / MLBS values. Scale bar indicates number of substitutions across branches. Colours indicate geographical

distributions. Red: Asia, Blue: South America/Central America, Green: Africa.

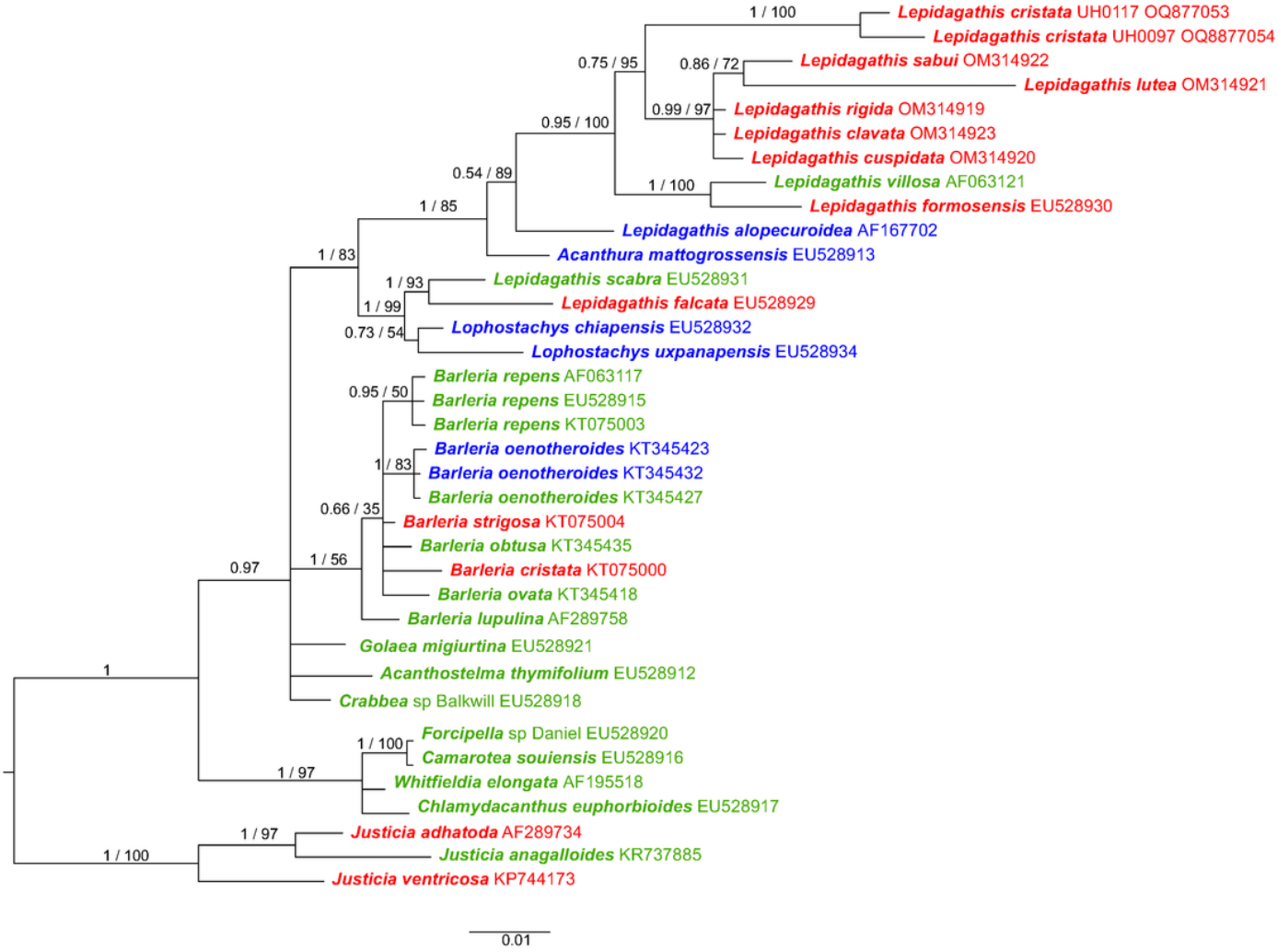

Figure 3

Bayesian phylogenetic tree of plastid *trnL-trnF* intron. The numbers above branches indicate BPP / MLBS values. Scale bar indicates number of substitutions across branches. Colours indicate geographical distributions. Red: Asia, Blue: South America/Central America, Green: Africa.

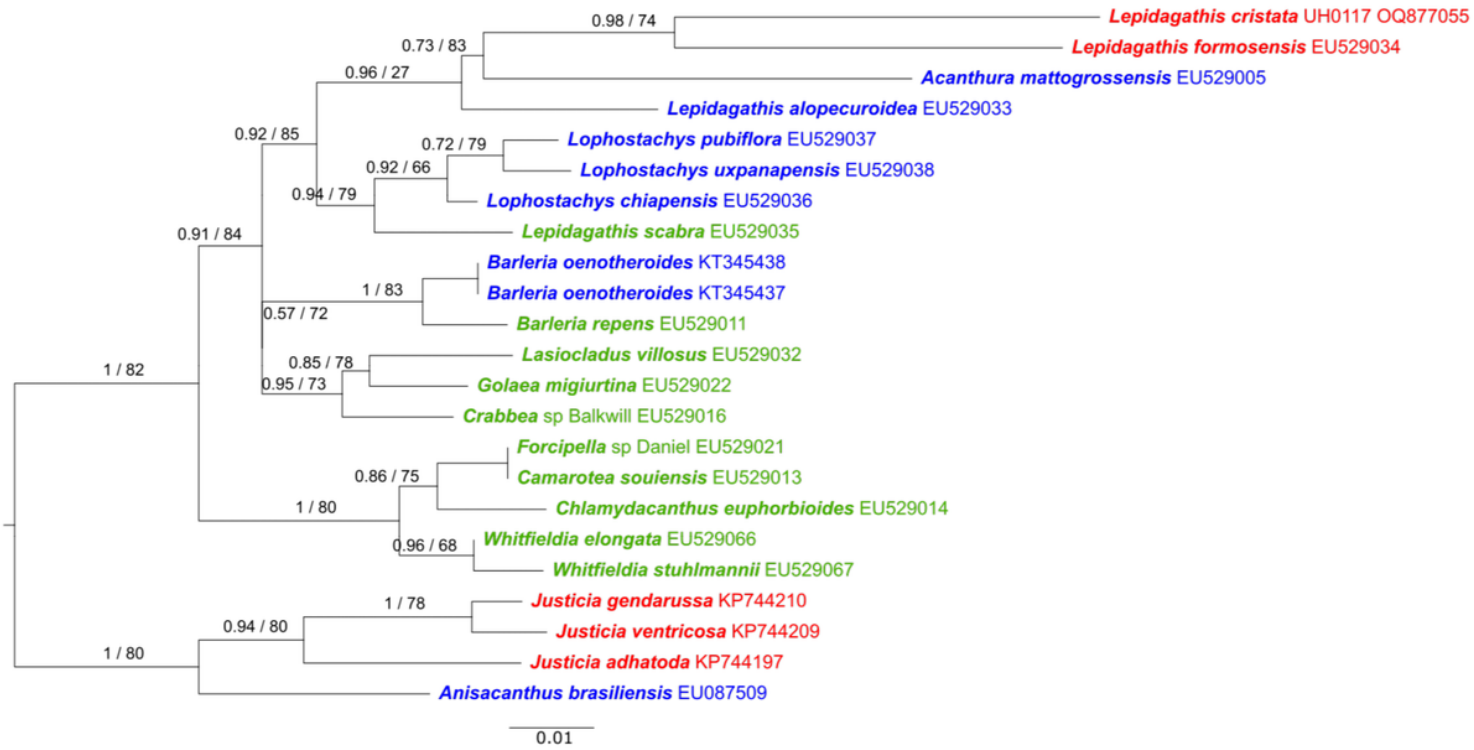

Figure 4

Bayesian phylogenetic tree of plastid *rps16* intron. The numbers above branches indicate BPP / MLBS values. Scale bar indicates number of substitutions across branches.Colours indicate geographical distributions. Red: Asia, Blue: South America/Central America, Green: Africa.

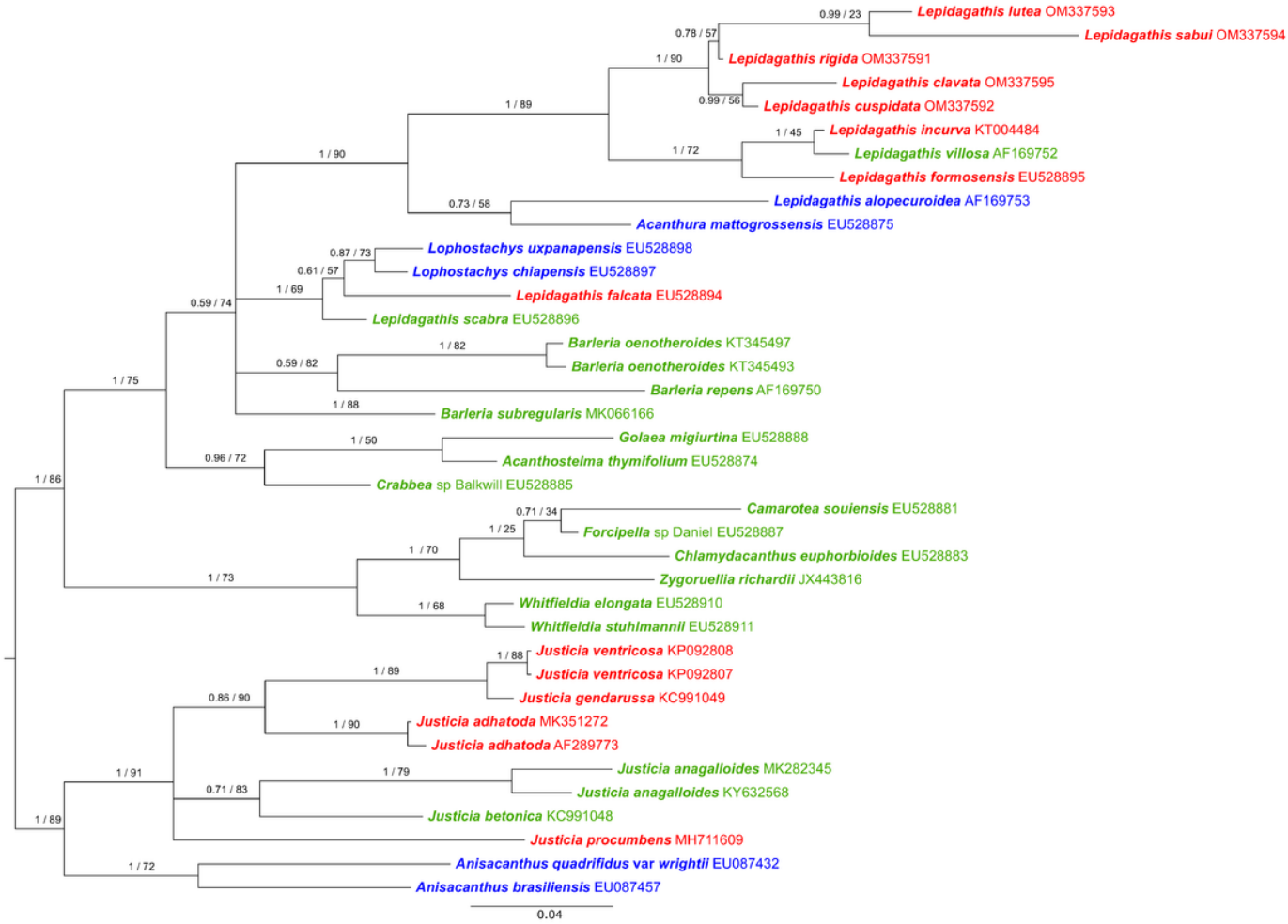

Figure 5

Bayesian phylogenetic tree of nuclear ITS. The numbers above branches indicate BPP / MLBS. Scale bar indicates number of substitutions across branches. Colours indicate geographical distributions. Red: Asia, Blue: South America/Central America, Green: Africa.

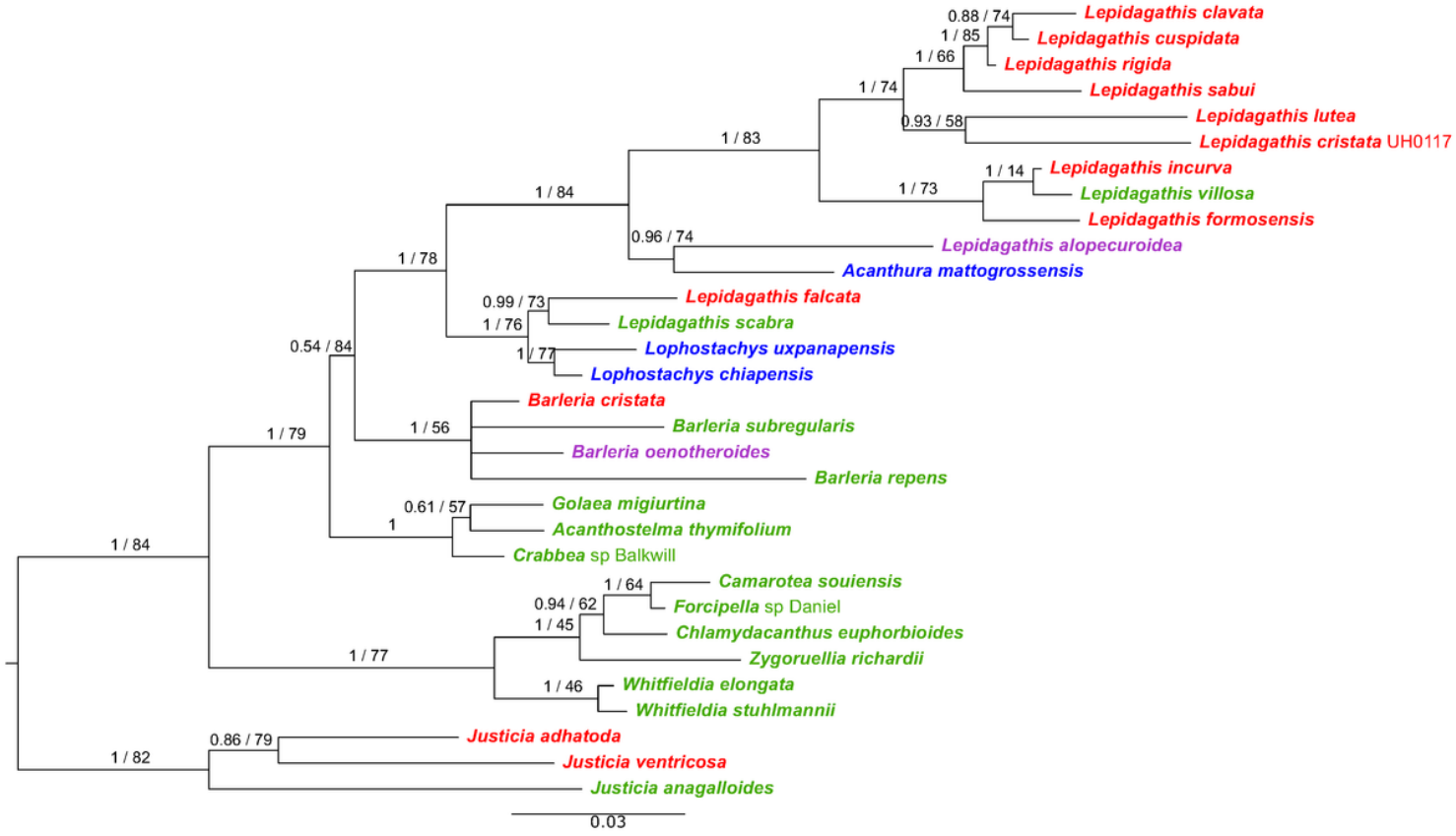

Figure 6

Bayesian phylogenetic tree of *trnS-trnG* intron, *trnL-trnF* intron, and nuclear ITS. The numbers above branches indicate BPP / MLBS values. Scale bar indicates number of substitutions across branches. Colours indicate geographical distributions. Red: Asia, Blue: South America/Central America, Purple: South America and Africa, Green: Africa.

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