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

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**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* Bent., first described by Bentham (*Labiatarum Genera et Species* 36, 1832) based on the type specimen from the island. While maintaining its endemcity, 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 *Aecolanthus* 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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(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 congener 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; Kubinová 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. *tomentosus* 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

![Figure 1](Image)

**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).
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 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: K004068025; 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*F (partial *trnL-trnf*F intergenic spacer, complete *trnl*, complete *trnL-trnf*F intergenic spacer, and partial *trnf*F), *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 × Phusion Green HF HS PCR Master Mix with 1.5 mM MgCl₂ (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 H₂O 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*F, (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 (21%) 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<sub>mp</sub>) and ML (BS<sub>ml</sub>) 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* Bentham., *P. glabratus* (Benth.) Alston, *P. fruticosus* L’Hér., and *P. malabaricus* (Benth.) R.H.Willemse (Fig. 2; 1: BS<sub>mp</sub> 74, BS<sub>ml</sub>-; 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. hadiensis*.
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       | KM877357 | KM877386 | AJ505380 | na      | na      | na              | na         | na         |
| Plectranthus caninus          | GB         | GB      | GB       | KM877357 | KM877386 | AJ505380 | KM877446 | KM877416 | na              | na         | na         |
| Plectranthus glabratius       | GB         | GB      | GB       | KM877354 | KM877382 | na      | KM877442 | KM877412 | na              | na         | na         |
| Plectranthus fruticosus       | GB         | GB      | GB       | KM877361 | KM877390 | na      | KM877450 | KM877420 | GU381473         | na         | na         |
| Plectranthus hadiensis var. tomentosus | GB | GB | GB | KM877369 | KM877398 | na      | KM877458 | KM877428 | ga              | na         | na         |
| Plectranthus malabaricus      | GB         | GB      | GB       | KM877354 | KM877382 | na      | KM877442 | KM877412 | na              | na         | na         |
| Plectranthus mollis           | GB         | GB      | GB       | KM877354 | KM877382 | na      | KM877442 | KM877412 | na              | na         | 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 (ITS18Sscf)    | GAAATGGTCCGGTGAAAGTGTTCC | PCR and sequencing | 70         | Barfuss, 2012      |
| ITS     | ITS26Sra (ITS26Scsr)    | GACGCTTCTCCGACTCAAATTCG | PCR and sequencing | 70         | Barfuss, 2012      |
| ITS    | ITS5.8Sfa (ITS5.8Scsf)  | GACTCTCGGCAACGGATATCTCG | Sequencing      | 70         | Barfuss, 2012      |
| ITS     | ITS5.8Sra (ITS5.8Scsr)  | GATGCGTACGCCCCAGGCAG | Sequencing      | 70         | Barfuss, 2012      |
| matK   | matK-413f-1             | TAATTACRATCAATTCATATAATTTCC | PCR and sequencing | 55         | Heckenhauer et al., 2016 |
|         | matK-PlecR1 (ratio:1:1:1): |                       |                |            |                    |
| matK   | matK-1227r-1            | GARGAYCRCRCTRATATAATGAAAGATTT | PCR and sequencing |             | Heckenaher et al., 2016 |
| matK   | matK-1227r-2            | GAAAGCGCTATGATAATGAAAGGTTT |                |             | Heckenaher et al., 2016 |
| matK   | matK-1227r-5            | GARGATCRCTRATATAATGAAATATT |                |             | Heckenaher et al., 2016 |
| rps16  | rpsF                    | GTGGTAGAAGAAGCAACGTCGACTT | PCR and sequencing | 70         | Oxelman et al., 1997 |
|        | rpsR2                   | TCGGGATCGAACATCAATGCAAC | PCR and sequencing |             | Oxelman et al., 1997 |
| rpoC1  | rpoC1 1                 | GTGGATACACTTCTTGATAATGG | PCR and sequencing | 55         | Ford et al., 2009   |
|        | rpoC1 4                 | CCATAAAGCATATCTTGAGTTGG | PCR and sequencing | 55         | Ford et al., 2009   |
| rpoB   | rpoB 2                  | ATGCAACGTCAGAGCTTCC | PCR and sequencing | 65         | Ford et al., 2009   |
|        | rpoB 4                  | GATCCACAGTCAATGCTTCC | PCR and sequencing | 65         | Ford et al., 2009   |
| trnT-   | a_mod                   | CATTACAATTCGAGATCTGCTTAAC | PCR and sequencing | 68         | Heckenaher et al., 2017 |
| trnL-   | f_mod                   | ATTTGAACCTGACGAGGAT | PCR and sequencing |             | Heckenaher et al., 2017 |
| trnF   | c                      | CGAAATCGGTAGACGCTACG | Sequencing      |             | Taberlet et al., 1991 |
|        | h                      | CCATTAGTCTCTGCACTTAC | Sequencing      |             | Taberlet et al., 2007 |
| ndhC-   | ndhC                   | TATTATTAGAATGCGGACGTTAGTCC | PCR and sequencing | 60         | Shaw et al., 2007   |
| trnV    | trnV(UAC)cy2            | GTGCACGGTCTCGAARTCCGA | PCR and sequencing | 60         | Shaw et al., 2007   |
| rpl32-  | rpl32-F                 | CAGTTCCAAAAAAGCCTACCTTC | PCR and sequencing | 60         | Shaw et al., 2007   |
| trnL(UAG) |                   | CTGCTTCTCAGAGCGAGCGT | PCR and sequencing | 60         | Shaw et al., 2007   |

1 Primer matK-PlecR was obtained by multiplexing several degenerate primers from Heckenaher 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  | 68   | 70    | 17    | 18   | 92            | 59        | 115        | 526      |
| (9.5)            | (7.9) | (7.6) | (3.0) | (3.5) | (5.4) | (5.2)          | (12.6)    | (7.0)      |          |
| Number of parsimony-informative characters (%) | 85  | 18   | 11    | 4     | 11   | 13            | 3         | 13         | 158      |
| (9.2)            | (2.1) | (1.2) | (0.7) | (2.2) | (0.8) | (0.3)          | (1.4)     | (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 recognizing 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 introduction 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.

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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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