



# *Brachystelma mahendragiriense* (Apocynaceae), a new species from Odisha, India

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## Abstract

A tuberous herb with yellow flowers collected from the grassy slopes of the Mahendragiri hills (Odisha, India) is described as a new species of *Brachystelma*. Morphological and molecular data are furnished in support of this novelty.

**Keywords:** Asclepiadoideae, *Brachystelma mahajanii*, *Ceropegia*, Ceropegieae, Phylogenetic Tree, Tuberous Species

## Introduction

*Brachystelma* R. Br. comprises of *c.* 160 species has distribution in sub-Saharan Africa, India, Sri Lanka, Southeast Asia and northern Australia (Prasad *et al.*, 2016). All 27 species reported from India (20 from peninsular India), are hitherto known only from India (Kambale *et al.*, 2014; Venu & Prasad, 2015; Britto & Bruyns, 2016; Prasad & Prasanna, 2016; Prasad *et al.*, 2016). The genus is represented by nine species in the Eastern Ghats. The authors while exploring open grassy slopes of Mahendragiri hills (Odisha), the northern stretch of Eastern Ghats, collected a tuberous herb with yellow flowers. It is described here as *Brachystelma mahendragiriense sp. nov.* The article draws significance for being the first report of presenting a *Brachystelma* species from the state of Odisha.

## Taxonomy

*Brachystelma mahendragiriense* K. Prasad, Chorghe & Venu, **sp. nov.** **Fig. 1**

**Type:** INDIA, **Odisha:** Gajapati district, Mahendragiri hills, 18°58'05.3" N, 084°22'08.6" E, 1393 m, 17.9.2014, K. Prasad & Alok Chorghe 4221a (holo, CAL), 4221b–d (iso, BSID).

Herb, *c.* 20 cm high, perennial; tubers subglobose, *c.* 3 cm diam., pale brown. Stems erect, unbranched, *c.* 1.5 mm thick, pubescent when young, glabrescent on maturity; internodes *c.* 4 cm long. Leaves

opposite-decussate, sessile, linear or linear-lanceolate, 3–6.5 × 0.15–0.2 cm, margins bent inward, apex acute; margins and midrib puberulent. Inflorescences cymes, reduced to two flowers; bract 1, ovate-lanceolate, 1–1.1 mm long, apex acute, glabrous, greenish pink, caducous; bracteoles 2, linear, *c.* 0.5 mm long, apex obtuse, glabrous. Flowers upright, on 4–6 mm long pedicels, patent. Sepals 5, triangular-acuminate, 0.9–1.25 mm long, sparsely ciliate, apex acute or obtuse visible between corolla lobes, greenish pink. Corolla rotate, glabrous, yellow, mottled dull/bright pink at base; tube shallow, 0.5–0.75 mm long, mottled pink; lobes rotate, oblong-ovate, 3.5–4 × 1.4–1.7 mm, margins slightly recurved, apex mucronate with a translucent mucro. Corona biseriate, fused, cupular, 0.5–0.75 × 1.5–1.75 mm, exceeding gynostegium in length, blackish yellow, 5-lobed; upper margins undulate or obscurely 5-lobed, adaxially with horizontally spreading white trichomes in 10 clusters altogether; staminal corona lobes reduced to scales; anther appendages procumbent on stigma head, oblong, *c.* 4 mm long, apex obtuse, yellowish. Pollinia ovoid, 0.2–0.25 × 0.1–1.5 mm, yellow with pellucid margin along inner side, attached to brown corpusculum by short caudicle. Follicles in pairs or solitary, tapering towards apex, 6–8 cm long; seeds comose, narrowly elliptic, *c.* 4 × 2 mm, light brown at centre.

*Flowering & fruiting:* May–September.

*Habitat:* Grass dominated rocky areas, 1350–1400 m.

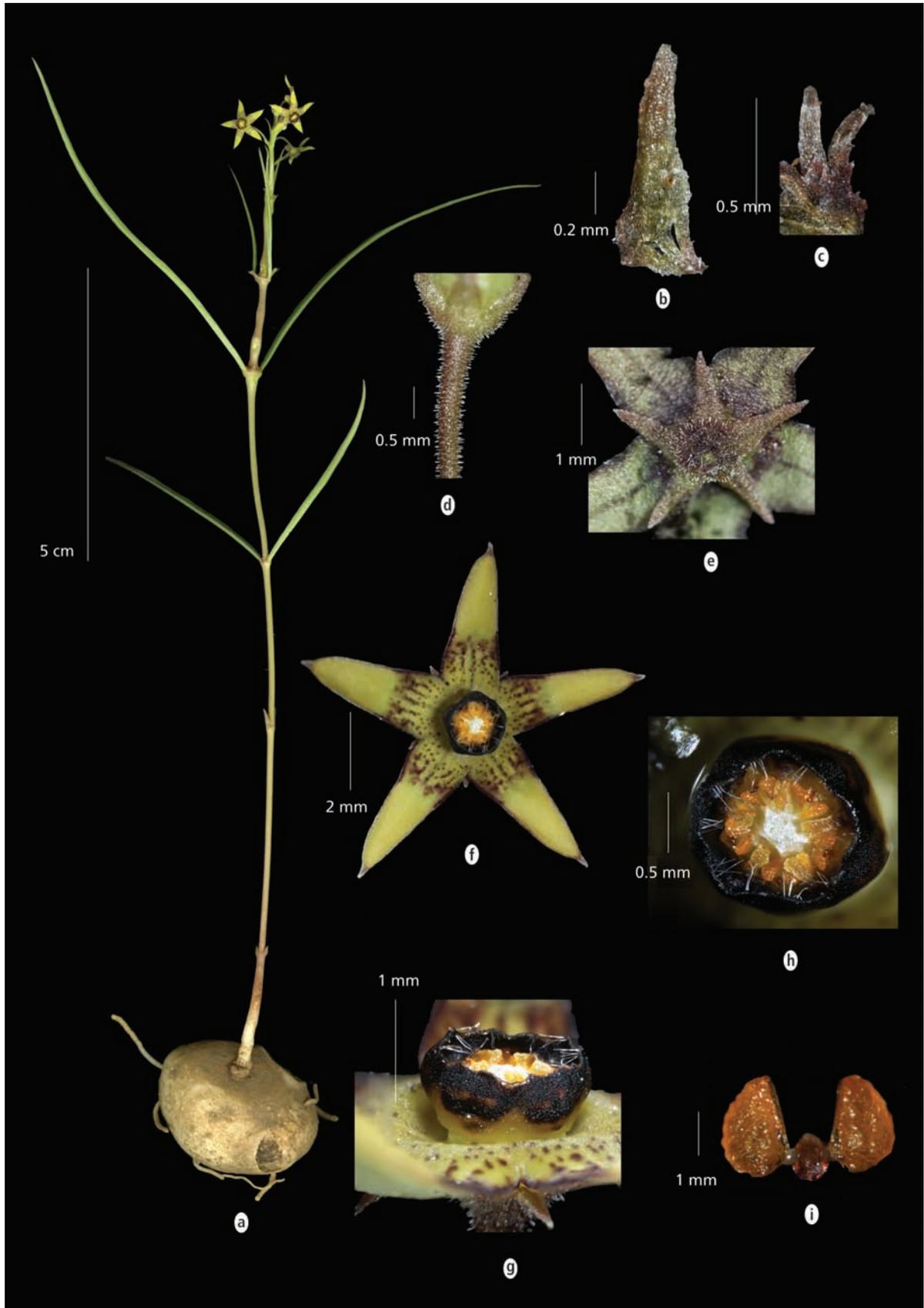


Fig. 1. *Brachystelma mahendragiriense* K. Prasad, Chorgha & Venu: a. Habit; b. Bract; c. Bracteoles; d. Pedicel; e. Calyx; f. Corolla; g, h. Corona side/top views; i. Pollinarium.

**Distribution:** Known from the type locality, Mahendragiri hills, Gajapati district, Odisha, India.

**Etymology:** The new species is named after Mahendragiri hills, its type locality.

**Conservation Status:** Since not much is known about its population and the species is described based on single location collections, this species is placed under 'Data Deficient' (DD) category (IUCN, 2014).

**Recognition:** Since the present collection is an erect species, the known climbers in *Brachystelma* and also species that produce flowers with slender corolla lobes and tips united at bud or even in flowering stage were considered remote for comparison. All the known erect *Brachystelma* species that produce flowers with indistinct corolla tube and lobes shorter (< 5 mm long), fleshy and rotate, were closely examined with reference to material under report for novelty evaluation. All such species as well were found to have their own distinctiveness in some important features: *Brachystelma matthewianum* Bruyns & Britto (deeply indented outer corona) and *B. saldanhae* Bruyns & Britto (corona raised on a short stipe) from Tamil Nadu; *B. vartakii* Kambale & S.R. Yadav (densely ciliated corolla lobes with bilobed corona) from Kerala; *B. swarupa* Kishore & Goyder (dangling inflorescence with 20 or more flowers at each node and corona cup ridged/grooved alternately at base and grooves hairy outside) from Tamil Nadu and Kerala; *B. annamacharyae* K. Prasad et al. (corolla entirely hairy and lobes reflexed), *B. ciliatum* Arekal & T.M. Ramakrishna (corolla lobes ciliated at tips and entirely reflexed on maturity; the latter feature is seen even in *B. swarupa*), *B. glabrum* Hook.f. (umbels terminal, 4-flowered, corolla lobes puberulous) from Andhra Pradesh and *B. elenaduense* Sathyan. (corolla lobes lanceolate and tips reflexed) from Karnataka are not similar to the proposed new species. *Brachystelma mahajanii* Kambale & S.R. Yadav that has been reported from Nilgiris appears to have broad similarity with *B. mahendragiriense* but differs from it in certain characters as given below.

*Brachystelma mahendragiriense* is similar to *B. mahajanii*, but differs in plants attaining 20 cm height (vs. reach up to 10 cm in *B. mahajanii*); tubers subglobose (vs. tubers discoid in *B. mahajanii*) leaves never reflexed, margins bent inward (vs. leaves reflexed, margins flat in *B. mahajanii*) inflorescences 2-flowered at every node, upright (vs. 1–8-flowered, drooping in *B. mahajanii*); bract glabrous, greenish pink, caducous; (vs. bract green, hairy at tip, persistent in *B. mahajanii*); sepals triangular-acuminate (sepals subulate in *B. mahajanii*); corolla lobes oblong-ovate, yellow, mottled dull/bright pink at base, uniformly thick, apex ending into a translucent appendage

(vs. lobes ovate, yellow but green at base, broad gibbous base distinct from linear thicker upper half, apex acute and devoid of translucent appendage in *B. mahajanii*); coronal cup blackish yellow, margins wavy with obscure lobation and with spreading white trichomes at interstaminal positions (corona greenish yellow, blotched purple inside and outside, margins distinctly 5-lobed, glabrous in *B. mahajanii*).

## Molecular studies

Since the campanulate/rotate nature of the corolla in *Brachystelma* has evolved multiple times (Surveswaran et al., 2009; Bruyns et al., 2015) within the lineage of *Ceropegia/Brachystelma*, the authors intend to test the phylogenetic affinity of this new species. Molecular data from both chloroplast and nuclear genomes are used for construction of a phylogenetic tree.

## Materials and Methods

Leaf material was collected in the field and stored in ziplock pouches along with silica gel which acts as an effective desiccant. Dried leaf material was ground in a plastic pestle in a 1.5 ml eppendorf tube along with acid washed sand. DNA was extracted from the leaf material using Nucleospin plant II DNA extraction kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions. Two nuclear and two chloroplast DNA markers were used in this study. The DNA regions, primers and annealing temperatures are listed in **Table 1**.

## PCR amplification

Polymerase chain reaction (PCR) amplifications of targeted regions was done in 25 µl volume reactions consisting for 2× DreamTaq Green PCR Master Mix (Thermo Scientific, Waltham, MA, USA), 10 pmol/µl concentration of each primer and DNA template of about 1-100 ng. The PCR cycle consisted of an initial denaturation at 95°C for 2 minutes followed by 40 cycles each of 95°C for 15 seconds, 58°C (annealing) for 15 seconds and 72°C (extension) for 55 seconds. A final extension at 72°C was done for 5 minutes. The annealing temperature of 58°C was maintained for ITS, *trnL* intron + *trnL-trnF* spacer and *trnS-trnG* spacer. For NCPGS region an annealing temperature of 46°C was used.

PCR products were visualized on 1% agarose gels stained with Ethidium Bromide. The PCR products were enzymatically cleaned using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA). 2 µl of ExoSAP-IT was added to 20 µl of PCR product and incubated at 37°C for 45 minutes. The

Table 1. DNA markers and primers used in phylogeny and annealing temperatures for PCR

Marker	Gene name	Genome	Primer Forward/ Reverse	Annealing temperature	Reference
ITS	Internal transcribed spacer	Nuclear	ITS4, ITS5	58°C	White <i>et al.</i> , 1990
nCPGS	Intron of Nuclear encoded chloroplast glutamine synthase	Nuclear	GScp687f, GScp994r	46°C	Emshwiller & Doyle, 1999
<i>trnL</i> + <i>trnL-F</i>	<i>trnL</i> intron and <i>trnL-trnF</i> intergenic spacer	Chloroplast	c, f	58°C	Taberlet <i>et al.</i> , 1991
<i>trnS-G</i>	<i>trnS-trnG</i> intergenic spacer	Chloroplast	trn S (GCU), trn G (UCC)	58°C	Hamilton, 1999

ExoSAP-IT activity was stopped by incubating at 80°C for 15 minutes. The purified PCR products were sequenced on both strands using the forward and reverse primer on an ABI Prism 3100 DNA sequencer in National Centre for Biological Sciences (Bangalore, India).

### Sequence assembly and Phylogenetic analysis

Forward and reverse sequences were assembled using the pregap4 and gap4 modules of the Staden package ver. 2.0.0b10 (Staden *et al.*, 2000). Sequences of other *Brachystelma* and *Ceropegia* were downloaded as FASTA files from the Genbank database. Sequence assembly was performed using the Muscle algorithm using Seaview ver. 4.4.2 (Gouy *et al.*, 2010). Sequence editing was performed using Aliview ver. 1.17 (Larsson, 2014).

Maximum parsimony analysis was performed using PAUP\* 4.0 b10 (Swofford, 2003). The four datasets were combined and tested for homogeneity using the partition homogeneity test. Heuristic searches were performed using the tree bisection reconnection (TBR) branch swapping, random addition of sequences and multiple trees option. Bootstrap supports were inferred from 1000 replicates using a heuristic search with the same settings as above. The best-fit model of sequence evolution for each dataset was computed using jModelTest ver. 2.1.7 (Darriba *et al.*, 2012). Bayesian phylogenetic analysis was performed using MrBayes ver. 3.2.2 (Ronquist *et al.*, 2012). The separate models were applied to each partition. Two independent Metropolis coupled Markov chain Monte Carlo chains were for 20 million generations sampling every 1000 generations. The convergence of the runs were checked using

Tracer ver. 1.6 (Rambaut *et al.*, 2014) as the values of effective sample size (ESS) greater than 200. The first 25% of the trees were considered as the burnin and the remaining 75% were summarized in MrBayes.

### Results and Discussion

The total length of the DNA data set and individual datasets, their variability, parsimony informative sites and best-fit DNA sequence evolution models are summarized in **Table 2**. The heuristic search for the most parsimonious trees yielded two trees with a length of 170 steps, consistency index (CI) of 0.912 and a retention index (RI) of 0.914. The topology of the trees was highly congruent and one of the two trees is shown on Figure 2. The Bayesian phylogenetic tree also yielded a similar tree topology but with some incongruent nodes which were not well supported (Bayesian posterior probability  $\geq 0.95$  and Maximum parsimony bootstrap greater than 90% were considered strong support). The new species was found closely associated with *B. mahajanii*. However, this sister relationship is not well supported because of the lack of sequence variation (pair wise genetic distance (p-distance) of 0.00073).

Overall, *B. mahendragiriense* falls in a clade containing most of the Indian *Brachystelmas* including *B. mahanjanii*, *B. ciliatum*, *B. kolarensis* Arekal & T.M. Ramakrishna, *B. vartakii* and *B. edule* Collett & Hemsl. complex (*B. edule*, *B. malwanense* S.R. Yadav & N.P. Singh and *B. naorojii* P. Tetali *et al.*). All these taxa are sister to *B. brevitubulatum* (Bedd.) Gamble. *Brachystelma bourneae* Gamble, however, appears as a separate lineage of *Brachystelma* and is not closely sister to this clade.

Table 2. Sequence characteristic of the nucleotide markers used in phylogeny

Marker	Total	Variable/ non-phylogenetically informative characters	Phylogenetically informative Characters (PIC)	Best-fit model of sequence evolution
ITS	633	27	28	SYM+G
nCPGS	864	43	19	HKY
<i>trnL+trnLF</i>	818	6	7	GTR
<i>trnSG</i>	558	14	4	F81
Total	2873	90	58	GTR+I

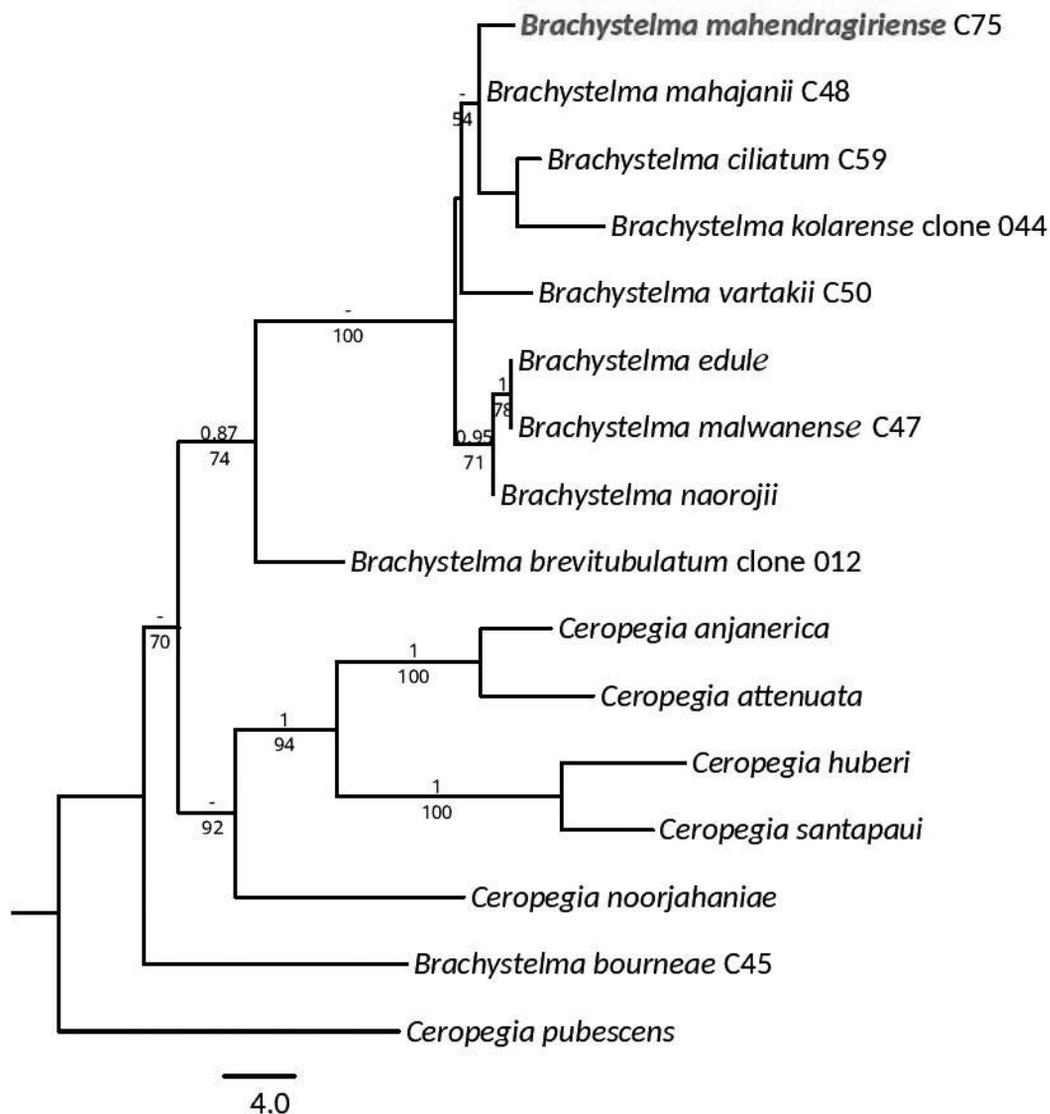


Fig. 2. One of the two most parsimonious trees obtained from a heuristic search in PAUP\*. The numbers above branches indicate Bayesian posterior probability support and the number below branches indicate bootstrap support percentages under parsimony method. Scale bar indicates numbers of nucleotide changes per 100 sites.

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