Ceylon Journal of Science 48(2) 2019: 133-141
DOI: http://doi.org/10.4038/cjs.v48i2.7617

RESEARCH ARTICLE

Molecular phylogeny helps to delimit *Plectranthus hadiensis* from its related morph occurring in Sri Lanka

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Received: 26/01/2019 ; Accepted: 28/04/2019

Abstract: *Plectranthus hadiensis* is an important medicinal plant in Sri Lanka. It was considered a separate species, *P. zeylanicus*, endemic to the island until its inclusion, as *P. hadiensis* var. *tomentosus,* together with morphs from southern Africa in the revised species concept of *P. hadiensis*. However, there are morphological, chemical, and therapeutic differences between the African and Sri Lankan morphs. We used eight molecular markers in a phylogenetic study to clarify the species concept of *P. hadiensis* and to investigate whether it should include the Sri Lankan morph. We examined the position of the two *P. hadiensis* morphs in relation to eight other *Plectranthus* species. The maximum likelihood tree revealed three clades: a weakly supported clade including *P. calycinus, P. glabratus, P. fruticosus,* and *P. malabaricus*; a highly supported clade including *P. amboinicus* and African and Sri Lankan specimens of *P. hadiensis*; and a highly supported clade formed by *P. barbatus*, *P. caninus*, and *P. hadiensis* var. *tomentosus*. The African *P. hadiensis* specimens form a highly supported subclade sister to a subclade containing the Sri Lankan *P. hadiensis*, suggesting that the subclades correspond to either two sister species or two subspecies. We propose that they are more likely to be sister species given the differences in morphology, chemistry, and chromosome number.

Keywords: *Plectranthus hadiensis*, *Plectranthus zeylanicus*, Lamiaceae, Ocimae, molecular phylogeny.

INTRODUCTION

Plectranthus hadiensis (Forssk.) Schweinf. ex Sprenger (Lamiaceae) is a medicinal plant whose native range extends from southern and eastern Africa to the southern Arabian Peninsula (Codd, 1985). It also occurs in Sri Lanka (Thwaites, 1864; Trimen, 1895; Cramer, 1981) and is known as *iriweriya* in Sinhala and *valakan* in Sanskrit (Jayaweera, 1981). In revising the species concept of *P. hadiensis*, Codd (1985) recognised three separate varieties of *P. hadiensis* and included the Sri Lankan morph in var. *tomentosus,* which he named *P. zatarhendi* var. *tomentosus* in an earlier treatment (Codd, 1975; Fig. 1a, b). Until Codd's revisions (1975, 1985), the morph occurring in Sri Lanka was thought to be a separate endemic species, *P. zeylanicus* Benth.*,* first described by Bentham (*Labiatarum Genera et Species* 36, 1832) based on the type specimen from the island. While maintaining its endemicity, Cramer (1978, 1981) reclassified the Sri Lankan morph as *Coleus zeylanicus* (Benth.) L.H.Cramer based on fused stamens, a trait originally used by Bentham to distinguish *Coleus* Lour. from *Plectranthus* L'Hér. Both Thwaites (1864) and Trimen (1895) found *P. zeylanicus* only as a cultivated plant in Sri Lanka and the latter did not believe that it grew wild on the island. Codd (1985) speculated that the Sri Lankan plant was an introduction from southern Africa where *P. hadiensis* var. *tomentosus* occurs naturally in dry woodland and rocky grassland.

In a molecular, morphological, and phytochemical phylogenetic analysis of the Ocimae, Paton *et al.* (2004) found that the *Plectranthus* species in their study separated into two clades, one clade that included *Plectranthus* species previously placed within *Coleus* and the genera *Pycnostachys* Hook., *Holostylon* Robyns & Lebrun, and *Anisochilus* Wall. ex Benth., and a sister clade including the remaining *Plectranthus* species and *Tetradenia* Benth., *Thorncroftia* N.E.Br., and *Aeollanthus* Mart. ex Spreng. All species in the *Coleus* clade had a sigmoid corolla tube and a horizontal anterior corolla lobe. The corolla tube of the Sri Lankan morph of *P. hadiensis* is distinctly sigmoid with a horizontal lower lip (Fig. 1c). In the South African morph, Codd (1985) described the corolla tube as bent, without specifying the degree of geniculation. In addition, the *Coleus* clade displayed fusion of all stamens, although in some species within the clade this trait was lost (Paton *et al.*, 2004). In the Sri Lankan morph of *P. hadiensis,* Cramer (1978) described the filaments as fused somewhat less than half-way along their length. However, in Codd's (1985) description, the stamens of *P. hadiensis* are free to the base. There are also dissimilarities in leaf pubescence of the Sri Lankan and South African morphs of *P. hadiensis*; Cramer

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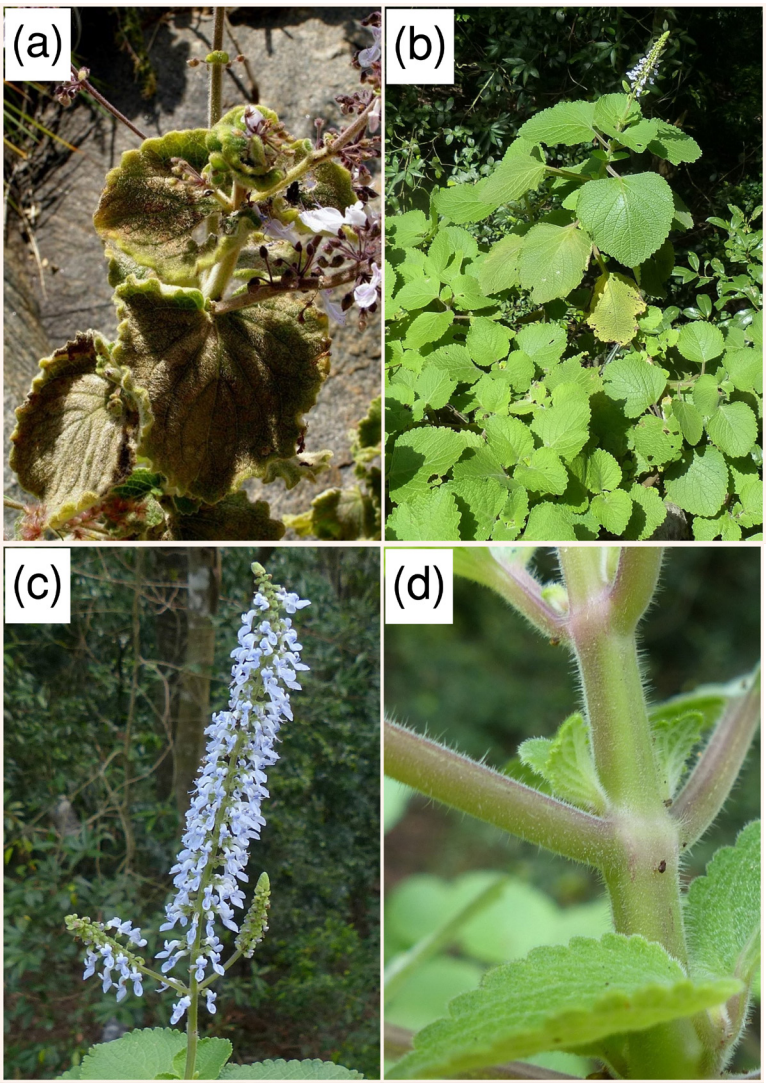

Figure 1: Southern African and Sri Lankan morphs of *Plectranthus hadiensis* var. *tomentosus*. (a) The morph from Swaziland, southern Africa (image: K.Braun, Swaziland's Flora Database, http://www.sntc.org.sz/flora/photo.asp?phid=5646, accessed 14 April 2019). (b) Habit, (c) inflorescence, and (d) stem and leaf pubescence of the morph from Sri Lanka (images: P. D. H. Prins).

(1981) described the leaves of the Sri Lankan morph as sparsely hirtellous (see Fig. 1d), whereas Codd (1985) used the densely tomentose nature of leaves of *P. hadiensis* var. *tomentosus* (Benth.) Codd and var. *hadiensis* as a diagnostic trait to distinguish them from var. *woodii* (Gürke) Codd.

The presence of quinonoid diterpenes was a trait used in Paton *et al.*'s (2004) phylogenetic analysis of the Ocimae; they found that most *Plectranthus* species in the *Coleus* clade produced these compounds whereas their congeners in the sister clade did not. Both the Sri Lankan and South African morphs of *P. hadiensis* contain royleanones, a type of quinonoid abietane diterpenoid, which are known to have antimicrobial properties (van Zyl *et al.*, 2008; Rijo *et al.*, 2010; Kubínová *et al.*, 2014). However, there are differences in the composition of royleanones between the two morphs. Both morphs have 7α-acetoxy-6β-hydroxyroyleanone (Mehrotra *et al.*, 1989; van Zyl *et al.*, 2008; the latter authors give the systematic name 7α-acetoxy-6β,12-dihydroxy-abieta-8,12-diene-11,14-dione), 7β-acetoxy-6β-hydroxyroyleanone, and 7β,6β-dihydroxyroyleanone (Mehrotra *et al.*, 1989; Dukhea, 2010). The South African morph also has 7α-formyloxy-6β-hydroxyroyleanone (7α-formyloxy-6β,12-dihydroxy-abieta-8,12-diene-11,14-dione; van Zyl *et al.*, 2008). The aroma of the leaves of the Sri Lankan morph was described by Trimen (1895) as sweet-scented and resembling that of lemon verbena (*Aloysia citrodora* Palau). On the other hand, the South African *P. hadiensis* var. *tometosus* is known as the 'Vicks Plant' with an odour similar to that of Vicks® VapoRub™ or mentholatum (Llifle Encyclopedia of Living Forms, www.llifle.com, accessed 1 Jan 2019), suggesting that the plant's dominant aroma is of camphor and menthol.

The Sri Lankan morph of *P. hadiensis* has many therapeutic uses in Ayurveda and folk medicine, mainly for treatment of gastrointestinal disorders such as diarrhoea and dysentery (Jayaweera, 1981; Mehrotra *et al.*, 1989; Arambewela and Wijesinghe, 2006; Waldia *et al*., 2011). In contrast, in South African Zulu medicine *P. hadiensis* var. *tomentosus* is employed as an enema (Hutchings, 1989, 1996). Given that the Sri Lankan morph is an ingredient in over 20 Ayurvedic preparations (Arambewela and Wijesinghe, 2006), its correct identification is important. The differences in morphology, chemistry, and therapeutic

use of the two morphs suggest that the species concept of *P. hadiensis* needs further consideration. Therefore, we undertook a molecular phylogenetic analysis using eight chloroplast markers to ascertain whether Codd's delimitation of *P. hadiensis* should include the Sri Lankan morph of the species.

MATERIALS AND METHODS

Sampling, DNA extraction, and PCR amplification

In this study, several accessions of *Plectranthus* were included. Accessions for ten species, including one for *P. zeylanicus*, were obtained from GenBank (Table 1). DNA of one *P. hadiensis* accession (DNA Bank ID: 19767) was obtained from Royal Botanic Gardens, Kew, DNA Bank (apps.kew.org/dnabank/, last accessed 2018-04-25). Genomic DNA was extracted from *c.* 20 mg of silica gel-dried (Chase and Hills, 1991) leaves of four *P. hadiensis* samples from Sri Lanka and one *P. hadiensis* herbarium specimen (Herbarium ID: K000468025, provided by Kew Herbarium) using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. To avoid degradation, material was frozen in liquid nitrogen and then ground to a fine powder using glass beads. Detailed information on accessions can be found in Table 1.

One nuclear region, the internal transcribed spacer (ITS: ITS 1-5.8S-ITS 2), and seven plastid regions: partial *matK,* partial *rps16,* partial *rpoC1,* partial *rpoB, trnT-trnL-trnF* (partial *trnT-trnL* intergenic spacer, complete *trnL*, complete *trnL-trnF* intergenic spacer, and partial *trnF*), *ndhC-trnV* (partial *ndhC,* partial *ndhC-trnV* sequence), and *rpl32-trnL* (partial *rpl32*, partial *rpl32-trnL* intergenic spacer), were amplified and sequenced. PCRs included 7.5 µL ٢× Phusion Green HF HS PCR Master Mix with 1.5 mM $MgCl_2$ (Life Technologies, LT, Vienna, Austria), 0.15 µL bovine serum albumin (0.2 g/L), 1.5 µL each primer (3.2 µM), 1 µL template DNA, and H_2O up to a final volume of 15 µL. The primers used in this study are provided in Table 2. Thermal cycle conditions were as follows: initial denaturation at 98 °C for 30 s, 35 cycles of denaturation at 98 °C for 10 s, annealing at 55–70 °C (depending on the primers, Table 2) for 30 s and extension at 72 °C for 45 s, followed by final extension of 5 min at 72 °C. PCR products were cleaned with 1.5 µL exonuclease I and FastAP thermosensitive alkaline phosphatase mixture (7 U Exo I, 0.7 U FastAP) at 37 °C for 45 min and 85 °C for 15 min. Sequencing reactions were performed with the BigDye Terminator Kit v3.1 (LT) using the same primers that were used for amplification or with internal primers (Table 2) according to the manufacturer's instructions. Sanger sequencing was carried out using a 3730 DNA analyser (LT).

Sequence alignment and phylogenetic analyses

Sequences were assembled and edited using Geneious (version 8.0.5, Kearse *et al.*, 2012). The alignment of sequences was performed in Geneious using the MAFFT plugin and inspected manually with BioEdit v7.0.4. Unsequenced regions were coded as missing data in the combined matrix. To infer phylogenetic relationships, maximum parsimony (MP) and maximum likelihood (ML) analysis were performed. MP analyses were conducted in PAUP version 4.0a149 (Swofford, 2016). For each data set, heuristic searches were conducted using 1000 replicates of random addition sequence, tree-bisection–reconnection (TBR) branch-swapping, and 'keeping multiple trees' (MulTrees) but saving only 20 trees per replicate. Clade support was estimated by the bootstrap (Felsenstein, 1985) with 1000 replicates, TBR branch swapping, and simple addition sequence. To explore the variability of each marker, nine matrices were analysed with MP: (1) ITS, (2) *matK,* (3) *rps16,* (4) *rpoC1,* (5) *rpoB*, (6) *trnT-trnL-trnF*, (7) *ndhC-trnV,* (8) *rpl32-trnL*, and (9) all regions combined. Information about the alignment characteristics and number of variable and potentially parsimony informative sites were obtained for each marker from PAUP. ML analysis was conducted using the combined data only. An ML rapid bootstrap analysis (1000 replicates) with search for best-scoring ML tree in one run was conducted in RAxML v8.2.0 (Stamatakis, 2014). The general time reversible (GTR+GAMMA) model with six substitution types (one for each pair of nucleotides) and gamma-distributed rate variation across sites and a proportion of invariable sites was used for the analysis. *Mentha longifolia* obtained from GenBank was used as the outgroup. Trees were visualized and edited in FigTree v1.4.1 (http://tree.bio.ed.ac.uk/software/figtree/, last accessed 2018-06-14).

RESULTS AND DISCUSSION

Our main aim in this study was the clarification of the species concept of *P. hadiensis*, specifically investigation of whether it should include the morph of *P. hadiensis* from Sri Lanka which differs from the African morph not only in its morphology and chemistry, but also in its therapeutic uses. We also examined the position of the two *P. hadiensis* morphs in relation to other *Plectranthus* species.

We sequenced both plastid and nuclear regions for our phylogenetic analysis. Of the plastid regions, *rpl32-trnL* exhibited the highest percentage of variable characters (12.6%). The nuclear *ITS* region was the most informative region with 85 (9.2%) potentially parsimony-informative sites. All eight markers combined resulted in a 7525 bp alignment and included 526 variable characters (7%) of which 158 (2.1%) were parsimony informative. Further parsimony statistics are given in Table 3.

Maximum likelihood (ML) and maximum parsimony (MP) analyses using the combined data set revealed similar results. The ML tree with bootstrap percentages from the MP (BS_{MP}) and ML (BS_{ML}) analyses is shown in Figure 2. Besides *P. mollis* (Aiton) Spreng., which is sister to all other *Plectranthus* taxa included in this study, our analyses revealed three clades: (1) a weakly supported clade including *P. calycinus* Benth., *P. glabratus* (Benth.) Alston, *P. fruticosus* L'Hér., and *P. malabaricus* (Benth.) R.H.Willemse (Fig. 2; 1: BS_{ML} 74, BS_{MP} -; this order will be used throughout; a hyphen indicates support < 70%); (2) a highly supported clade consisting of *P. amboinicus* (Lour.) Spreng., the two African taxa of *P. hadiensis* (one from Tanzania and one from South Africa) and the four *P.*

Table 1: Specimens and accessions used in this study. The collection number, herbarium voucher, and location are given for newly generated sequences in this study. GenBank accession numbers of species used for phylogenetic analysis are stated. Abbreviations: DL, D. Large (field collector); na, not available; NMPG, National Medicinal Plants Garden, Ganewatte, Sri Lanka. Herbarium codes: K, Royal Botanic Gardens Kew, U.K; GB, sequence obtained from GenBank.

Species	**Collection**	**Voucher**	**Location**	**ITS**	***matk***	***rps16***	***rpoC1***	***rpoB***	***trnT-trnL-trnF***	***ndhC-trnV***	***rpl32-trnL***
Plectranthus amboinicus	GB	GB	GB	KM877368	KM877397	AJ505377	KM877457	KM877427	KX364780	na	na
Plectranthus barbatus	GB	GB	GB	KM877375	KM877405	AJ505378	KM877465	KM877435	KX364783	na	na
Plectranthus calycinus	GB	GB	GB	KF855455	na	AJ505380	na	na	na	na	KF855675
Plectranthus caninus	GB	GB	GB	KM877357	KM877386	na	KM877446	KM877416	na	na	na
Plectranthus glabratus	GB	GB	GB	na	JF357828	AJ505387	na	na	AJ505508	na	na
Plectranthus fruticosus	GB	GB	GB	KM877361	KM877390	na	KM877450	KM877420	GU381473	na	na
Plectranthus hadiensis var. *tomentosus*	GB	GB	GB	JX974581	KC211323	na	na	na	na	na	na
Plectranthus malabaricus	GB	GB	GB	KM877369	KM877398	na	KM877458	KM877428	na	na	na
Plectranthus mollis	GB	GB	GB	KM877354	KM877382	na	KM877442	KM877412	na	na	na
Plectranthus hadiensis	Ward, C.J. 9794	K	Natal, South Africa	MH884557	MH884577	MH884594	na	MH884583	MH884562	MH884567	na
	Bidgood, S.; Hoenselaar, K.; Leliyo, G.; Vollesen, K. 6400		Tanzania	na	MH884578	MH884595	MH884589	MH884584	MH884563	MH884568	MH884572
	DL; R.S.1		Matale, Sri Lanka	MH884553	MH884573	MH884590	MH884585	MH884579	MH884558	na	MH884569
	DL; R.S.2		Matale, Sri Lanka	MH884554	MH884574	MH884591	MH884586	MH884580	MH884559	MH884564	MH884570
	DL; R.S.3		NMPG, Sri Lanka	MH884555	MH884575	MH884592	MH884587	MH884581	MH884560	MH884565	MH884571
	DL; R.S.4		NMPG, Sri Lanka	MH884556	MH884576	MH884593	MH884588	MH884582	MH884561	MH884566	na
Plectranthus zeylanicus	GB	GB	GB	KM877366	KM877395	na	KM877455	KM877425	na	na	na
Mentha longifolia	GB	GB	GB	KY072949	KU956042	KU956042	KU956042	KU956042	KU956042	KU956042	KU956042

Table 2: Details of primers used in this study**.**

Region	Primer	Sequence (5'-3')	Usage	T_A (°C)	Reference
ITS	ITS18Sfa (ITS18Scsf)	GAATGGTCCGGTGAAGTGTTCG	PCR and sequencing	70	Barfuss, 2012
	ITS26Sra (ITS26Scsr)	GGACGCTTCTCCAGACTACAATTCG	PCR and sequencing		Barfuss, 2012
	ITS5.8Sfa (ITS5.8Scsf)	GACTCTCGGCAACGGATATCTCG	Sequencing		Barfuss, 2012
	ITS5.8Sra (ITS5.8Scsr)	GATGCGTGACGCCCAGGCAG	Sequencing		Barfuss, 2012
matK	matK-413f-1	TAATTTACRATCAATTCATTCAATATTTCC	PCR and sequencing	55	Heckenhauer *et al.*, 2016
	matK-PlecR[1] (ratio:1:1:1):				
	matK-1227r-1	GARGAYCCRCTRTRATAATGAGAAAGATTT	PCR and sequencing		Heckenhauer *et al.*, 2016
	matK-1227r-2	GAAGAYCCGCTATGATAATGAGAAAGGTTT			Heckenhauer *et al.*, 2016
	matK-1227r-5	GARGATCCRCTRTRATAATGAGAAATATTT			Heckenhauer *et al.*, 2016
rps16	rpsF	GTGGTAGAAAGCAACGTGCGACTT	PCR and sequencing	70	Oxelman *et al.*, 1997
	rpsR2	TCGGGATCGAACATCAATTGCAAC	PCR and sequencing		Oxelman *et al.*, 1997
rpoC1	rpoC1 1	GTGGATACACTTCTTGATAATGG	PCR and sequencing	62	Ford *et al.*, 2009
	rpoC1 4	CCATAAGCATATCTTGAGTTGG	PCR and sequencing		Ford *et al.*, 2009
rpoB	rpoB 2	ATGCAACGTCAAGCAGTTCC	PCR and sequencing	65	Ford *et al.*, 2009
	rpoB 4	GATCCCAGCATCACAATTCC	PCR and sequencing		Ford *et al.*, 2009
trnT-trnL-trnF	a_mod	CATTACAAATGCGATGCTCTAAC	PCR and sequencing	68	Heckenhauer *et al.*, 2017
	f_mod	ATTTGAACTGGTGACACGAGGAT	PCR and sequencing		Heckenhauer *et al.*, 2017
	c	CGAAATCGGTAGACGCTACG	Sequencing		Taberlet *et al*., 1991
	h	CCATTGAGTCTCTGCACCTATC	Sequencing		Taberlet *et al*., 2007
ndhC-trnV	ndhC	TATTATTAGAAATGYCCARAAAATATCATATTC	PCR and sequencing	60	Shaw *et al.*, 2007
	trnV(UAC)x2	GTCTACGGTTCGARTCCGTA	PCR and sequencing		Shaw *et al.*, 2007
rpl32-trnL	rpL32-F	CAGTTCCAAAAAAACGTACTTC	PCR and sequencing	60	Shaw *et al.*, 2007
	trnL(UAG)	CTGCTTCCTAAGAGCAGCGT	PCR and sequencing		Shaw *et al.*, 2007

[1] Primer *matK*-PlecR was obtained by multiplexing several degenerate primers from Heckenhauer *et al*. (2016).

hadiensis specimens from Sri Lanka and the *P. zeylanicus* specimen from GenBank (Fig. 2; 2: 92, 96); and (3) a highly supported clade formed by *P. barbatus* Andrews, *P. caninus* Roth, and *P. hadiensis* var. *tomentosus* (= *P. zeylanicus*) (Fig. 2; 3: 100, 100).

With respect to *P. hadiensis* accessions, the two African specimens form a highly supported subclade (Fig. 2; clade 2, green: 100, 96). This subclade is sister to a subclade containing the four specimens of *P. hadiensis* from Sri Lanka newly sequenced in this study and *P. zeylanicus* obtained from GenBank (Fig. 2; clade 2, red: 100, 99). The accessions of Sri Lankan *P. hadiensis* and *P. zeylanicus* form a polytomy. Our phylogenetic trees suggest that the subclade containing African *P. hadiensis* and the subclade containing Sri Lankan *P. hadiensis* specimens (and *P. zeylanicus*) may correspond to either sister species or subspecies. However, based on the morphological and chemical differences between the African and Sri Lankan *P. hadiensis* morphs we discussed earlier and the differences in chromosome number, we propose that it is more likely that the two subclades correspond to sister species rather than subspecies. *P. zeylanicus* is a tetraploid with a haploid chromosome count of n = 14 (2n = 28; x = 7; Thoppil, 1993) whereas African *P. hadiensis* is a hexaploid with n = 21 (2n = 42; x = 7; de Wet, 1958). Among African species of *Plectranthus*, the commonest chromosome number for

Table 3: Alignment and parsimony characteristics.

	ITS	*matk*	*rps16*	*rpoC1*	*rpoB*	*trnT-trnL-trnF*	*ndhC-trnV*	*rpl32-trnL*	Combined
Total number of accessions	15	16	11	13	14	11	6	6	16
Length of alignment bp	920	856	916	574	508	1704	1132	915	7525
Number of variable characters (%)	87 (9.5)	68 (7.9)	70 (7.6)	17 (3.0)	18 (3.5)	92 (5.4)	59 (5.2)	115 (12.6)	526 (7.0)
Number of parsimony-informative characters (%)	85 (9.2)	18 (2.1)	11 (1.2)	4 (0.7)	11 (2.2)	13 (0.8)	3 (0.3)	13 (1.4)	158 (2.1)
Tree length of best parsimonious tree (steps)	290	95	86	21	32	107	62	135	290
Trees saved (parsimony analysis)	9730	5436	4990	4880	1907	6573	6630	7400	9730
Consistency index	0.81	0.937	1	1	0.938	1	1	0.993	0.81
Retention index	0.783	0.793	1	1	0.929	1	1	0.993	0.783
Rescaled consistency index	0.635	0.743	1	1	0.871	1	1	0.926	0.635
Homoplasy index	0.19	0.063	0	0	0.063	0	0	0.007	0.19

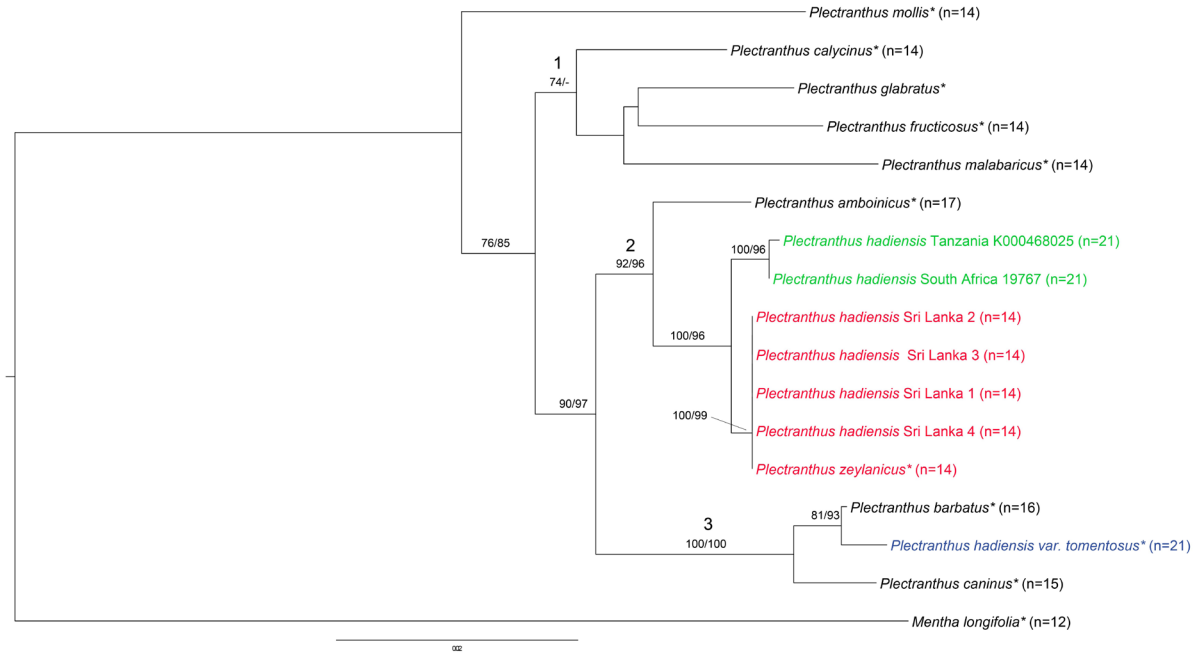

Figure 2: Maximum likelihood tree showing three clades (1-3) for *Plectranthus* taxa included in the study. Bootstrap percentages (≥ 70%) from maximum likelihood and maximum parsimony analyses are shown for each node. A hyphen indicates bootstrap support < 70%. The mean haploid chromosome count (n) is given for each species. The chromosome count for *P. zeylanicus* was obtained from Thoppil (1993). All other chromosome counts are from the Chromosome Counts Database (CCDB, version 1.46, Rice *et al*., 2015). As the Sri Lankan morph of *P. hadiensis* was formerly known as *P. zeylanicus*, we have assumed that the chromosome count for the Sri Lankan specimens of *P. hadiensis* is the same as that for *P. zeylanicus*. Sequences obtained from GenBank are indicated with an asterisk.

the genus is 2n = 28, with a basic number of 7 (de Wet, 1958; Morton, 1962; see also Fig. 2). The chromosome count of *Plectranthus* species in the Chromosome Counts Database (http://ccdb.tau.ac.il/Angiosperms/Lamiaceae/Plectranthus/; Rice *et al.*, 2015) ranges from n = 7 to n = 42, with a median of n = 14 (44% of the species). Whole genome duplication (polyploidy) has an important role in the evolution of angiosperms (Soltis *et al.*, 2014). Recent phylogenetic analysis suggests that polyploidy is a key mechanism for cladogenesis and for speciation within plant genera (Zhan *et al.*, 2016). Polyploidy may also introduce phenotypic and ecological diversity in plant lineages leading to niche differentiation and enhanced responses to environmental stress (Soltis *et al.*, 2014). It is likely that whole genome duplication has played a role in speciation within the large genus of *Plectranthus* (currently 300 species, including *Coleus*; Stevens, 2001 onwards).

Interestingly, the GenBank accessions of *P. hadiensis* var. *tomentosus,* within which Codd (1985) included *P. zeylanicus,* are separated from the other *P. hadiensis* specimens and *P. zeylanicus* in our phylogenetic analysis (Fig. 2; clade 3, blue). However, our analysis was based on only two markers for *P. hadiensis* var. *tomentosus*. It is important that a larger number of individuals and markers are used to understand the phylogenetic affinities of *P. hadiensis* var. *tomentosus* and to ascertain whether its inclusion within the *P. hadiensis* species concept is justified.

CONCLUSION

Our results suggest that the Sri Lankan and African morphs of *P. hadiensis* are phylogenetically distinct enough to be considered either sister species or subspecies. However, given the differences in morphological, chemical, and cytological traits, it is more likely that they are sister species. We therefore believe that reinstatement of Cramer's nomenclature, *Coleus zeylanicus* (Benth.) L.H.Cramer, to the Sri Lankan morph of *P. hadiensis* (*iriweriya*) may be warranted. The genus *Coleus* is currently synonymized with *Plectranthus*. However, in their most recent phylogenetic study of the subtribe Plectranthinae, Paton *et al.* (2018) show that the *Coleus* clade, which includes *P. amboinicus* (the type of *Coleus*), *Solenostemon*, *Pycnostachys*, and *Anisochilus*, forms a well-defined sister group to the rest of the species in the subtribe. Therefore, they recommend recognising *Coleus* as a separate genus. The circumscription of the new *Coleus* genus would include *P. hadiensis* (see Fig. 1 in Paton *et al.*, 2018). We believe that this new finding justifies restoration of the Cramer rather than the Bentham nomenclature to *iriweriya*.

Proper identification of *P. hadiensis* taxa is important for future researchers and medical practitioners since the pharmacological properties of the Sri Lankan plants may be wrongly attributed to *P. hadiensis* from southern Africa and vice versa. Our study looked at only molecular markers, with only two markers for *P. hadiensis* var. *tomentosus*. Our results demonstrate the need for further phylogenetic analyses involving chemical constituents (particularly those of medicinal value) and morphological and cytological features, in addition to molecular markers, to confirm whether the two morphs of *P. hadiensis* are the same or different species. Such an analysis should include multiple individuals collected from different regions of south and southeast Asia (including Sri Lanka and India) and Africa, as well as a greater number of individuals of *P. hadiensis* var. *tomentosus* than we have used in our study, for a more precise delimitation of the *P. hadiensis* species concept. *C. zeylanicus* may well be an introduction from Africa as is *P. amboinicus*, which is also used in Sri Lanka for its medicinal properties. Trimen (1895) stated that *C. zeylanicus* is morphologically similar to *P. parviflorus* Willd., a species native to Oceania. On the other hand, *C. zeylanicus* may be an example of speciation following migration from Africa, as is the case for some Asian *Plectranthus* species (Paton *et al.*, 2018). Ideally, any future analysis should include *P. parviflorus* and other African and Asian *Plectranthus/Coleus* species with morphological similarities to *C. zeylanicus,* as well as members of the Ocimae in the Sri Lankan flora, to understand the phylogenetic affinities and putative origins of *C. zeylanicus*.

ACKNOWLEDGEMENTS

This work was supported by the Austrian Science Fund FWF (P26548-B22) to R. S. Kew DNA Bank is acknowledged for providing DNA aliquots of *Plectranthus hadiensis* (DNA Bank ID: 19767). We thank Kew Herbarium for allocating the *P. hadiensis* herbarium specimen (herbarium ID: K000468025).

AUTHOR CONTRIBUTIONS

P.D.H.P. conceived the idea, J.H. and M.H.J.B. did the laboratory work, J.H. carried out the phylogenetic analysis, D.L., J.H., and R.S. wrote the manuscript.

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