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Strobilanthes medahinnensis (Acanthaceae) a new species, based on morphological and molecular data, from the Peak Wilderness Nature Reserve, Sri Lanka

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Abstract

Strobilanthes medahinnensis, a new species of Acanthaceae is described and illustrated from Sri Lanka. The new species is similar to *S. anceps* in having ovate leaves, yellow gland dots of abaxial leaf surface and reflexed bracts but differs by rounded stem, leaves with acuminate apex, elongated spikes, lanceolate outermost bract with long acuminate apex. The establishment of the new species is supported by complete plastome genome analyses.

Keywords: chloroplast genome, barcoding, bract, habitat, distribution

Introduction

Strobilanthes Blume (1826: 781), one of the largest genera in the family Acanthaceae, is mostly distributed in the evergreen forests of south and southeast of Asia and Melanesia and comprises about 450 species (Carine & Scotland 1998, Carine & Scotland 2002, Mabberley 2017, POWO 2019, Thomas *et al*. 2020). The plietesial flowering of many species in the genus makes it difficult to study and many species are poorly known and rarely collected. In Sri Lanka, many species have a rather restricted distribution in undisturbed moist montane, submontane, and lowland forests of the central and southwestern parts of the island. *Strobilanthes exserta* Clarke (1884: 445), *S. willisii* Carine in Carine *et al.* (2000: 971), and *S. cordifolia* Vahl (1794: 84) Wood (2014: 390) are confined to the dry zone while *S. lupulina* Nees (1832: 85) is distributed widely at low and high altitudes of the wet zone. During the plant explorations at Peak Wilderness Nature Reserve, on the 1st of September 2015, the first author found an interesting *Strobilanthes* population at Medahinna at 1430 m elevation, consisting of about 25 individuals, growing in the shade and along the nature trail. Although the population was not flowering at that time, this appeared distinct in leaf characters of its morphologically closest ally *S. anceps* Nees (1837: 312). We conducted extensive plant explorations across the entire distribution range of *Strobilanthes* in Sri Lanka covering 21 administrative districts from January 2012 to September 2020. However, this new plant was not found in any other location except Medahinna and we visited Medahinna several times to study this population since 2015. Finally, on 29th June 2020, we could observe flowers in the population at Medahinna. After a closer examination of the specimens and critical study of the literature, comparison of the specimens at National Herbarium, Royal Botanic Gardens, Peradeniya (PDA), and online herbaria, we found that it is different from all known species of *Strobilanthes* in the world. In addition to the preparations of distribution map, superlative photographs, and meticulously illustrated line drawings, the complete chloroplast genomes of this interesting material and *S. anceps* were also analyzed. According to morphological and molecular evidence, it could not be assigned to any previously published species; hence it is described as a new species below.

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Material and methods

Sample collection

Herbarium specimens were prepared following the standard protocol (Alexiades 1996) and the type material was deposited at the National Herbarium at Royal Botanic Gardens, Peradeniya, Sri Lanka, (PDA). Flowers were also preserved in ethanol for detailed morphological study. Young leaves were harvested from new species for molecular work, along with materials of *S. anceps*.

Morphological observations

A detailed morphological comparison was accomplished using published *Strobilanthes* descriptions (Wood 1994, 1995, 1998, Venu 2006, Wood & Scotland 2009), as well as herbarium specimens at the National Herbarium, Royal Botanic Gardens, Peradeniya (PDA), online herbaria such as Kew (K), University of Graz, Institute of Plant Sciences (UGPS) and online databases of digitized herbarium specimens (JSTOR).

Studies of both micro and macro morphological characters were carried out on field-collected materials. Dimensions were measured using a ruler with 0.5 mm accuracy, tape and, digital caliper readings from $0.01x10^{-3}$m. The species was photographed *in situ* and also from ethanol preserved floral material using a Nikon D850 camera with AF-S VR MICRO NIKKOR 105 mm f/2.8 G-ED and AF-S VR NIKKOR 70-200 mm f/2.8E FLED lenses. We also recorded the character states of our specimens in a matrix from the population of Medahinna at Peak Wilderness Nature Reserve and populations of *S. anceps* at Horton plains National Park, Hakgala Strict Nature Reserve, Mandaran Nuwara, Reverstern, Namunukula. Measurements were taken from five samples of each population. Plant habitat, life form, leaf, inflorescence, bract, calyx, corolla, and stamens were observed. The quantitative measurements such as plant height, petiole length, inflorescence length, peduncle length, pedicel length, bract length, corolla tube length, and diameter were taken. The line illustration of the new species was prepared from alcohol-preserved material and digital photos. The GPS coordinates of the collection sites were recorded from sample collection sites using GARMIN GPSmap 78s and mapped on a floristic map of Sri Lanka using ARC GIS version 10.8.1 ESRI, 2020 (Figure 1).

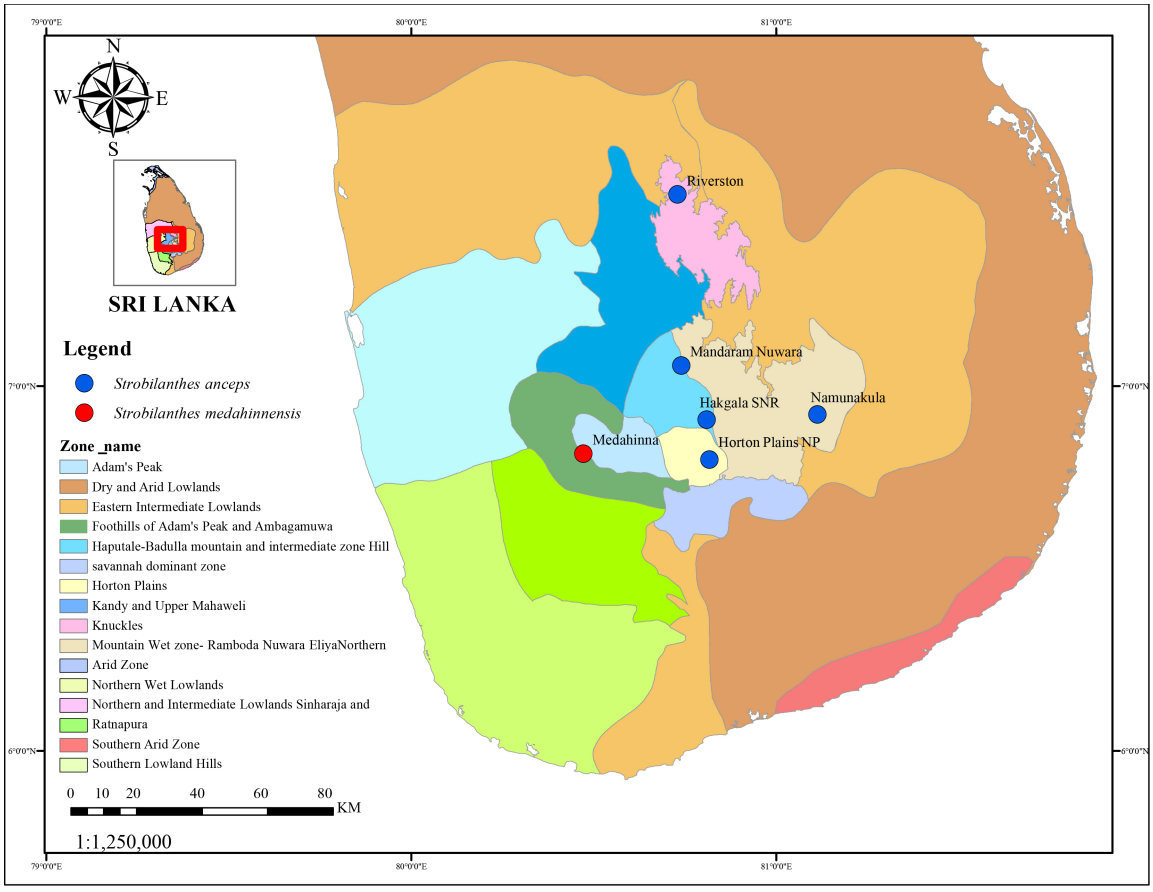

FIGURE 1. *Strobilanthes medahinnensis* and *Strobilanthes anceps* recorded localities in Sri Lanka.

DNA extraction and sequencing

Genomic DNA from fresh leaf samples was extracted using a plant DNAeasy mini kit (Qiagen 69106) following the manufacturer's guidelines. The quality and quantity of extracted DNA were assessed with Nanodrop 2000 spectrophotometer (Thermo Scientific) and Agarose gel electrophoresis. About 1µg of good quality genomic DNA was sent to Admera Health, the USA for high throughput sequencing. Prior to sequencing, the quality of genomic DNA was quantified with Qubit 2.0 DNA HS Assay (ThermoFisher, Massachusetts, USA) and quality assessed by Tapestation genomic DNA Assay (Agilent Technologies, California, USA). Library preparation was performed using KAPA Hyper Prep kit (Roche, Basel, Switzerland) following the manufacturer's recommendations. Library quality and quantity were assessed with Qubit 2.0 DNA HS Assay, Tapestation High Sensitivity D1000 Assay, and QuantStudio 5 System (Applied Biosystems, California, USA). Illumina 8-nt dual-indices were used. Equimolar pooling of libraries was performed based on QC values and sequenced on an Illumina NovaSeq S4 with a read length configuration of 150 PE for 80 M PE reads (40M in each direction) per sample.

Plastome assembly and analysis

Raw reads were *de novo* assembled using GetOrganelle v1.7.1 (Jin *et al.* 2020), with organelle type *embplant_pt.* The assembled contigs were confirmed after mapping raw reads again to the contigs. The gaps observed between the contigs were filled at this stage to build the consensus cp genome. The assemblies were annotated using the online annotation tool Geseq v1.84 (Tillich *et al*. 2017) and examined using the visualization tool OrganellarGenomeDRAW (OGDRAW) v1.3.1 (Greiner *et al*. 2019). The annotated assemblies were loaded to Geneious v11.0.5 (Kearse *et al.* 2012) and aligned using MAFFT v7.450 (algorithm FFT-NS-2) (Katoh & Standley 2013). The alignment was further analyzed to find DNA polymorphism using DnaSP v6.12.03 (Rozas *et al*. 2017) by calculating the nucleotide variability (Pi) adopting a sliding window mechanism (window length - 600, step size - 200). To find perfect Simple Sequence Repeats (SSR), Krait v1.3.3 (Du *et al*. 2018) was used with the minimum number of repeats set to 8, 4, 3, 3, and 3 for mono-, di-, tri-, tetra-, and pentanucleotide SSRs, respectively. The assembled chloroplast genomes were submitted to GenBank (Accession Numbers: *S. anceps,* MW411188; *S. medahinnensis*, MW411189).

The divergence time between the two species was estimated using the BEAST2 v2.6.3 software package (Drummond *et al.* 2012) based on the Bayesian phylogenetic analysis. Additionally, *S. cusia* (Nees) O. Kuntze (1891: 499) (NC_037485), *Oryza Sativa* Linnaeus (1753: 333) Indica Group (NC_008155), *O. nivara* Sharma & Sahstry (1965: 161) (NC_005973), *Arabidopsis lyrata* Linnaeus (1753: 665) O'Kane & Al-Shehbaz (1997: 325) (LN877383) and *A. thaliana* Linnaeus (1753: 665) Heynhold in Holl & Heynhold (1842: 538) (NC_000932) were included in the analysis. While the site substitution rate was estimated using a GTR model with a relaxed lognormal clock, the divergence was determined using the Yule node calibration technique by assuming a normal distribution with a mean of 5.97 Mya between *A. thaliana* and *A. lyrata* (Hohmann *et al.* 2015). The Markov chain Monte Carlo process was set to run for 100,000,000 generations sampling at every 10,000 generations. The output of BEAST2 was examined using Tracer v1.7.1 (Rambaut *et al.* 2018). The maximum clade credibility tree after 10% postburn-in was generated using TreeAnnotator v2.6.3 and visualized using FigTree v1.4.4 (Rambaut 2017).

Results

Morphological characters

Morphological investigations confirmed that the newly collected material is allied to *S. anceps* in having ovate leaves, yellow gland dots present abaxial leaf surface, reflexed bracts, corolla covering with glandular hairs as well as corolla gradually expanded from the base but differs several features such as having shrubs less branched, rounded stem, elongated internodes, purplish-brown petioles, above leaf surface less pubescent and granulate, leaf apex acuminate, purplish-brown elongated spikes which are longer than 3.5 cm with long peduncles and purplish-brown outer surfaces of inner bracts, lanceolate outermost bracts which are longer than 2 cm with long purplish-brown stalk , long acuminate, androecium with glabrous filaments, anther thecae dark brown, glabrous style and ovary (Table 1, Figure 2). The collection localities for the new taxon are restricted to an undisturbed evergreen rain forest at a high altitude below 1500 m in a wet zone (Figure 1).

TABLE 1. Diagnostic morphological characters of *Strobilanthes medahinnensis* and *Strobilanthes anceps.*

Character	***S. medahinnensis***	***S. anceps*†**
Habit	shrubs, 1–1.5 m high, less branched	shrubs, 0.6–1.0 m high, much branched
Stem	rounded, villous, brownish green below, purplish-brown above	weakly quadrangular, glabrescent below, pubescent above, stem green
Leaves	lamina ovate, 3.0–12.0 × 1.5–2.0 cm, base rounded, apex acuminate, margin entire, above and beneath same green colour, few yellow gland dots present abaxially, midvein densely villous.	lamina ovate or elliptic, 2–13.5 × 1.5–4.5 cm, base cuneate or rounded, apex long-acuminate or caudate, margin subentire or crenulate, above dark green, beneath paler, yellow gland dots present abaxially, veins pubescent.
Petiole	purplish-brown outer surface, 4.0–6.0 cm long	green outer surface, 0.2–3.5 cm long
Inflorescence	pedunculate spikes born on bifurcate or trifurcate axillary and terminal branchlets, 3.5–6.5 cm long, 1 cm wide, peduncle 2.5–6.5 cm long purplish-brown colour	pedunculate heads borne on simple or 3-forked axillary branchlets. heads 1–3.5 cm long, 1 cm wide, peduncle 0.5–3 cm long green colour
Outermost bracts	lanceolate, midrib prominent, 2.0–3.0 cm long, long acuminate, long purplish-brown stalk	ovate, midrib not prominent, 1.5–2.0 cm long, acute, short green stalk
Inner bracts	1.2–1.5 × 0.4–0.6 cm long, purplish-brown outer surface	1.5–2 × 0.9–1.5 cm long, outer surface of upper part green, lower part pale
Calyx	4.0–6.5 mm long, purplish-brown outer surface	6.0–10.0 mm long, green outer surface
Corolla	corolla pale pinkish white, tube 2.1–2.4 cm long. corolla lobes 2.5 mm long & 4mm wide	corolla white, tube 2.1–2.5 cm long. corolla lobes3 mm long & 4mm wide
Androecium	stamens included, filaments glabrous, anther thecae ca 1.5 mm, dark brown	stamens included, filaments sparsely pubescent, anther thecae ca 1 mm, white
Gynoecium	style c. 2.2 cm long, glabrous, ovary glabrous.	style c.2.2 cm long, sparsely hairy, ovary sparsely hairy
Capsule	not seen	oblong

†Wood, 1998

Molecular evidence

DNA libraries for *S. anceps* and *S. medahinnensis* contained approximately 100,000,000 and 123,000,000 raw reads of the full plant genome respectively. A complete cp genome could be obtained for *S. medahinnensis* with the single contig assembled from the raw reads of *S. medahinnensis* using GetOrganelle. However, the same procedure resulted in five non-overlapping contigs from *S. anceps* and four gaps were identified by aligning the five contigs against the complete chloroplast assembly of *S. medahinnensis.* The lengths of the gaps were 878 bp (within rpoB gene), 291 bp (within atpB gene), 291 bp (within atpB gene), and 1456bp (between rpl16 and rps3 genes). In the raw reads remapping step, these gaps were filled by exclusively mapping raw reads to the respective regions. After the complete assembly, the length of the two cp genomes differed by 63 nucleotides (Table 2). Both cp genomes had the typical quadripartite structure consisting of two inverted repeat regions (IRA and IRB), short single-copy (SSC), and large sequence copy (LSC) regions. The GC contents, protein-coding genes, tRNA, and rRNA genes between the two species were very similar (Figure 4). The pairwise alignment had a total of 373 base pair differences caused by 101 polymorphic sites (substitutions) and 14 indels; minimum and maximum indel lengths were 1 bp and 27 bp, respectively.

TABLE 2. The characterization of two chloroplast genomes of *Strobilanthes medahinnensis* and *Strobilanthes anceps.*

Species	*S. medahinnensis*	*S. anceps*
Genome size (bp)	144893	144951
LSC length (bp)	94424	94482
SSC length (bp)	17817	17817
IR length (bp)	16326	16326
GC content (%)	38.2	38.2
GC content in LSC (%)	36.6	36.5
GC content in SSC (%)	32.5	32.5
GC content in IR (%)	45.1	46.1
Protein-coding genes	89	89
tRNA genes	62	62
rRNA genes	12	10

TABLE 3. DNA polymorphism of three common DNA barcodes and two variable regions between *Strobilanthes medahinnensis* and *Strobilanthes anceps.*

Region (size - bp)	trnFGAA-ndhJ (200)	atpB-rbcL (600)	matK (1550)	psbA-trnH (452)	rbcL (1434)
Base pair difference	13	78	1	0	10

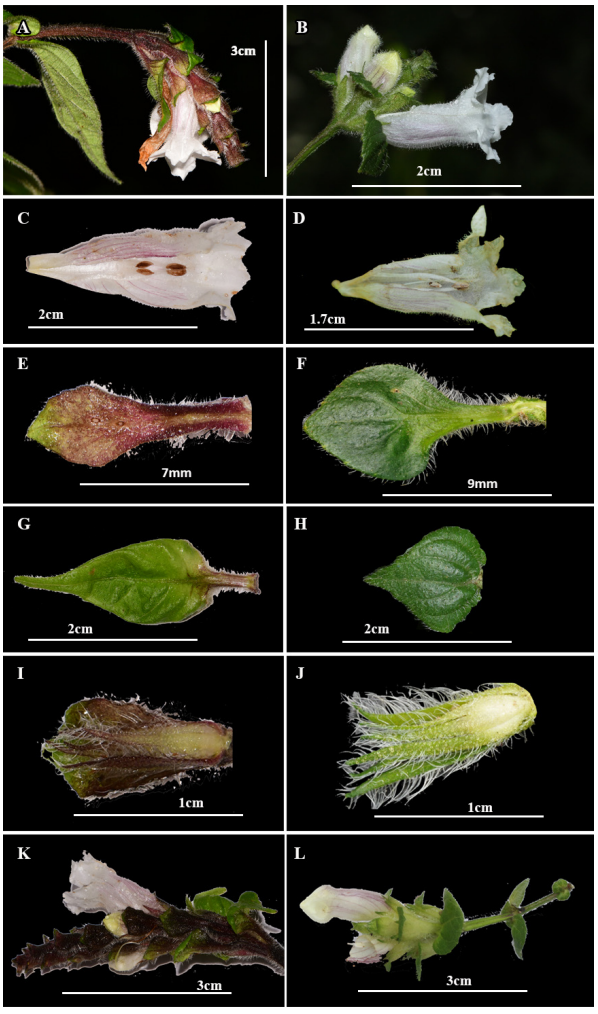

FIGURE 2. Diagnostic morphological characters of *Strobilanthes medahinnensis* and *Strobilanthes anceps.*
S. medahinnensis—**A.** side of the corolla, **C**. half flower, **E.** inner bract, **G.** outermost bract **I.** calyx, **K.** inflorescence. *S. anceps*—***B.*** side of the corolla, **D.** half flower, **F.** inner bract, **H.** outermost bract. **J.** calyx, **L.** inflorescence

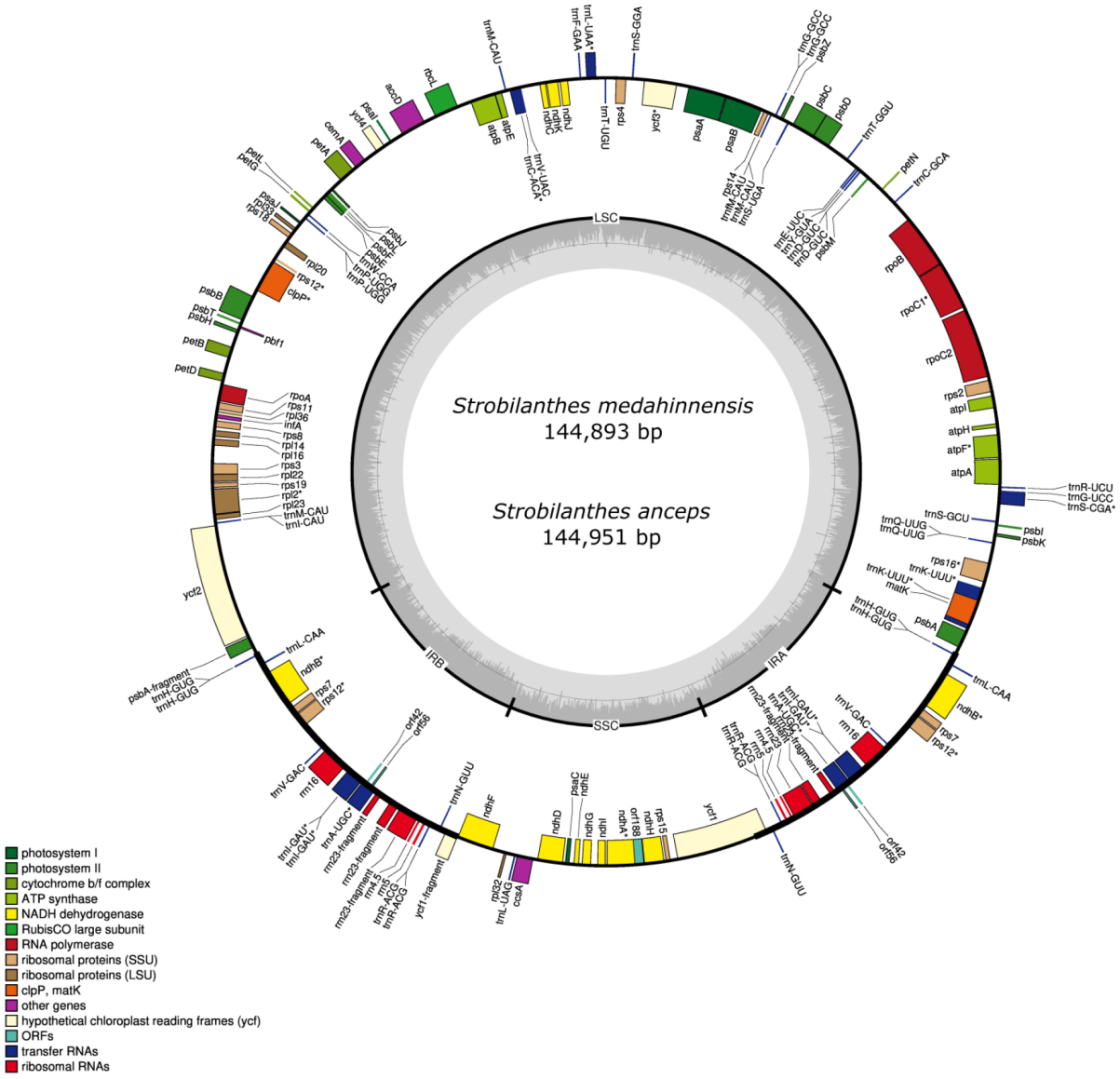

FIGURE 3. Chloroplast genome maps of *Strobilanthes medahinnensis* and *Strobilanthes anceps*. Genes inside the outer ring are transcribed clockwise, while genes outside the outer ring are transcribed counterclockwise. Genes of different functional groups are shown in colored bars. The inner-circle (dashed gray area) indicates the proportional GC content of the corresponding genes. Regions of the large single-copy (LSC), small single-copy (SSC), and inverted repeats (IRA and IRB) are indicated.

Among common cp barcodes, the rbcL gene had the highest amount of variability with 10 sites, while matK had only a single base pair difference (Table 3). Further, psbA-trnH regions were identical between the two species. The universal barcodes such as matK, rbcL, and psbA-trnH have failed in the identification of species due to their low variability and other highly variable regions are proposed in such circumstances (Cheng *et al.* 2020). It is recommended to use one of the highly variable regions to distinguish the two species. DNA polymorphism analysis revealed two highly variable regions (trnF-GAA-ndhJ and atpB-rbcL) between the two species with a significant nucleotide variability, Pi > 0.02. Interestingly, the both regions were intergenic spacers (trnF-GAA-ndhJ and atpB-rbcL), of them, the region between atpB and rbcL genes reported the highest nucleotide variability with 78 polymorphic sites. We propose atpB-rbcL region as a suitable DNA barcode for the identification of these two species in particular and possibly for distinguishing other *Strobilanthes* species after a comprehensive analysis. The atpB-rbcL region is about 600pb in length which makes it ideal for PCR amplification and Sanger sequencing. The SSR distribution between the two cp

genomes is shown in Table 4. The most common SSR type was A/T mononucleotide microsatellites. This observation matched with previous studies that have reported a higher AT content and a lower GC content in cp genomes (Eguiluz *et al*. 2017). Trinucleotide microsatellites were also observed in the genomes more frequently than di/tetra/penta SSR types. The distribution of SSRs based on their location was similar between the two plastid genomes. Many of the SSRs were found in coding regions. These SSRs are also beneficial in developing molecular markers to discriminate the two species in the future.

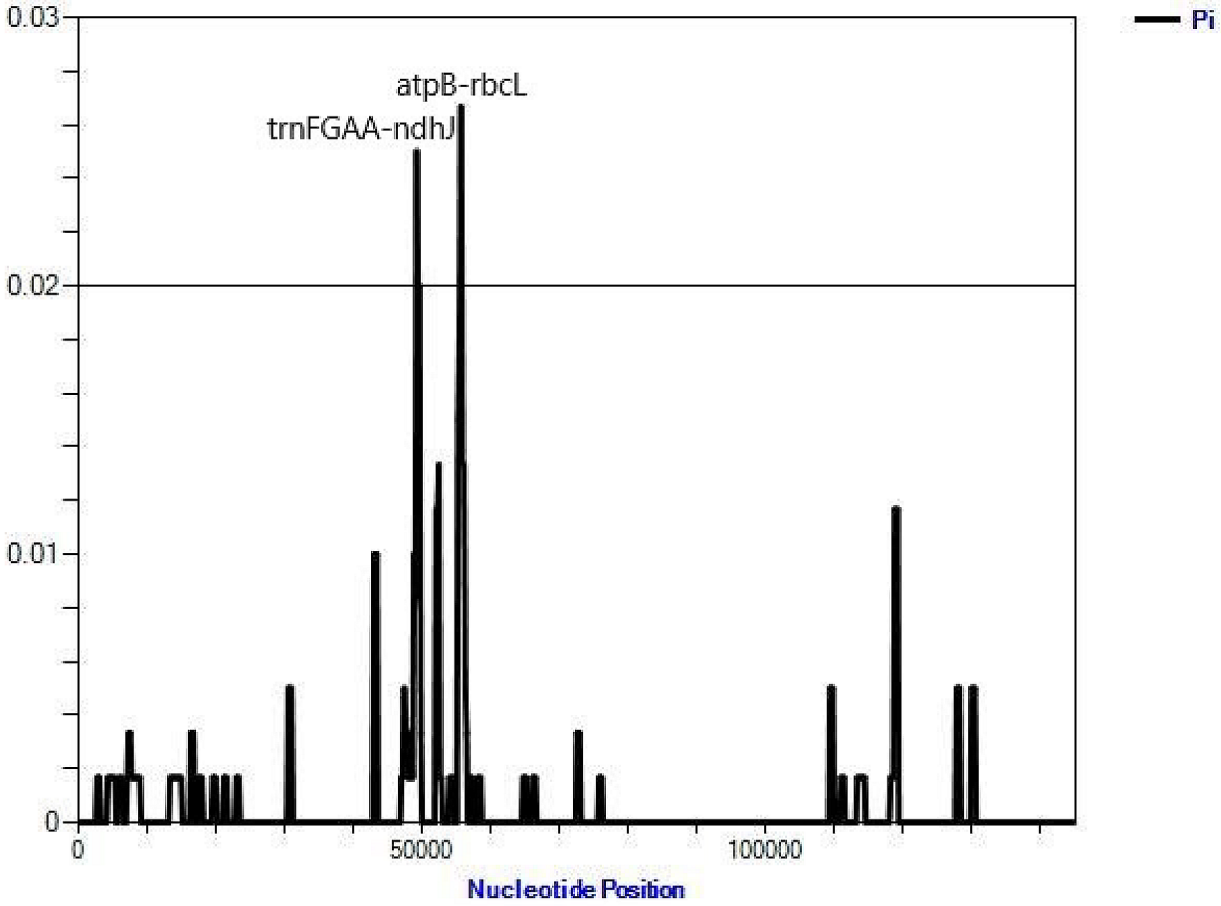

FIGURE 4. Nucleotide variability (Pi) comparison between *Strobilanthes medahinnensis* and *Strobilanthes anceps* using the window sliding analysis (window length: 600 bp and step size: 200 bp). The X-axis indicates the position of the midpoint of the window, while Y-axis indicates the nucleotide diversity (Pi) of each window.

TABLE 4. The SSR type, location, and count in the two chloroplast genomes.

Species	SSR type					Location			
	Mono	Di	Tri	Tetra	Penta	Intergenic	Genes	Introns	Total
S. medahinnensis	88	48	67	8	2	82	89	42	213
S. anceps	90	49	67	8	2	85	89	42	216

The contraction and the expansion of the two chloroplast IR regions have been considered as strong evidence for evolutionary studies (Shahzadi *et al.* 2019). The IR regions of two species coincide with each other except a 3bp long substitution found in an intergenic spacer (IGS) region. The divergence time of *S. medahninnensis* and *S. anceps* was determined by using a fossil evidence based speciation event between *Arabidopsis thaliana* and *A. lyrata.* Based on the divergence time (Figure 5) the speciation between *Strobilanthes medahinnensis* and *S. anceps* happened about 0.33 million years ago (Mya). This suggests recent divergence of two species, possibly due to habitat isolation in restricted environment. Nevertheless, it is more than the time taken to diverge cultivated rice, *Oryza sativa* Indika from one of its wild relative *O. nivara* (Figure 5).

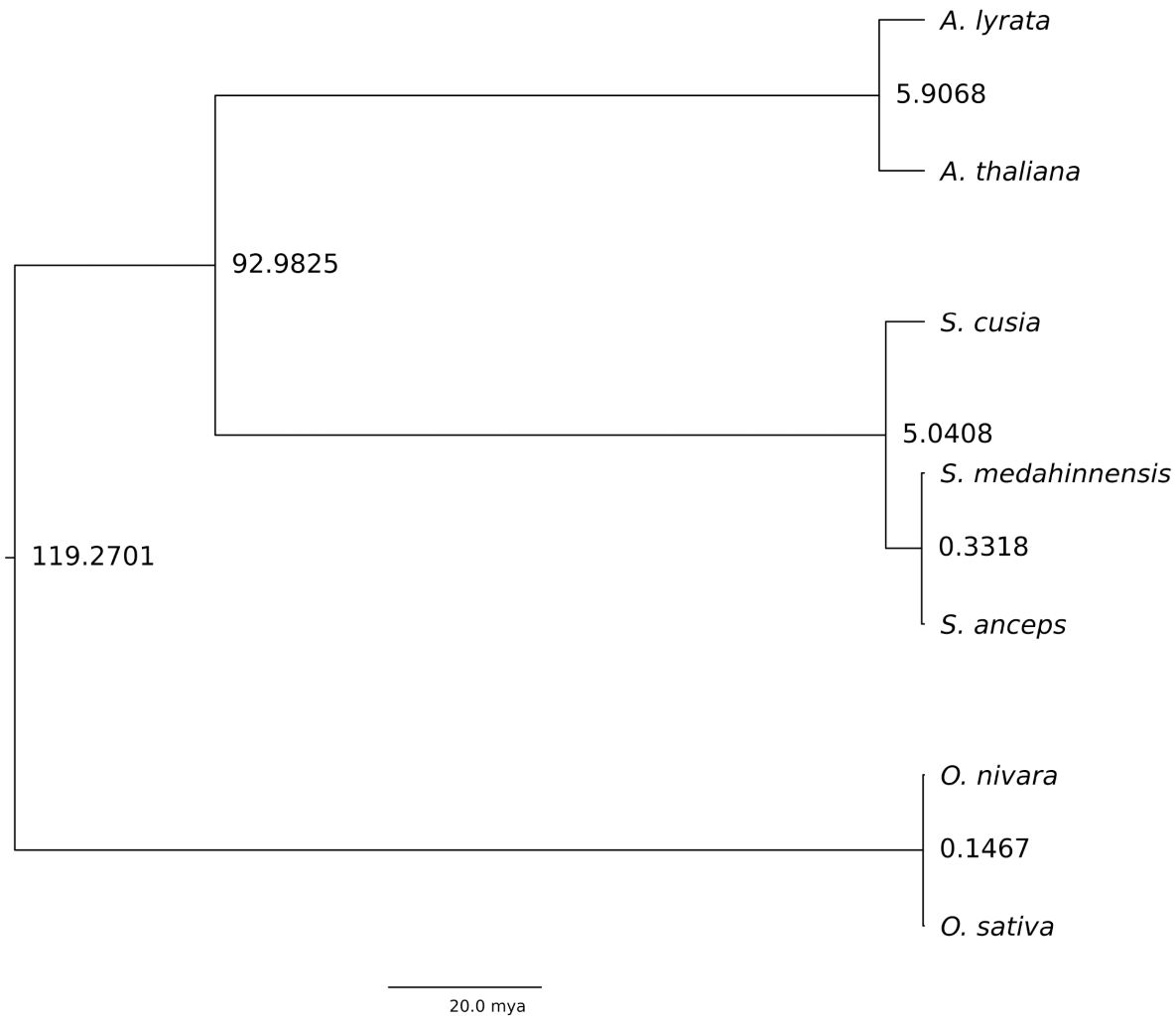

FIGURE 5. The divergence times of 7 species based on complete chloroplast sequences. Values at nodes indicate divergence dates in millions of years. Divergence time of *A. thaliana* and *A. lyrata* was modeled by a normal distribution with a mean of 5.97 Mya.

Taxonomic treatment

Strobilanthes medahinnensis Nilanthi, *sp. nov.* (Figure 6 & 7)

Type:—SRI LANKA, Sabaragamuwa Province, Ratnapura District, Peak Wilderness Nature Reserve, Medahinna, E 166892, N 179540, 1430 m, 29 June 2020, *Nilanthi 149* (holotype PDA!).

Diagnosis:—The new species is similar to *Strobilanthes anceps*, but differs by shrubs with less branched (not much branched), rounded stem (not quandrangular), leaves with acuminate apex (not long acuminate or caudate), elongated spikes (not short spikes), lanceolate outermost bracts (not ovate), outermost bract with long acuminate apex (not acute), outermost bract with long purplish-brown stalk (not short green stalk), inner bracr with purplish-brown outer surface (not green outer surface), androecium with glabrous filaments (not pubescent filaments), dark brown anther thecae (not white anther thecae), glabrous style (not hairy style), glabrous ovary (not hairy ovary).

Description:—Shrub, 1.0–1.5 m high. Stem erect, rounded, swollen just above the nodes, brownish green below, purplish-brown above, villous, internodes 2.5–5.0 cm long. Leaves opposite, slightly unequal; petiole 4.0–6.0 cm long, villous, blades ovate, 3.0–12.0 × 1.5–2.0 cm, base rounded, margin ciliate, apex acuminate, indumentum granular adaxially, abaxial less pubescent, midvein densely villous, few yellow gland dots present abaxially, lateral nerves 5-8 pairs, prominent on both surfaces, raised beneath. Inflorescence bifurcate or trifurcate in axillary and terminal

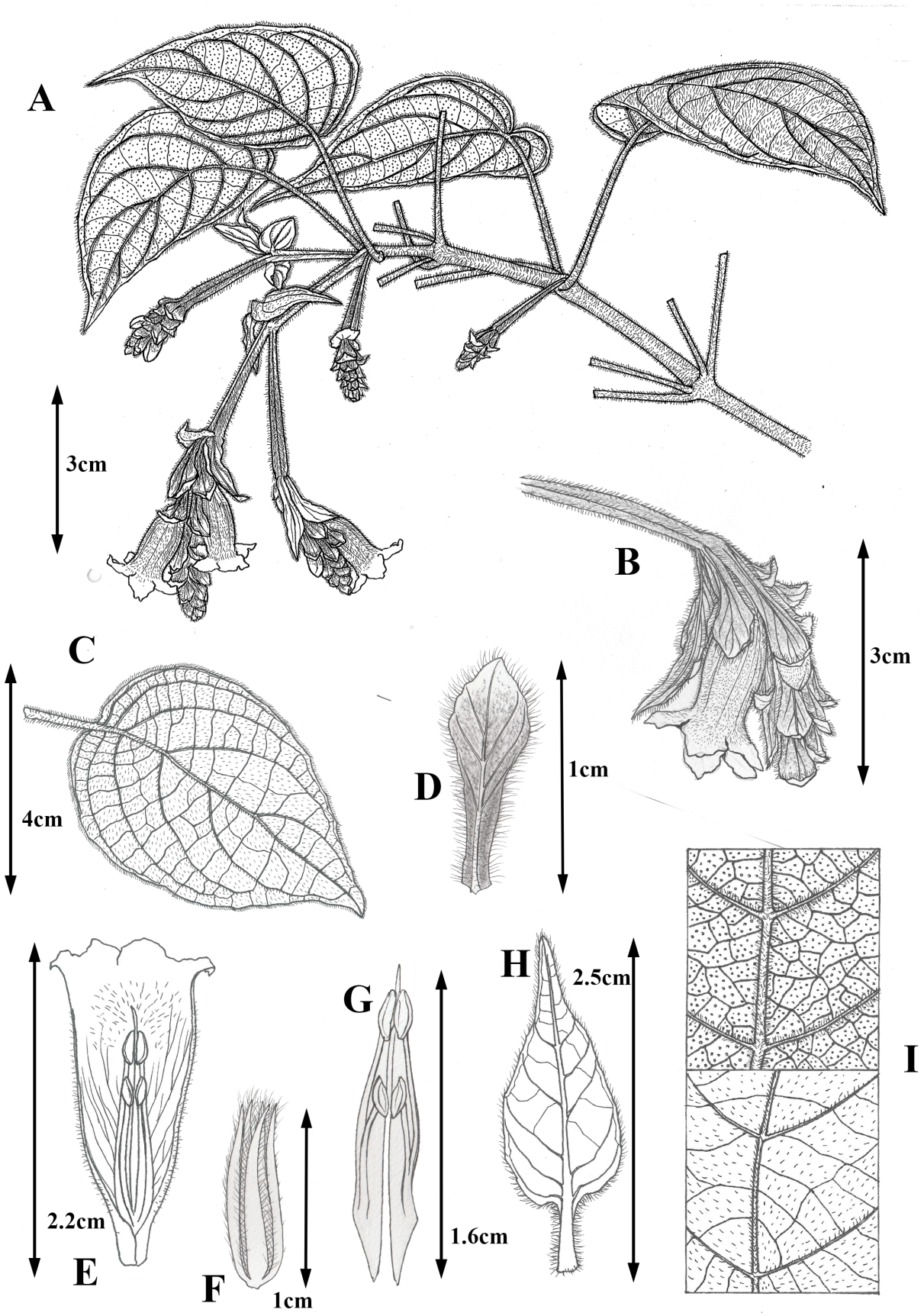

FIGURE 6. *Strobilanthes medahinnensis.* A. Flowering branch; B. Inflorescence; C. Leaf D. Inner bract; E: Half flower; F. Calyx; G. Stamens; H. Outermost bract; I. Close up of the leaf surfaces adaxial and abaxial. (Drawn by Rukmal Ratnayake on the holotype).

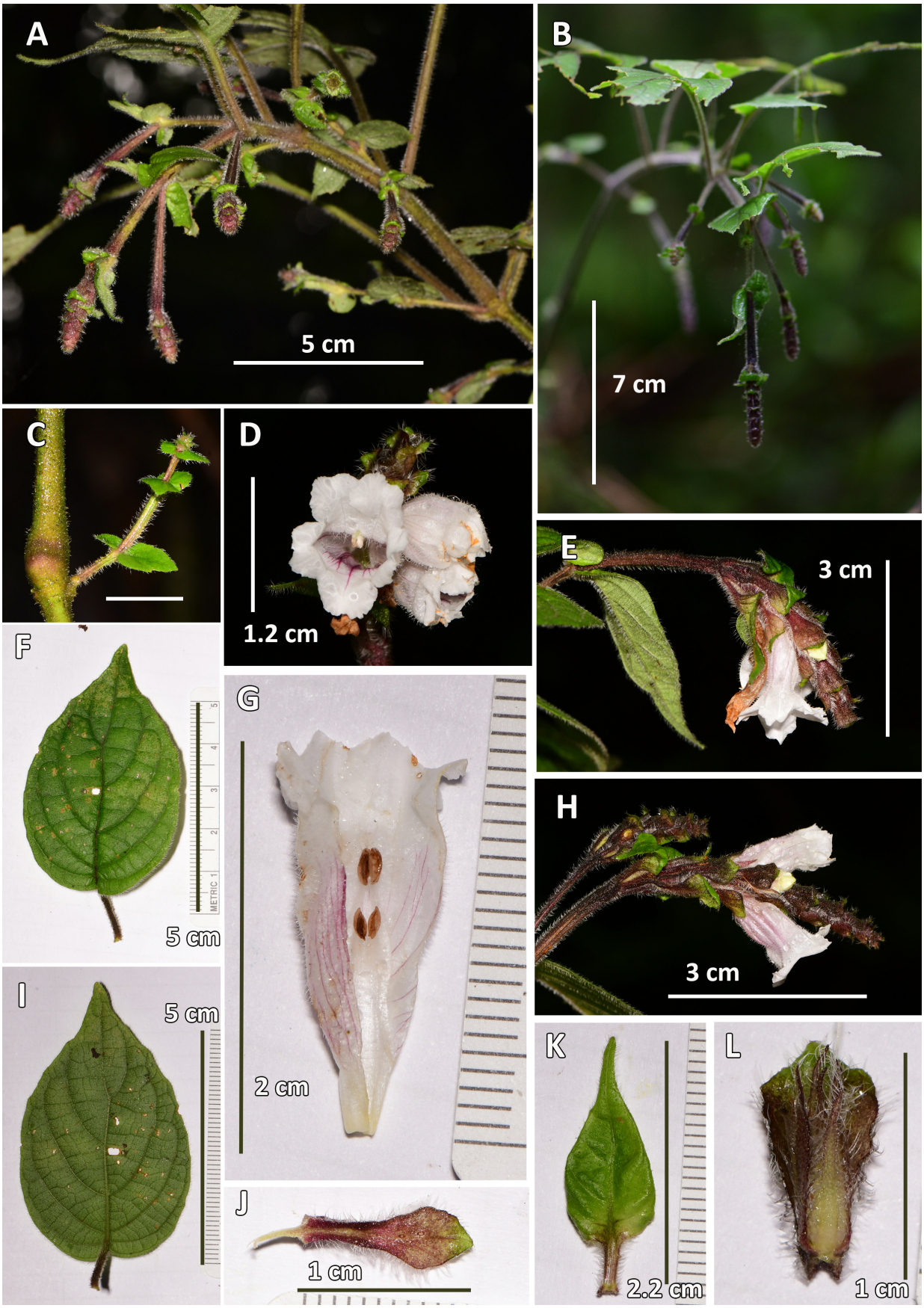

FIGURE 7. *Strobilanthes medahinnensis*. A-B. Habit; C. Stem; D. Front view of the corolla; E–H. Inflorescence; F. Leaf (adaxial); G. Half flower; I. Leaf (abaxial); J. Inner bract; K. Outermost bract; L. Calyx with Bractiole.

pedunculate spikes, 3.5–6.5 cm long, 1 cm wide, peduncle purplish-brown. Outermost bracts lanceolate, 2.0–3.0 × 0.6–0.8 cm, base cuneate, margin ciliate, apex acuminate, 4.5–5.8 mm long stalked, midrib prominent; Inner bracts obovate, 1.2–1.5 × 0.4–0.6 cm, outer surface purplish-brown, recurved, gland-dotted dorsally, ciliate on the margins; bractioles linear-oblong, narrowly acute 6.0–0.9 mm long, ciliate, narrowly acute at apex, slightly shorter than the calyx, outer surface purplish-brown. Calyx 6-9 mm long, 5-lobed to the base, the lobes equal, linear-lanceolate, ciliate on margins, acute at apex, outer surface purplish-brown. Corolla 2.1–2.4 cm long, pale pinkish white, dark purple lines on inner surface of corollainner side pubescent, corolla gradually expanded from the base, lobes ovate, ca. 2.5 mm long, ca. 4 mm wide, apex obtuse. Stamens 4, included, didynamous, basally attached to corolla tube, filaments of shorter pair 8.0–8.5 mm long, longer pair 12.0–12.5 mm long, anthers dark brown. Ovary ovate-oblong, 2 mm long, glabrous; style slender; stigma simple. Capsule unseen.

Etymology:—The specific epithet "*medahinnensis*" refers to the type locality.

Phenology:—It was in flowering from June to August.

Habitat and Distribution:—This species inhabits at Medahinna growing in the shade and along the nature trail of Erathna in an evergreen rain forest at an elevation of 1400–1500 m in a wet zone. This area belongs to the Peak Wilderness Nature Reserve where many endemic species of flowering plants were recorded, and it is legally administered by the Department of Wildlife Conservation. The allied species *S. anceps*, which has a wide distribution in Sri Lanka, is also mostly found in montane forests at around 1450–2300 m altitudes.

Conservation status:—The new species was only discovered at Medahinna from the Peak Wilderness Nature Reserve in Sri Lanka. About 50 individuals were observed and the extent of occurrence was ca. 1 km^2. Further explorations to obtain the precise population status are needed to assess its conservation status. Based on available data, the new species is assigned to the category 'Data Deficient' (DD) of the International Union for Conservation of Nature (IUCN, 2019).

Specimens of *S. anceps* examined:—SRI LANKA.**Matale District**: Sudagalle Kande, Sep 1893, *Trimens, 00011051* (PDA). **Kandy District**: Rangala, Apr 1886, *Trimens, 00011051* (PDA); **Ratnapura District**: Adam's Peak, 17 Oct 1927, *Alston 940, 00011054* (PDA);1700m, 21 Sep 1969, *Van Beusekom 1554* (PDA); 2 Oct 1969, *Reitz 30012* (PDA); Eknaligoda, Sep 1855, *Thwaites C.P. 2000*, 00011050 (PDA); Sinharaja forest, Sep 1891, *White*, 00011049 (PDA); Handepan Ella, 1200 feet, 5 Jul 1975, *Waas 1402* (PDA); Medahinna, E 166892, N 179540, 1430 m, 29 June 2020, *Nilanthi 149* (PDA). Nuwara Eliyadistrict: Horton Plains, Nov 1893, *Nock*, 00011053 (PDA); 2300 m, 3 Dec 1970, *Theobald & Krahulik 2741* (PDA); 2400m, 20 Oct 1993, *Cramer 6864* (PDA); Jungle above Hakgala, 4 Apr 1906, *A.M.S.,* 00011047 (PDA); 5 Oct 1906, *J.C. Willis* 00011052 (PDA); 1700m, 21 Sep 1969, *Coll.CoF & R J Van Beusekon 1554*, (PDA); Pundaluoya to NuwaraEliya,1600m, 26 Dec 1970, *Theobald & Krahulik 2819* (PDA); Godamaditta, Kotmale, 1300 m, 27 Oct 1972, *Jayasuriya 968* (PDA); Ramboda, 2000 m, Nov.1978, Horton Plains, 20 Oct 1993, *L. Cramer 6864* (PDA); *Kostermans 27096* (PDA); Kikiliyamana, 1530 m, 29 Oct 1994, *Jayasuriya 8397* (PDA); Mipilimana, 1845 m, 16 Oct 1994, *A. H. M. Jayasooriya 8435* (PDA); **Hambantota District**: Ussangoda near Ambalantota, 29 Oct 1993, *Clayton & Jayasekara, 6100* (PDA).

Discussion

Strobilanthes is an important floristic element of the Peak Wilderness Nature Reserve in Sri Lanka and our recent field study demonstrated that thirteen species of *Strobilanthes*, i.e., *S. anceps, S. calycina* Nees (1837: 312)*, S. hookeri* Nees (1837: 312)*, S. lupulina, S. pulcherrima* Anderson (1860: 229)*, S. punctata* Nees (1847: 183)*, S. sexennies* Nees (1837: 312)*, S. viscosa* (Arn. ex Nees) Anderson (1860: 226)*, S. laxa* Anderson (1860: 228)*, S. walkeri* Arn. ex Nees (1837: 312)*, S. helicoides* Anderson (1860: 229), *S. habracanthoides* Wood (1995: 22) and *S. medahinnensis* are recorded. The last record of *Strobilanthes laxa* was reported by Robin Foster from Piduruthalagala in 1975. After then, we recorded it again from Peak Wilderness Nature Reserve in 2019. *S. sexennis* var. *cerinthoides* Nees (1837: 312) Clarke (1884: 474) was recorded there for the first time in 2017. The discovery of *S. medahinnensis* from Sri Lanka highlights the importance and need for further fieldwork in Sri Lanka.

The chloroplast genomes are important pieces of information when deciding evolutionary traits and determining the identity of a species. Here we used the complete Chloroplast genome variation and its divergent analysis to further confirm speciation event. The chloroplast region, atpB-rbcL could be a DNA barcode for differentiation of *S. medahinnensis* from its close relative *S. anceps*.

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