



Ceropegia mizoramensis and *C. murlensis* (Asclepiadaceae) - Two new species from Northeast India with Phylogenetic and morphological evidence support

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ABSTRACT: Based on phylogenetic and morphological evidence, two hitherto unknown species under genus *Ceropegia* viz. *Ceropegia mizoramensis* and *C. murlensis* (Asclepiadaceae) are described here for the first time. The species are collected from Mizoram, Northeast India. The present communication deals with the brief description and photo illustrations of both the species along with key to species found in the region for their easier identification.

KEY WORDS: Asclepiadaceae, *Ceropegia*, India, ITS, Mizoram, New taxa.

INTRODUCTION

The genus *Ceropegia* instated by Linnaeus in 1753 includes approximately 260 species, distributed in South-east Asia, India, Madagascar, Tropical Arabia, Canary Islands, Africa except Mediterranean region, New Guinea and Northern Australia (Ansari 1984, Bruyns 1997, 2003, Jagtap and Singh 1999, Mabberly 1987, Maurya *et al.* 2018, Punjani *et al.* 2017, Rahangdale and Rahangdale 2012, Sachin *et al.* 2006, The Plant List 2013, Yadav and Shendage 2010). In India, the genus is represented by 56 species and 10 varieties (Murthy *et al.* 2012, Kullayiswamy *et al.* 2013). Out of 56 species, 7 taxa are synonymous viz., *C. bulbosa* Roxb. var. *lushi* Hook.f. with *C. bulbosa* Roxb.; *C. candelabrum* L. var. *biflora* (L.) Ansari with *C. candelabrum* L.; *C. longifolia* var. *sinensis* Huber with *C. dolichophylla* Schltr.; *C. panchganiensis* Blatter & McCann, *C. rollae* Hemadri and *C. sahyadrica* Ans. & Kulk. with *C. lawii* Hook.f. and *C. intermedia* Wight var. *wightii* with *C. omissa* H. Huber. In North-east India (including Sikkim) 9 species (viz., *C. angustifolia* Vahl ex Decne., *C. arnottiana* Wight, *C. dolichophylla* Schltr., *C. hookeri* C.B. Clarke ex Hook.f., *C. kachinensis* Prain, *C. longifolia* Wall., *C. lucida* Wall., *C. macrantha* Wight and *C. pubescens* Wall.) have been recorded (Jagtap and Singh 1999, Nautiyal *et al.* 2009). Here we describe and illustrate two new species of *Ceropegia* from Northeast India.

MATERIALS AND METHODS

Morphology

During floristic explorations (2012-2014) to Murlen National Park, Champhai, Mizoram, several specimens

of *Ceropegias* were collected. The study of the vegetative as well as flowering stages of the collected plants revealed two unusual specimens. We compared the specimens with morphologically similar *Ceropegia oculata* and *C. dolichophylla* and observed a number of differences (Table 1, 2).

DNA extraction, polymerase chain reaction and sequencing

Genomic DNA was extracted from silica dried leaves using a DNeasy Plant Mini Kit (Qiagen, Amsterdam, Netherlands). DNA amplification and sequencing of the entire ITS region (ITS1, 5.8S and ITS2) were performed using the Primers ITS 1 and ITS 2 (White *et al.* 1990). The polymerase chain reaction (PCR) was performed with standard methods using Red Dye Master mix (25 µl), PCR amplification was performed with 35 cycles (Denaturation for 1 min at 94°C, annealing for 1 min at 51°C, and 1 min of extension at 72°C followed by a last cycle of final extension for 5 min at 72°C). PCR products were checked for the presence of appropriate bands on a 0.8 % agarosegel, purified, and sequenced at SciGenome Labs., Cochin India. Sequences were comprised of ITS1, 5.8S and ITS2 regions. Forward and reverse sequences were edited and assembled using the computer program Geneious v. 6.1.8 (Drummond *et al.* 2010). All sequences have been deposited in GenBank.

Phylogenetic Analysis

A total of 64 nucleotide sequences (including all out groups) were assembled, aligned followed by manual adjustments in Geneious 6.1.8 (Drummond *et al.* 2010). Phylogenetic analyses were done using Maximum Likelihood approach, analyses were performed using



RAxML v. 8.1.18 (Stamatakis, 2014) on CIPRESS Science Gateway v.3.3. While analyzing dataset GTRGAMMA is checked which uses 25 rate categories instead of 4 as used in most other implementations of the gamma shape parameter for capturing rate heterogeneity (Stamatakis 2008, 2014) parameter in lower versions (RAxML 7.0.4). Parameters for the evolutionary model were set to default and the state frequency parameter for stationary nucleotide frequency of the rate matrix was fixed. Relative support for the clades recovered was assessed via bootstrap analysis using 1,000 replicates in ML analyses. The following criteria were used to assess bootstrap support percentages (BP): 50–70 %, low; 71–84 %, moderate; 85–100 %, strong. The final tree was drawn using Fig Tree 1.4.0 (Rambaut 2006-12).

RESULTS

ITS characteristic and out-group information

We obtained ITS sequences of *Ceropegia mizoramensis*, *C. murlensis* and *C. lushiansis* and added to a dataset comprising of 66 species of *Ceropegia* retrieved from GenBank. *Heterostemma tanjorensis* Wight & Arn., *Anisostoma cordifolia* Fenzi and *Sisyranthus compactus* N.E. Br. were included as outgroups. The sequence alignment is available from the communicating author on request. Multiple sequence alignment: ITS sequences retrieved (GenBank + Sequenced) are 724 base pair long and consists of 322 identical sites and rest were informative (see Appendix).

Molecular Diagnosis

The analyses of ITS sequences of *Ceropegia* species revealed that the two new species described (below) from North Eastern part of India (Mizoram) are grouped together along with other North East Himalayan species viz. *Ceropegia monticola* and *C. longifolia* and form a clade (Fig 3). *C. mizoramensis* is sister to *C. murlensis* and differs at nine positions: 16 (A/C), 19 (A/T), 29 (A/C), 41 (C/T), 56 (A/T), 59 (G/C), 93 (A/T), 261 (A/T), 650 (A/C). *C. monticola* is sister to newly described *C. mizoramensis* and *C. murlensis* and differs at 5 positions: 16 (A/C), 97 (T/C), 261 (A/T), 267 (A/G), 543 (G/T) in ITS alignment. *C. oculata* (GenBank ID KP244970 and EU106679) is grouped together along with species from peninsular India.

Taxonomic Treatment

Ceropegia mizoramensis Ram. Kumar & S. Sharma, *sp. nov.* **Fig. 1**

Diagnosis: *Ceropegia mizoramensis* is morphologically close to *Ceropegia oculata* Hook. but is dissimilar in roots being fascicled, stem hairy in 2 rows, leaves elliptic-lanceolate, pedicel sparsely hairy, corolla tube and outer corona hairy inside while *C. oculata* bears

tuberous roots, stem glabrous, leaves ovate to ovate-oblong, pedicel glabrous, corolla tube and outer corona glabrous inside (Table 1).

Type: INDIA, Mizoram, Champhai, Murlen National Park, ca. 1,100 m, 18 Sep. 2014, R. Kumar & S. Sharma 131485 (holotype: ASSAM; isotype: CAL).

Twining herb, up to 1 m tall; rootstock a cluster of fusiform roots; stem wiry, terete, hairy (prominent at nodes). Petiole 4–6 mm long, narrowly winged, puberulent. Leaves elliptic, elliptic-lanceolate, 60–75 × 5–7 mm, glaucous and glabrous above except for mid-vein, appressed pubescent beneath, base cuneate, apex acuminate, margins minutely ciliate; lateral veins 4–6 pairs, obscure. Inflorescence umbel-like, 3-flowered, 10–15 mm long peduncle, slightly pubescent. Pedicel 5–15 mm long, sparsely puberulent. Calyx 5-lobed, linear-lanceolate, 4–7 mm long, 5-veined, glabrous, apex acuminate. Corolla purplish white with dark purple markings inside, 25–32 mm long; basal inflation 7–10 mm wide; tube 18–22 mm long, 3–5 mm wide at the base of lobes with dark purple markings inside; lobes oblong, 10–12 × 2–3 mm wide, increasing gradually to 4–6 mm wide at throat with dark purple markings inside up to 1/2 of the length rest upper portion green, glabrous outside; apical part of incurved tip 5–6 mm, densely pubescent inside. Outer corona divided into pairs of triangular lobes; lobes outside glabrous, hairy inside; inner corona lobes erect, linear, obtuse, hairy at base, larger than outer lobes. Pollinaria 5, reddish brown, waxy with pellucid layer; pollinium 0.5 × 0.37 mm, attached with light brown, 0.15 mm long caudicels; corpuscle 0.3 × 0.2 mm, dark brown. Follicles not seen.

Phenology: flowers from August to October.

Distribution and habitat: currently known only from the type locality and found growing in limestone quarrying area along the margins of open forests at ca. 1, 100 m elevation.

Etymology: this new species is named after the Indian state Mizoram from where the specimens of this species were collected.

Table 1. Comparison of diagnostic morphological characters of *Ceropegia mizoramensis* with its allied species *C. oculata*.

	<i>C. mizoramensis</i>	<i>C. oculata</i>
Root	Fascicled	Tuberous
Stem	Hairy in two rows	Glabrous
Leaves	Elliptic-lanceolate	Ovate or ovate-oblong
Pedicel	Sparsely hairy	Glabrous
Corolla tube	Hairy	Glabrous
(inside)		
Outer corona	Hairy	Glabrous

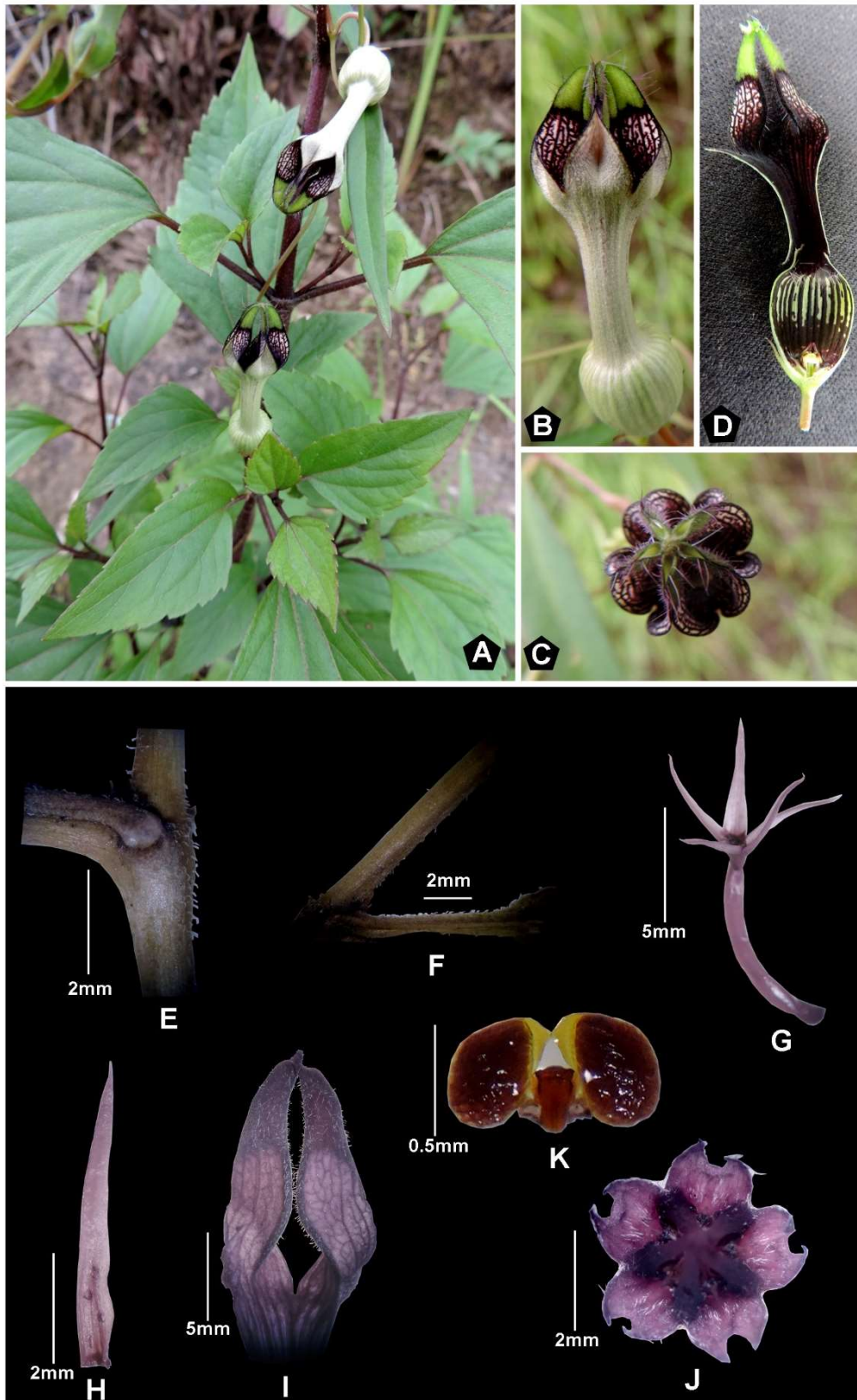


Fig 1: *Ceropegia mizoramensis* Ram. Kumar & S. Sharma, sp. nov. **A.** Habit; **B.** Single flower; **C.** Apex of terminal; **D.** L. S. of flower; **E.** Stem node; **F.** Petiole; **G.** Calyx with pedicel; **H.** Single sepal; **I.** terminal; **J.** Corona; **K.** Pollinaria. (Voucher: Ramesh Kumar & S. Sharma 131485; Photo: Ramesh Kumar).



Ceropegia murlensis Ram. Kumar & S. Sharma, *sp. nov.*

Fig. 2

Diagnosis: *Ceropegia murlensis* is morphologically is close to *C. dolichophylla* Schlechter, but differs in being sparsely pilose, stem purplish, inflorescence 6 – 12-flowered, corolla tube with a ring of hairs at neck of basal inflation, outer corona lobes glabrous outside and hairy on purplish tip whereas *C. dolichophylla* is glabrous, stem pale, inflorescence 2 – 7-flowered, corolla tube lack of hairs at neck of basal inflation, outer coronalobes glabrous outside or some time ciliate (Table 2).

Type: INDIA, Mizoram, Champhai, Murlen National Park, ca. 1,100 m, 18 September, 2014, R. Kumar & S. Sharma 131486 (holotype: ASSAM; isotype: CAL).

Extensive, twining herb, 2–3.5 m tall; rootstock a cluster of fusiform roots; stem wiry, terete, 2 mm in diam., purplish, sparsely pilose along 1–2 sides, hairs prominent at nodes. Petiole 10–12 mm long, narrowly winged, puberulent. Leaves linear, linear-lanceolate, 70–140 × 6–9 mm, glaucous and glabrous above except mid-vein, appressed pubescent beneath, base cuneate, apex acuminate, margins minutely ciliate; lateral veins 4–7 pairs, obscure. Inflorescence umbel-like, 6–12-flowered, peduncle 15–22 mm long, slightly pubescent. Pedicel 5–18 mm long, glabrous. Calyx 5, linear, 4–7 mm long, glabrous. Corolla greenish with dark maroon tip and purple markings inside, 35–45 mm long; corolla tube 18–22 mm long, 4–5 mm wide at the base of lobes, greenish outside and dark marron inside, corolla lobes oblong, 20–22 × 3–4 mm, increasing gradually 5–6mm wide at throat, greenish with purple markings half way and dark maroon tip, apical part of incurved tip 3–5 mm long, hairy outside and along margins; purplish veined inside and a ring of hairs at neck of basal inflation; basal inflation 4–6 mm wide; Outer corona divided into pairs of triangularly lanceolate lobes; lobes outside glabrous, hairy and purplish on tip; inner corona lobes erect, purplish, linear-lanceolate, obtuse, glabrous at base, larger than outer lobes. Pollinaria 5, creamy white, waxy with pellucid layer; pollinium 0.5 × 0.25 mm, attached with dark yellow, 0.15 mm long caudicle to dark brown, 0.25 × 0.1 mm corpuscle. Follicles not seen.

Table 2. Comparison of diagnostic morphological characters of *Ceropegia murlensis* with its allied species *C. dolichophylla*.

	<i>C. murlensis</i>	<i>C. dolichophylla</i>
Habit	2–3.5 m tall	1–1.5 m tall
Stem	Purplish	Pale grey
Inflorescence	6–12 flowered	2–7 flowered
Corolla tube	18–22 mm long, a ring of hairs at neck of basal inflation	22 – 45 mm long, hairs absent at neck of basal inflation (Jagtap & Singh 1999)
Outer corona lobes	Glabrous outside, hairy on purplish apex	Glabrous or some time ciliate throughout

Phenology - flowers from August to October.

Distribution and habitat - currently known only from the type locality and found growing in a limestone quarrying area along the margins of open forests at ca. 1, 100 m elevation.

Etymology - this new species is named after the protected area (Murlen National Park, Mizoram) within buffer region of which the species was found growing.

Keys to the species of *Ceropegia* from North-East India

- 1a. Corolla lobes shorter than tube 2
- 1b. Corolla lobes almost equal or longer than tube 5
- 2a. Corolla lobes broadly ovate or ovate-oblong or as long as broad *C. longifolia*
- 2b. Corolla lobes oblong, longer than broad 3
- 3a. Peduncle pubescent; corolla tube much inflated at base 4
- 3b. Peduncle almost glabrous; corolla tube slightly inflated at base *C. kachinensis*
- 4a. Roots tuberous; stem glabrous; leaves ovate or ovate-oblong; corolla tube glabrous in side *C. oculata*
- 4b. Roots fascicled; stem hairy bifariously; leaves elliptic-lanceolate, corolla tube hairy inside *C. mizoramensis*
- 5a. Corolla tube apically funnel-shaped 6
- 5b. Corolla tube apically subcylindrical, hardly dilated 9
- 6a. Leaves ovate-cordate, ovate-oblong or ovate-lanceolate 7
- 6b. Leaves linear-lanceolate, elongate or elliptic-oblong 8
- 7a. Corolla lobes oblong, apex acute, tube moderately inflated at base; flowers minutely hairy outside *C. micrantha*
- 7b. Corolla lobes deltoid at base, apex spatulate, tube slightly inflated at base; flowers glabrous outside *C. lucida*
- 8a. Plants 1–1.5 m tall; inflorescence 2–7-flowered; corolla tube without a ring of hairs at neck of basal inflation, corolla lobes linear-lanceolate *C. dolichophylla*
- 8b. Plants 2–3.5 m tall; inflorescence 6–12-flowered; corolla tube with a ring of hairs at neck of basal inflation, corolla lobes oblong *C. murlensis*
- 9a. Corolla tube glabrous inside *C. arnottiana*
- 9b. Corolla tube hairy inside 10
- 10a. Corolla more than 35 mm long, tube hairy within towards apical section, lobes ovate at base, linear, pubescent outside *C. pubescens*
- 10b. Corolla less than 35 mm long, tube with a thin ring of hairs about the middle within, lobes linear at base, sub-spatulate, glabrous outside *C. hookeri*

DISCUSSION

The two new species described herewith are part of the *Ceropegia longifolia* complex, a very variable species complex occurring over a large distribution area in the Indo-Malayan hotspot region. There have been extensive phylogenetic studies of *Ceropegia* based on up to six molecular markers which have revealed numerous insights about the group but the most important thing, from the present study point of view is that the relationship obtained follows the geographic origin than morphological relatedness (Bruyns *et al.* 2014, 2015; Meve and Liede-Schumann 2007, Meve *et al.* 2016). The ITS (ITS1+5.8s+ITS2) phylogeny presented herewith (Fig 3) is the first attempt to characterise the *Ceropegia* from North-east India. The additional data generated for the *C. longifolia* complex is however not

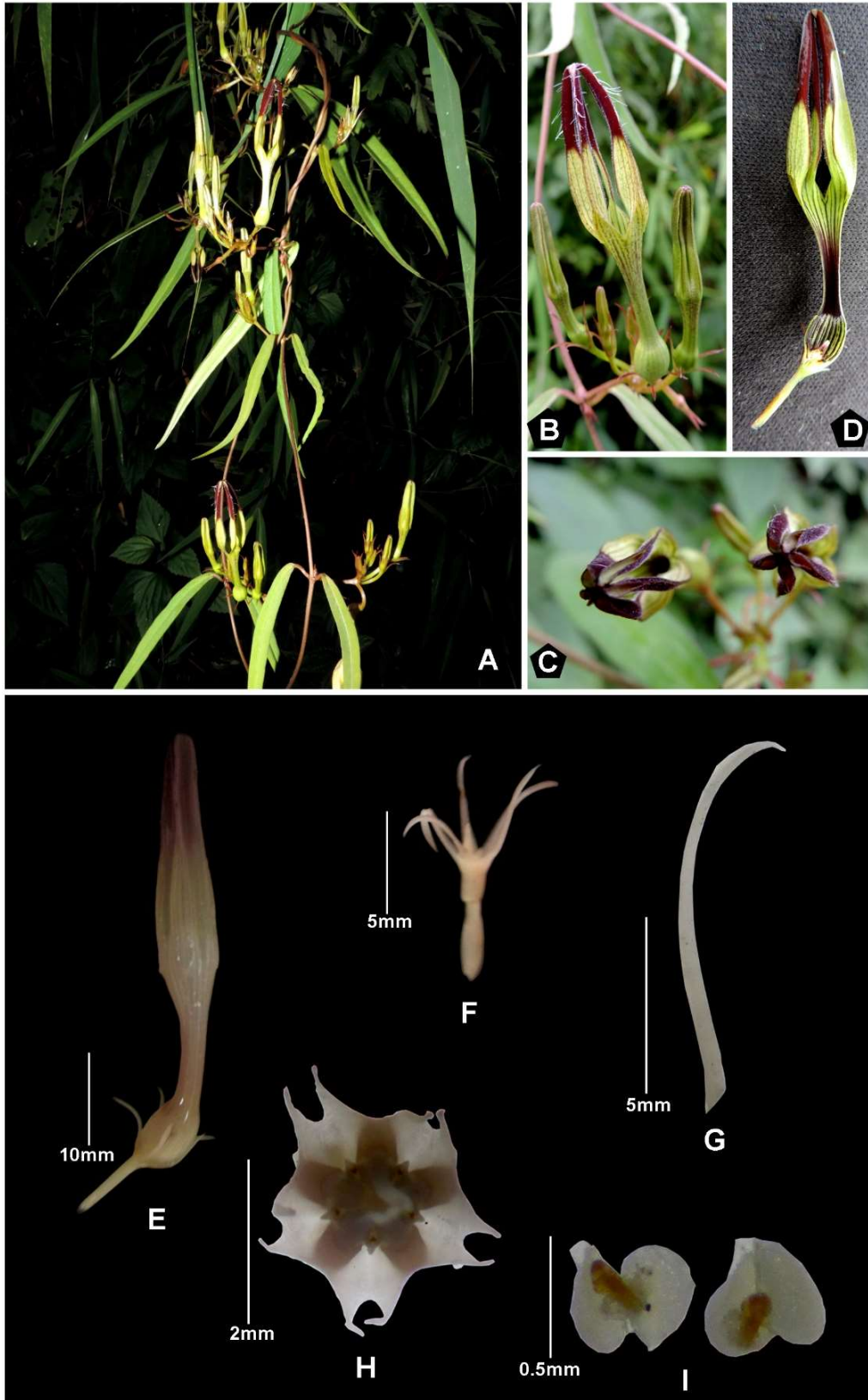


Fig 2: *Ceropegia murlensis* Ram. Kumar & S. Sharma, *sp. nov.* **A.** Habit; **B.** Single flower; **C.** Apex of terminal; **D.** L. S. of flower; **E.** Single flower; **F.** Calyx; **G.** Single sepal; **H.** Corona; **I.** Pollinaria. (Voucher: *Ramesh Kumar & S. Sharma 131486*; Photo: Ramesh Kumar).

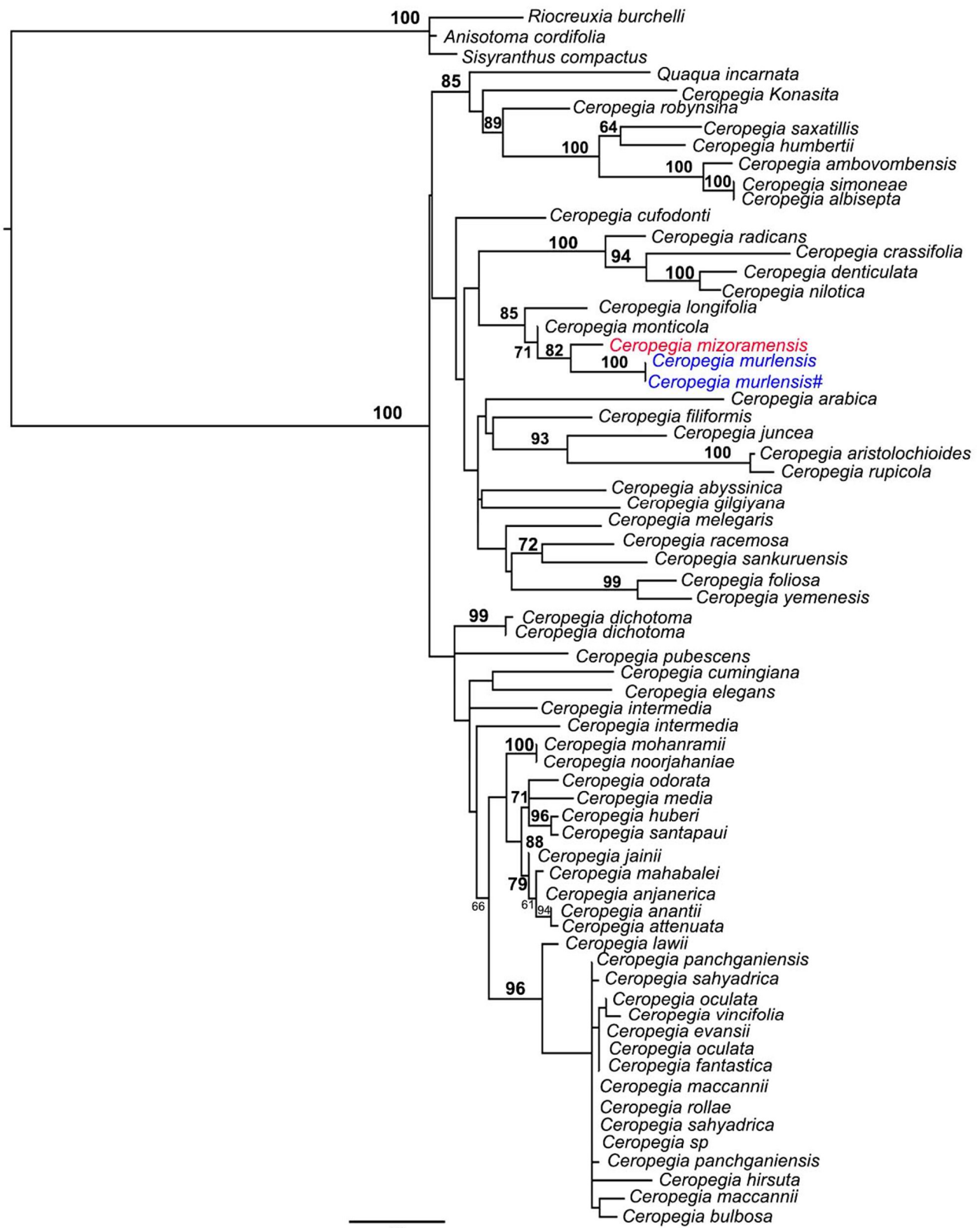


Fig 3: Best ML tree inferred from analyses of nrDNA (ITS). 67 accessions from GenBank, newly described taxa and 3 outgroups analysed under GTRGAMMA model of substitution (RaXML Bootstrap values are indicated above branches).



sufficient to resolve the complex, yet certainly help in adding morphological data in the form of diagnosis, description and key to the species from North-East India and molecular data which would further help in providing better understanding of the complex.

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Appendix: Accession numbers of nrDNA ITS (ITS 1, 5.8s and ITS2) sequences retrieved from GenBank/EMBL database for 61 species including two newly sequenced *Ceropegia* spp. (Rf: Meve & Liede-Schumann 2007).

Taxa	GenBank accessions
<i>Anisotoma cordifolia</i> Fenzl	AJ310780
<i>Ceropegia abyssinica</i> Decne.	AM493298
<i>Ceropegia albisepta</i> Junn. & H. Perrier	AM493299
<i>Ceropegia ambovombensis</i> Rauh & Ge'rold	AM493300
<i>Ceropegia anantii</i> S.R. Yadav, Sardesai & S.P. Gaikwad	EU106699
<i>Ceropegia anjanerica</i> M.Y. Kamble & S.R. Yadav	EU106690
<i>Ceropegia arabica</i> H. Huber	AM493301
<i>Ceropegia aristolochiodies</i> Decne.	AM493302
<i>Ceropegia attenuata</i> Hook.	EU106700
<i>Ceropegia bulbosa</i> Roxb.	EU106687
<i>Ceropegia crassifolia</i> Schltr.	AM493303
<i>Ceropegia cufodontii</i> Chiov.	AM493304
<i>Ceropegia cumingiana</i> Decne.	AM493294
<i>Ceropegia denticulata</i> K. Schum. ex Engl.	AM493291
<i>Ceropegia dichotoma</i> Haw.	AM493290
<i>Ceropegia dichotoma</i> Haw.	EU312082
<i>Ceropegia elegans</i> Wall.	EU106677
<i>Ceropegia evansii</i> McCann	EU106680
<i>Ceropegia fantastica</i> Sedgw.	EU312083
<i>Ceropegia filiformis</i> (Burch.) Schlter.	AM493289
<i>Ceropegia foliosa</i> Bruyns	AM493288
<i>Ceropegia gilgiana</i> Werderm.	AM493287
<i>Ceropegia hirsuta</i> Wight & Arn.	EU106688
<i>Ceropegia huberi</i> Ansari	EU106694
<i>Ceropegia humbertii</i> H. Huber	AM493286
<i>Ceropegia intermedia</i> Wight	AM493285
<i>Ceropegia intermedia</i> Wight	EU106678
<i>Ceropegia jainii</i> Ansari & B.G.P. Kulk	EU106693
<i>Ceropegia juncea</i> Roxb.	EU106691
<i>Ceropegia konasita</i> Masinde	AM493284
<i>Ceropegia lawii</i> Hook.f.	EU106689
<i>Ceropegia longifolia</i> Wall.	AM493283
<i>Ceropegia maccannii</i> Ansari	EU106685
<i>Ceropegia maccannii</i> Ansari	HQ154108
<i>Ceropegia mahabalei</i> Hemadri & Ansari	EU106692
<i>Ceropegia media</i> (Huber) Ansari	EU106696
<i>Ceropegia meleagris</i> H. Huber	AM493282
<i>Ceropegia mizoramensis</i> Ram. Kumar & S. Sharma	MH428807
<i>Ceropegia mohanramii</i> S.R. Yadav, S.P. Gaikwad & Sardesai	EU106698
<i>Ceropegia monticola</i> W.W. Sm	AM493306
<i>Ceropegia murlensis</i> Ram. Kumar & S. Sharma	MH428808
<i>Ceropegia murlensis</i> Ram. Kumar & S. Sharma	MH428809
<i>Ceropegia nilotica</i> Kotschy	AJ402161
<i>Ceropegia noorjahaniae</i> M. A. Ansari	EU106697
<i>Ceropegia occulata</i> R. A. Dyre	EU106679
<i>Ceropegia oculata</i> Hook.	HQ154110
<i>Ceropegia odorata</i> Nimmo	EU106701
<i>Ceropegia panchganiensis</i> Blatt. & McCann	EU106682
<i>Ceropegia panchganiensis</i> Blatt. & McCann	HQ154107
<i>Ceropegia pubescens</i> Wall.	AM493280
<i>Ceropegia racemosa</i> N.E. Br.	AM493279
<i>Ceropegia radicans</i> Schlter	HM475344
<i>Ceropegia robynsi</i> Werderm	AM493278
<i>Ceropegia rollae</i> Hemadri	EU106686
<i>Ceropegia rupicola</i> Deflers	AM493277
<i>Ceropegia sankuruensis</i> De. Wilde	AM493276
<i>Ceropegia santapau</i> Wadhwa & Ansari	EU106695
<i>Ceropegia saxatilis</i> Jum. & H. Perrier	AJ310786
<i>Ceropegia shayadrica</i>	EU106684
<i>Ceropegia shayadrica</i>	HQ154106
<i>Ceropegia simoneae</i> Rauh	AM493275
<i>Ceropegia</i> sp.	EU106683
<i>Ceropegia vincifolia</i> Hook.	EU106681
<i>Ceropegia yemenesis</i> Meve & Mangelsdorff	AM493293
<i>Quaqua incarnate</i> (L.f.) Bruyns	AJ488821
<i>Riocreuxia burchellii</i> K. Schum.	AJ488771
<i>Sisyranthus compactus</i> N.E. Br.	AJ310795