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COMMUNICATION

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COMMUNICATION

Resolving taxonomic problems in the genus *Ceropegia* L. (Apocynaceae: Asclepiadoideae) with vegetative micromorphology

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Abstract: The genus *Ceropegia* L. of Family Apocynaceae, subfamily Asclepiadoideae comprises 213 accepted taxa distributed in tropical and sub-tropical regions of Africa, India, Australia and neighbouring regions. The taxa are mainly identified on the basis of flower morphology. A study was undertaken to reveal micromorphology of 26 taxa distributed in Western Ghats of India to solve the identity problems. The micromorphology is studied with standard microscopic methods in five replicates of each character and taxa to reveal the parameters, distribution of stomata, stomatal index, stomatal density, epidermal cell wall pattern and stomatal measurements. The data obtained was subjected to ANOVA to find out the experimental mean, standard deviation and standard error. A consensus phylogeny tree is constructed using the PAST on the basis of Jaccard similarity coefficient. Results of study revealed that, micro morphological characters, viz., type of stomata, number and characters of subsidiaries, anticlinal cell wall pattern, and stomatal index are very significant in delimitation of closely allied taxa. Beside 'paracytic' stomata, 'tetracytic', 'isotricytic', & 'anomocytic' stomatal types, and amphistomatic distribution are recorded for first time in *Ceropegia*. The taxa are separated easily from each other using vegetative micromorphology and can be identified even in absence of flowers. An identification key and phylogenetic tree is derived on the basis of vegetative micro-morphology and gross morphological characters.

Keywords: Epidermal cell wall pattern, identification key, phylogeny, stomata, stomatal index, subsidiaries.

Abbreviations: SD—Stomatal density | SI—Stomatal index | WG—Western Ghats.

हिंदी सारांशः अपोसायनेसी परिवार के उपपरिवार अस्क्लेपीडोयडी के सिरोपेजिया लिन.-प्रजाती में अफ्रीका, भारत, ऑस्ट्रेलिया और पडोसी क्षेत्रों के उष्णकटिबंधीय और उप-उष्णकटिबंधीय क्षेत्रों में वितरित २१३ पादप प्रजाती शामिल हैं | यह प्रजातीयां मुख्य रूप से फूलों के आकृती के आधार पर पहचाने जाते हैं | पहचान की समस्याओं को हल करने के लिए भारत के पश्चिमी घाटों में वितरित २६ प्रजातीयों के शाकिय सुक्ष्माकृती को प्रकट करने के लिए एक अध्ययन किया गया | इस शाकिय सूक्ष्मदर्शी अध्ययन के लिए प्रत्येक वर्ण के पांच प्रतिकृतियों को समावेशित किया गया | इनमें निम्न मापदंडों को जैसे की, पर्णवंध्रों के वितरण, पर्णवंध्रों का इंडेक्स, पर्णवंध्रों की घनता, उपत्वक कोशिकाओं के छिपाव की रचना, और पर्णवंध्रों के माप शामिल है | प्राप्त जाणकारी के प्रयोगात्मक माध्य, मानक विचलन, और मानक त्रुटि का पता लगाने के लिए एनोवा लागू किया गया | फायलोजेनेटिक वृक्ष साकारणे के लिए पास्ट कार्यप्रणाली का उपयोग किया गया | अध्ययन के परिणामों से पता चलता है कि, सूक्ष्म रूपात्मक वर्ण, अर्थात, पर्णवंध्र प्रकार, संख्या, और पर्णवंध्र सहायक कोशिकाओं के चरित्र, उपत्वक कोशिकाओं के छिपाव की रचना, और पर्णवंध्रों का इंडेक्स बारीकी से संबंधित प्रजातीयों के परिसीमन में बहुत महत्वपूर्ण हैं | पैरासाइटिक पर्णवंध्रों के अलावा टेट्रासाइटिक, आइसोट्राइसाइटिक, और एनेमोसाइटिक, प्रकार के साथ एम्फिस्टोमेटिक वितरण पहली बार सिरोपेजिया में दर्ज किये गये हैं | शाकिय सूक्ष्मदर्शी अध्ययन का उपयोग करके प्रजातीयों को एक दूसरे से आसानी से अलग किया एवं फूलों की अनुपस्थिति में भी पहचाना जा सकता हैं | शाकिय सुक्ष्माकृती और सकल आकारिकीय वर्णों के आधार पर एक पहचान कुंजी और फाइलोजेनेटिक वृक्ष की उत्पत्ति की गयी हैं |

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Author contribution: SSR has done the micro-morphology work of this study and presentation of the results. SRR has done the field work, collection and processing of samples, morphological characterization of the taxa, photography and manuscript editing. The work is done on a mutual benefit basis.

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INTRODUCTION

The genus *Ceropegia* L. of family Apocynaceae, subfamily Asclepiadoideae comprises many narrow endemic and threatened species distributed only in tropical and sub-tropical regions especially, Africa, India, Australia, and neighbouring region. The genus comprises of 213 accepted species, eight unplaced, and 36 un-assessed taxa of species and infra-specific ranks (Plant list 2019; Plant of the World Online 2020). Most of the taxa are endemic to the regions or locations from where they are described. In India, it is represented by about 68 taxa comprising of 61 species, two subspecies and five varieties with recent descriptions by Diwakar & Singh (2011), Rahangdale & Rahangdale (2012), Kamble et al. (2012), Sujanapal et al. (2013), and Kumar et al. (2018). Of these taxa, 40 species and six varieties are endemic to India, while 34 species and two varieties are endemic to Western Ghats (Jagtap & Singh 1999; Singh et al. 2015).

The taxa under this genus are mainly described on the basis of floral characters, while many vegetative characteristics are similar in many taxa. The taxonomists in the world have tried to resolve systematic crises among these species or taxa below rank of species. Since last decade many new taxa at specific and infra-specific rank are described (Singh et al. 2015). Still, many of the Indian taxa are kept under unresolved categories by international databases (such as The Plant List (2019) as well as POWO (2020)) due to insufficient data related to allied species and varieties. In India, there are two major regions of distribution of this genus, viz., Himalaya and peninsular India. Most species found in Western Ghats or peninsular India possess tubers, while those found in the Himalayan region do not have tubers and are non-succulent. In general, all these taxa are described on the basis of morphological characters; especially on floral morphology as the allied taxa do have many similarities in vegetative characters. The vegetative micro-morphology was neglected in most of the cases, may be because of morpho-similarities among the taxa in vegetative condition. There is negligible literature available on microscopic characters of vegetative organs; and *Ceropegia* species are generally identified on the basis of habit and flower characters. Therefore, it is a difficult task to identify the species without flowers. So, there is need of micro-morphological as well as anatomical characters that would be helpful in identification of taxa at species and infra-specific level in vegetative state. Considering these facts and lacunae in the literature a study was undertaken to reveal micro-morphological characters of taxa of this genus and provide identification key on the basis of vegetative and micro-morphological characters.

MATERIALS AND METHODS

Plant Materials

Materials for the present study comprise a total 26 taxa of the genus *Ceropegia* L. from Western Ghats. Fresh materials were collected from wild plants as well as plants maintained in the nursery for study. The locations of taxa included in the study range from Nashik in Maharashtra to Coimbatore in Tamil Nadu through Wayanad in Kerala. A list of taxa under study with their status, distribution, and locations of collection for the present study is given in the Table 1.

In the present study, *C. attenuata* Hook. var. *attenuata, C. attenuata* Hook. var. *mookambikae* Diwakar & Singh, *C. mahabalei* Hemadri & Ansari var. *hemalatae* Rahangdale & Rahangdale, *C. mahabalei* Hemadri & Ansari var. *mahabalei, C. maharashtrensis* Punekar et al. are retained because these taxa are merged without any solid reasons and without comparing the characters said in original protologues of above mentioned taxa. As the present study is aimed to find out more stable characters for correct distinction between the taxa, the above mentioned taxa are accepted.

Methods

Vegetative morphology

Total 14 vegetative morphological characters of each taxon are recorded by observation of living materials. They are mentioned in the Table 4 to avoid repetition. Only newly observed characters are mentioned in detail.

Micro-morphology

For epidermal study, five leaves per sample were picked at 4–6th nodes from base. Epidermal peelings of suitable size were made from both upper and lower surfaces of each leaf. Peelings were treated with 5% Sodium Hypochlorite for clearing. Then epidermal debris was brushed off under observation using Lawrence and Mayo stereo-zoom microscope NSZ-606 and the peel was finally mounted in 50% Glycerine. Thereafter, slides were observed under research microscope (Lawrence & Mayo Model No. XSZ-N107T) for epidermal details. For this, five leaves were taken and from each leaf five different fields were observed and data was recorded, making total observation size of 25 sample areas. The observed parameters are, type of stomata, distribution of stomata on leaf surface (hypostomatic/amphistomatic), stomatal density/mm^2, stomatal Length (µ), Width (µ), pore length

Table 1. Plant materials (taxa) used in the present study.

	Taxa name	Status*	Distribution*	Locality of collection	Specimen number
1	*Ceropegia anantii* Yadav et al.	EN	Endemic; MS	Konkan, Ratnagiri, MS	1122
2	*C. anjanerica* Malpure et al.	CR	Endemic; MS	Anjaneri, Nashik, MS	23926
3	*C. attenuata* Hook. var. *attenuata*	EN	Endemic; MS, Goa, KA, Raj.	Junnar, Pune, MS	907, 1116, 21441 (AHMA)
4	*C. attenuata* Hook. var. *mookambikae* Diwakar & Singh	EN	Endemic; MS, KA	Konkan, Ratnagiri, MS	1112
5	*C. bulbosa* Roxb. var. *bulbosa*	Common	Asia & Africa	Junnar, Pune, MS	18544, 18545 (AHMA)
6	*C. bulbosa* Roxb. var. *lushii* (Grah.) Hook. f.	Common	India, Pakistan	Junnar, Pune, MS	21685, 24312 (AHMA)
7	*C. candelabrum* L.	VU	India, Sri Lanka	Waynad, KL	23924
8	*C. concanensis* Kamble et al.	EN	Endemic; MS	Konkan, Ratnagiri, MS	1120, 1121
9	*C. elegans* Wall.	Occasional	India, Sri Lanka	Coimbatore, TN	23923
10	*C. evansii* McCann	EN	Endemic; MS	Ambegaon, Pune, MS	139, 1117, 1118
11	*C. fantastica* Sedgwick	CR	Endemic; MS, KA, Goa	Amboli, Sindhudurg, MS	23920
12	*C. hirsuta* Wight & Arn.	Common	India, Thailand	Junnar, Pune, MS	0466, 23908, 23909, 23910, 23911,
13	*C. jainii* Ansari & Kulkarni	EN	Endemic; MS	Kas, Satara, MS	23927
14	*C. juncea* Roxb.	Occasional	India, Sri Lanka	Coimbatore, TN	23925
15	*C. lawii* Hook. f.	EN	Endemic; MS	Junnar, Pune, MS	0316
16	*C. maccannii* Ansari	EN	Endemic; MS	Simhagarh, Pune, MS	0832
17	*C. mahabalei* Hemadri & Ansari var. *hemalatae* Rahangdale & Rahangdale	CR	Endemic; MS	Junnar, Pune, MS	0136, 0137 (Types), 1114
18	*C. mahabalei* Hemadri & Ansari var. *mahabalei*	CR	Endemic; MS	Junnar, Pune, MS	0138, 0908, 23915, 23916, 23918
19	*C. maharashtrensis* Punekar et al.	CR	Endemic; MS	Junnar, Pune, MS	23222
20	*C. media* (Huber) Ansari	EN	Endemic; MS	Junnar, Pune, MS	0774, 1119, 22735X, 23917
21	*C. oculata* Hook.	VU	Endemic; MS, KL, TN	Junnar, Pune, MS	23902, 23903, 23904, 23914
22	*C. odorata* Nimmo ex. Hook f.	CR	Endemic; MS, Raj, Guj.	Junnar, Pune, MS	22922
23	*C. panchganiensis* Blatt. & McCann	CR	Endemic; MS	Junnar, Pune, MS	18549 (AHMA)
24	*C. rollae* Hemadri	CR	Endemic; MS	Junnar, Pune, MS	1123, 23209, 23334, 23912, 23913
25	*C. sahyadrica* Ansari & Kulkarni	CR	Endemic; MS	Ambegaon, Pune, MS	0188, 23907
26	*C. vincifolia* Hook.	VU	Endemic; MS	Junnar, Pune, MS	0313

CR—Critically Endangered | EN—Endangered | VU—Vulnerable | Guj—Gujarat | KA—Karnataka | KL—Kerala | MS—Maharashtra | Raj—Rajastha | TN—Tamil Nadu | *—as per Singh et al. (2015). Specimens are deposited at AHMA—Agharkar Research Institute, Pune and Herbarium of Hon. Balasaheb Jadhav College, Ale.

(µ), characters and number of subsidiaries and epidermal cell wall characters as per the techniques described by Dilcher (1974) and Kotresha & Seetharam (2000). The classification of stomatal types given by Prabhakar (2004) was followed to recognize stomatal complex, with number and characteristics of subsidiaries.

Parameters studied

1. Stomatal index (SI):

Stomatal index = [S ÷ (E+S)] × 100

where, E = no. of epidermal cells, S = no. of stomata in an unit (mm^2) area taken for observation (0.022mm^2 under 400X magnification).

2. Stomatal Density (SD):

Stomatal Density = No. of stomata per unit area (1mm^2)

For this, number of stomata in a unit area is recorded for total sample size as that for stomatal index observations.

3. Stomatal length (µ):

The length of stomata is measured as the longitudinal end to end distance of guard cells, using ocular meter scale standardized for each magnification with the stage micrometer.

4. Stomatal width (µ):

This is the maximum length in the middle transect of stoma with the ocular meter scale.

5. Stomatal pore length (µ):

This is length of stomatal opening observed with the help of ocular meter scale.

Statistical analysis comprising of mean, variance, standard deviation, and standard error are applied for each parameter as per method given by Singh & Chaudhary (1985).

Phylogenetic analysis

Phylogenetic analysis was done on the basis of 42 vegetative morphological and micro-morphological characters (Table 4). For phylogenetic analysis PAST ver.03 was used as per Harper (1999), Hammer et al. (2001), Hammer & Harper (2006). In this, the characters were represented in the binary format (Table 5), which was used to generate a phylogenetic tree by Jaccard similarity coefficient model and data replication method - bootstrap method at 1,000 replications on the basis of a matrix of characters.

RESULTS

The observations regarding stomatal distribution, type, subsidiaries character & number, and the anticlinal wall characters of epidermal cells are presented in the Table 2 and Image 1. The results are described under the subheads as mentioned below.

Distribution and type of stomata

The stomata are distributed only on lower surface of the leaf (hypostomatic) in all the taxa studied except in *C. bulbosa*. In both varieties, *C. bulbosa* var. *bulbosa* and *C. bulbosa* var. *lushii*, the leaves are amphistomatic, i.e., stomata are present on both surfaces of the leaves. Four types of stomata, i.e., paracytic, anomocytic, tetracytic, and isotricytic are recorded in the taxa under study. Among these types, the paracytic stomata are most common and found in 16 taxa, the anomocytic are less common and recorded in four taxa; while the tetracytic stomata are observed in only *C. elegans*. Five taxa have mixed stomata; of which, four taxa viz., *C. anantii, C. attenuata* var. *attenuata, C. maharashtrensis*, and *C. sahyadrica* have isotricytic and tetracytic intermixed with each other, while in *C. rollae* paracytic stomata were found along with anomocytic stomata.

Characters and number of subsidiaries

The subsidiaries in studied taxa are either distinct (different than epidermal cells) or indistinct (same as epidermal cells). The stomatal complexes in 12 taxa have distinct subsidiaries while 13 taxa have indistinct ones. Among the 16 taxa having paracytic stomata, 12 taxa have distinct subsidiaries while remaining four have indistinct ones. In *C. rollae,* where stomata are of mixed type, i.e., paracytic and anomocytic, the subsidiaries were distinct along the paracytic stomata while with anomocytic stomata there were indistinct subsidiaries. The number of subsidiaries is also variable from two to five, but generally showed a fixed number or a range in all the specimens of same taxon. The number is fixed to two subsidiaries in nine taxa and four subsidiaries in two taxa. In other 15 taxa, the number of subsidiaries ranges 2–3 in three taxa, 2–4 in three taxa, 3–4 in seven taxa, and 4–5 subsidiaries in two taxa with anomocytic stomata. The taxa having mixed stomata with isotricytic and tetracytic types have indistinct subsidiaries.

Anticlinal wall pattern

The patterns of anticlinal walls of epidermal cells are observed and recorded in all the taxa under study. There are three patterns, 'straight', 'rounded (curved)', and 'undulate (wavy)'. Out of all 26 taxa, 10 have undulate anticlinal walls, eight taxa have rounded walls, and remaining eight have straight anticlinal walls. The results showed that, anticlinal wall pattern is not found to be associated with the other characters studied. This character is independent of stomatal type, size or number and type of subsidiaries. But, it is important for the differentiation between different taxa.

Stomatal characteristics

The experimental results about stomatal measurements are presented in Table 3 and described below.

Stomatal Index (SI)

The results revealed that, mean value of SI for the taxa under study (experimental mean) is 14.75±0.35. The highest SI is found in *C. attenuata* var. *attenuata* (21.80) followed by *C. mahabalei* var. *hemalatae* (20.32), and *C. sahyadrica* (19.94); while the lowest value of SI is recorded in *C. juncea* (8.18) along with *C. panchganiensis* (11.24), *C. vincifolia* (11.81), and *C. hirsuta* (11.88).

Stomatal Density (SD)

The mean value for stomatal density/mm^2 of leaf area is 252.31±8.50 with the highest value of 403.64±10.60 in *C. maharashtrensis* followed by *C. anantii* (390.91), and *C. jainii* (370.91), while the lowest SD 98.18±3.40 in *C. juncea* subtended by *C. bulbosa* var. *lushii* (105.45), *C. bulbosa* var. *bulbosa* (127.27), and *C. hirsuta* (152.73). *C. juncea* is a succulent taxon with highly reduced leaves

Table 2. Epidermal and stomatal characters observed in the taxa under study.

	Name of taxon	Stomatal distribution	Type of Stomata #	Subsidiary cells		Anticlinal cell wall pattern (Epidermal Cell outline) $
				Number	Character	
1	*Ceropegia anantii*	Hypostomatic	Isotricytic & Tetracytic	3–4	Indistinct	Rounded
2	*C. anjanerica*	Hypostomatic	Paracytic	2–3	Distinct	Undulate
3	*C. attenuata* var. *attenuata*	Hypostomatic	Isotricytic & Tetracytic	3–4	Indistinct	Undulate
4	*C. attenuata* var. *mookambikae*	Hypostomatic	Paracytic	2	Distinct	Undulate
5	*C. bulbosa* var. *bulbosa*	Amphistomatic	Anomocytic	3–4	Indistinct	Straight
6	*C. bulbosa* var. *lushii*	Amphistomatic	Anomocytic	3–4	Indistinct	Straight
7	*C. candelabrum*	Hypostomatic	Paracytic	2	Distinct	Undulate
8	*C. concanensis*	Hypostomatic	Paracytic	4	Indistinct	Undulate
9	*C. elegans*	Hypostomatic	Tetracytic	4	Indistinct	Rounded
10	*C. evansii*	Hypostomatic	Paracytic	2	Distinct	Undulate
11	*C. fantastica*	Hypostomatic	Paracytic	2	Distinct	Straight
12	*C. hirsuta*	Hypostomatic	Paracytic	3–4	Distinct	Rounded
13	*C. jainii*	Hypostomatic	Paracytic	2–3	Distinct	Rounded
14	*C. juncea*	Hypostomatic	Paracytic	2–4	Distinct	Straight
15	*C. lawii*	Hypostomatic	Paracytic	2	Distinct	Undulate
16	*C. maccannii*	Hypostomatic	Anomocytic	4–5	Indistinct	Straight
17	*C. mahabalei* var. *hemalatae*	Hypostomatic	Paracytic	2–4	Indistinct	Rounded
18	*C. mahabalei* var. *mahabalei*	Hypostomatic	Paracytic	2	Distinct	Undulate
19	*C. maharashtrensis*	Hypostomatic	Isotricytic & Tetracytic	3–4	Indistinct	Straight
20	*C. media*	Hypostomatic	Paracytic	2	Distinct	Straight
21	*C. oculata*	Hypostomatic	Paracytic	2	Distinct	Undulate
22	*C. odorata*	Hypostomatic	Paracytic	2	Indistinct	Rounded
23	*C. panchganiensis*	Hypostomatic	Anomocytic	4–5	Indistinct	Rounded
24	*C. rollae*	Hypostomatic	Paracytic & Anomocytic	2–4	Distinct & Indistinct	Straight
25	*C. sahyadrica*	Hypostomatic	Isotricytic & Tetracytic	3–4	Indistinct	Rounded
26	*C. vincifolia*	Hypostomatic	Paracytic	2–3	Indistinct	Undulate

#—as per Prabhakar (2004) | $—as per Dilcher (1974) | Curved—Rounded | Wavy—Undulate.

and also have lowest value of SI.

Stomatal Size

Regarding the stomatal dimensions, the largest stomata are recorded in *Ceropegia juncea,* while the smallest stomata in the *C. attenuata* var. *mookambikae*. The experimental mean for stomatal length is 29.54±0.73µ, stomatal width 19.68±0.67µ and the pore length 19.68±0.64 microns. The stomatal length is highest in *C. juncea* (39.0µ), followed by *C. vincifolia* (32.5µ), *C. bulbosa* (32.25µ), *C. oculata* (32.0µ), and *C. candelabrum* (31.5µ). The succulent species *C. juncea* have largest stomata with respect to length 39.0±1.5µ, width 26.5±1.7µ, and pore length 27.0±0.97µ; while those values for *C. attenuata* var. *mookambikae* are 23.0±0.5µ, 15.0±0.79µ, and 12.0±0.5µ, respectively having the smallest stomata. As per the ratio of length x width, the stomatal size is ranging from the smallest one of 345µ^2 in *C. attenuata* var. *mookambikae* to 1033.5µ^2 in *C. juncea* has largest stomata with the experimental mean value of 587.3µ^2.

Phylogeny

A consensus phylogenetic tree based on 42 morphological and micro-morphological characters obtained after 1,000 replications revealed that the dendrogram is divided into three clusters (Figure 1). Cluster I comprises seven taxa: *C. maccannii*, *C. panchganiensis*, *C. rollae*, *C. attenuata* var. *attenuata*, *C. sahyadrica, C. maharashtrensis*, and *C. anantii*. All these taxa are clustered with unit distance of 0.2–0.8.

Cluster II comprises total 15 taxa with unit distance of

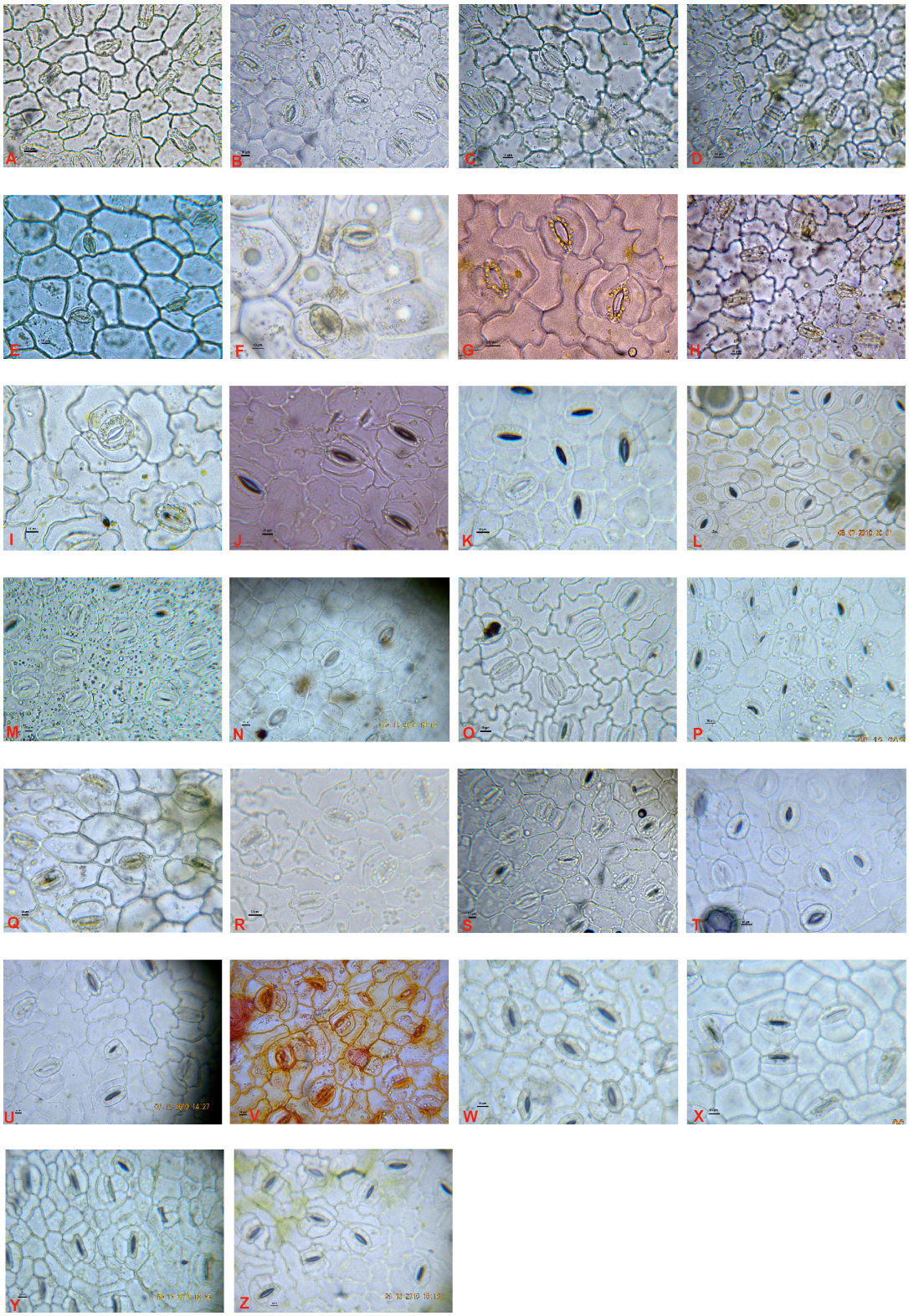

Image 1. Type of stomata and epidermal cell wall pattern in *Ceropegia* L. | A—*Ceropegia anantii* | B—*Ceropegia anjanerica* | C—*Ceropegia attenuata* var. *attenuata* | D—*Ceropegia attenuata* var. *mookambikae* | E—*Ceropegia bulbosa* var. *bulbosa* | F—*Ceropegia bulbosa* var. *lushii* | G—*Ceropegia candelabrum* | H—*Ceropegia concanensis* | I—*Ceropegia elegans* | J—*Ceropegia evansii* | K—*Ceropegia fantastica* | L—*Ceropegia hirsuta* | M—*Ceropegia jainii* | N—*Ceropegia juncea* | O—*Ceropegia lawii* | P—*Ceropegia maccannii* | Q—*Ceropegia mahabalei* var. *hemalatae* | R—*Ceropegia mahabalei* var. *mahabalei* | S—*Ceropegia maharashtrensis* | T—*Ceropegia media* | U—*Ceropegia oculata* | V—*Ceropegia odorata* | W—*Ceropegia panchganiensis* | X—*Ceropegia rollae* | Y— *Ceropegia sahyadrica* | Z—*Ceropegia vincifolia*. Scale bar in each picture = 10µm. © Sanjaykumar R. Rahangdale.

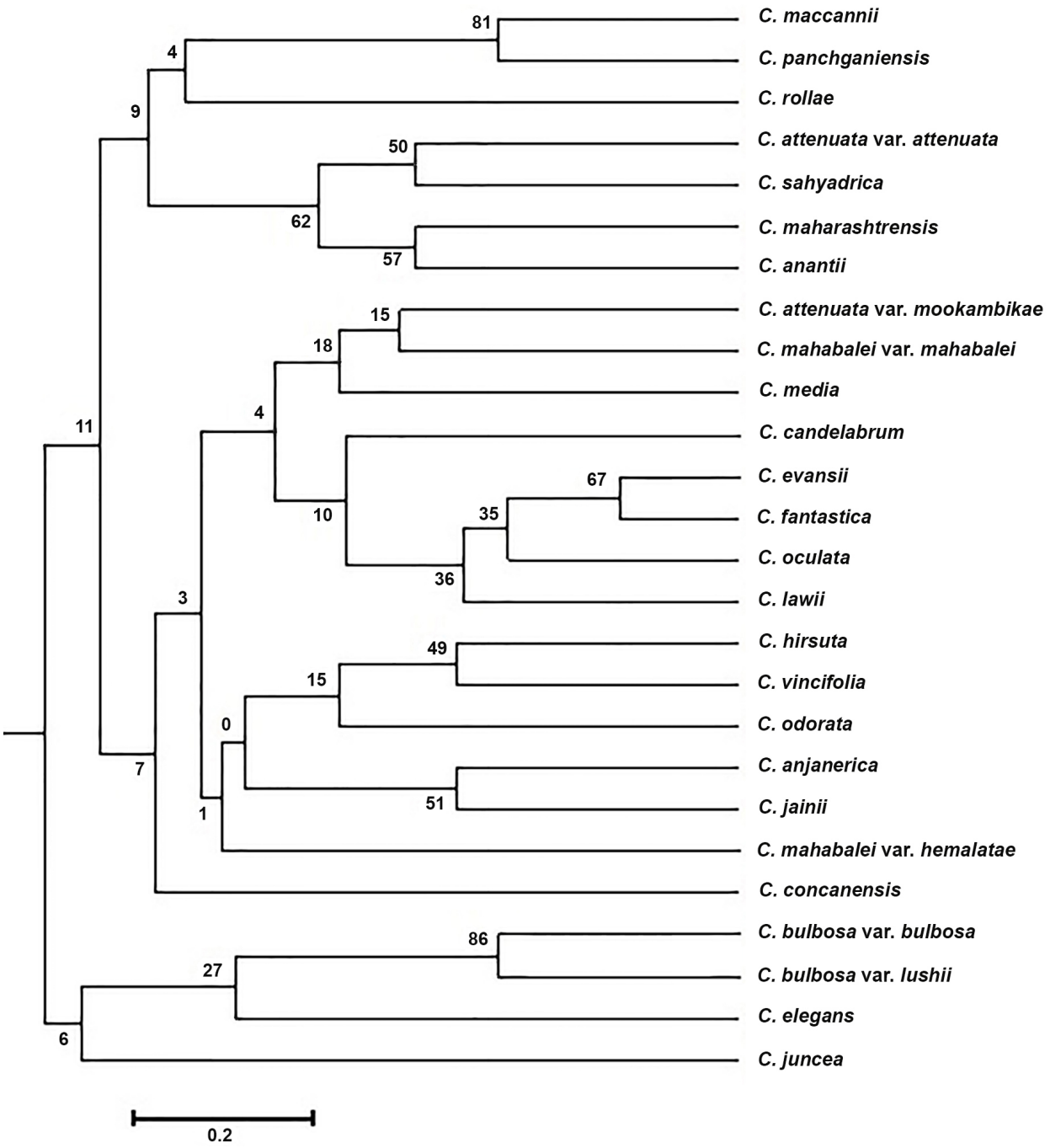

Figure 1. A consensus phylogenetic tree of 26 taxa of the genus *Ceropegia* L. (The numbers near branches represent the % of replications).

0.2–0.7. All the taxa of this cluster have paracytic stomata. This cluster further has two sub-clusters. The sub-cluster IIa comprises eight taxa: *C. attenuata* var. *mookambikae*, *C. mahabalei* var. *mahabalei*, *C. media, C. candelabrum, C. evansii, C. fantastica, C. oculata*, and *C. lawii*. Taxa in this sub-cluster have two distinct subsidiaries in all the taxa. From the dendrogram it is observed that the taxa, *C. evansii, C. fantastica, C. oculata*, and *C. lawii* are closely related having unit distance of 0.2–0.4. Another sub-cluster IIb comprises seven taxa having indistinct and variable subsidiaries: *C. hirsuta, C. vincifolia, C. odorata, C. anjanerica, C. jainii, C. mahabalei* var. *hemalatae*, and *C. concanensis*.

The Cluster III is an externally linked group having four taxa with unit distance of 0.2–0.8. It comprised two varieties of *C. bulbosa, C. elegans*, and *C. juncea.*

Table 3. The stomatal measurements recorded in the taxa.

	Name of taxon	Stomatal index		Stomatal density/ mm²		Stomatal length (µ)		Stomatal width (µ)		Stomatal pore length (µ)		Size of stomata (µ²)
		Mean	SE ±	Mean	SE ±	Mean	SE ±	Mean	SE ±	Mean	SE ±	
1	*C. anantii*	16.71	0.28	390.91	10.50	26.00	0.61	18.50	0.61	17.50	1.12	481.0
2	*C. anjanerica*	14.69	0.16	338.18	9.85	31.00	0.61	21.00	1.00	22.00	0.94	651.0
3	*C. attenuata* var. *attenuata*	21.80	0.47	300.00	12.03	28.50	1.00	20.50	0.50	16.50	0.61	584.3
4	*C. attenuata* var. *mookambikae*	15.05	0.16	329.09	7.99	23.00	0.50	15.00	0.79	12.00	0.50	345.0
5	*C. bulbosa* var. *bulbosa* ^	14.04	1.64	101.82	2.40	33.5	1.7	21.5	1.00	23.5	0.61	
	C. bulbosa var. *bulbosa* ˅	17.15	1.05	152.73	2.68	31.0	1.0	18.5	1.27	19.0	1.27	
	C. bulbosa var. *bulbosa* *	15.60	0.60	127.27	7.41	32.25	1.35	20.00	1.13	21.25	0.94	645.0
6	*C. bulbosa* var. *lushii* ^	13.80	1.04	96.36	1.70	31.5	0.61	20.0	0.0	20.5	0.5	
	C. bulbosa var. *lushii* ˅	12.41	1.13	114.55	2.19	29.5	0.50	19.5	0.5	19.5	0.5	
	C. bulbosa var. *lushii* *	13.10	0.48	105.45	4.17	30.50	0.55	19.75	0.25	20.00	0.50	602.4
7	*C. candelabrum*	14.39	0.28	145.45	3.71	31.50	0.61	20.50	0.50	20.00	0.00	645.8
8	*C. concanensis*	12.11	0.30	258.18	9.36	26.00	0.61	15.50	0.50	13.00	0.50	403.0
9	*C. elegans*	12.39	0.23	198.18	5.80	30.00	0.79	21.00	0.61	20.50	0.50	630.0
10	*C. evansii*	15.04	0.52	209.09	12.03	33.00	0.50	19.50	0.50	19.50	0.50	643.5
11	*C. fantastica*	16.94	0.46	269.09	7.84	28.50	1.27	23.00	1.22	16.00	0.61	655.5
12	*C. hirsuta*	11.88	0.40	152.73	5.17	30.00	0.00	22.00	1.46	19.50	0.50	660.0
13	*C. jainii*	12.26	0.30	370.91	8.58	31.50	1.00	20.00	0.00	20.50	0.50	630.0
14	*C. juncea*	8.18	0.37	98.18	3.40	39.00	1.50	26.50	1.70	27.00	0.94	1033.5
15	*C. lawii*	14.49	0.46	263.64	11.13	29.00	1.00	15.50	0.94	18.50	0.61	449.5
16	*C. maccannii*	17.36	0.41	254.55	8.30	28.00	0.50	23.00	0.50	18.50	0.61	644.0
17	*C. mahabalei* var. *hemalatae*	20.32	0.33	341.82	10.54	30.50	0.50	20.00	0.00	24.00	1.00	610.0
18	*C. mahabalei* var. *mahabalei*	16.50	0.12	325.45	10.05	29.50	0.50	19.50	0.50	24.50	0.94	575.3
19	*C. maharashtrensis*	15.48	0.18	403.64	10.60	28.00	1.46	21.00	0.61	15.50	0.50	588.0
20	*C. media*	14.85	0.46	314.55	17.39	28.00	1.66	16.25	1.12	16.25	1.12	455.0
21	*C. oculata*	14.76	0.52	230.91	8.27	32.00	0.94	16.75	0.50	20.00	1.37	536.0
22	*C. odorata*	13.51	0.43	250.91	8.35	28.00	0.94	21.50	0.61	21.00	0.61	602.0
23	*C. panchganiensis*	11.24	0.28	205.45	5.33	29.50	0.50	21.00	0.61	19.50	0.50	619.5
24	*C. rollae*	12.99	0.22	201.82	4.61	30.00	0.00	18.50	0.61	19.00	0.61	555.0
25	*C. sahyadrica*	19.94	0.60	285.45	15.19	30.00	0.00	16.00	0.61	20.00	0.00	480.0
26	*C. vincifolia*	11.81	0.20	189.09	3.40	32.50	0.00	20.00	0.00	22.50	0.00	650.0
	Experimental mean @ 25*df*	**14.75**	**0.35**	**252.31**	**8.50**	**29.84**	**0.73**	**19.68**	**0.67**	**19.40**	**0.64**	**587.3**

*—the data of amphistomatic taxa are taken from mean of both the surfaces and then the experimental mean is calculated | ^—upper surface of leaf | ˅—lower surface of leaf.

DISCUSSION

As the genus *Ceropegia* is considered to be xerophytic because of its escape mechanism against the hot period by perennial tubers. It should be preferably hypostomatic and the results are in corroboration with the concept, but the exceptions are both the varieties of *C. bulbosa* as they have amphistomatic leaves. Both the varieties of this taxon are usually distributed in dry rain-shadowed area of the Western Ghats, even though they are amphistomatic; this may be due to more succulent leaves than other taxa. Metcalfe & Chalk (1950) reported isobilateral leaves in the species of *Ceropegia* and *Hoya* R.Br. having fleshy leaves. Therefore, present results also confirm the amphistomatic nature of some taxa in *Ceropegia* which have fleshy leaves. The presence of stomata only on

Key for identification of species on the basis of vegetative and micro-morphological characters

1. Plants erect 2
1. Plants twiner 3
2. Leaves broadly ovate 4
2. Leaves lanceolate/linear 5
4. Stomata paracytic or anomocytic 6
4. Stomata mixed 7
6. Stomata paracytic, subsidiaries 2 distinct, epidermal cell wall undulate ..... *C. lawii*
6. Stomata anomocytic, subsidiaries 4–5 indistinct, epidermal cell wall straight / rounded 8
7. Stomata para & anomocytic, subsidiaries 2–4, epidermal cell wall straight ..... *C. rollae*
7. Stomata isotricytic & tetracytic, subsidiaries 3–4 indistinct 9
8. Epidermal cell wall straight, SI 17.36 ..... *C. maccannii*
8. Epidermal cell wall rounded, SI 11.24 ..... *C. panchganiensis*
9. Epidermal cell wall rounded, SI 19.94, SD 285.45 ..... *C. sahyadrica*
9. Epidermal cell wall straight, SI 15.48, SD 403.64 ..... *C. maharashtrensis*
5. Plants robust up to 1m tall, stomata paracytic 10
5. Plants small / delicate, shorter than 0.5m tall, stomata paracytic / other types 11
10. Stem stout, leaf margin straight, subsidiary 2, distinct, epidermal cell wall undulate ..... *C. mahabalei* var. *mahabalei*
10. Stem scandant, leaf margin undulate, subsidiary 2–4, indistinct, epidermal cell wall rounded ..... *C. mahabalei* var. *hemalatae*
11. Stomata paracytic, subsidiaries distinct 12
11. Stomata isotricytic & tetractytic, subsidiaries 3–4 indistinct 13
12. Subsidiaries 2 or 4, epidermal cell wall undulate 14
12. Subsidiaries 2–3, epidermal cell wall rounded / undulate 15
14. Subsidiaries 2, SI 15.05, SD 329.09 ..... *C. attenuata* var. *mookambikae*
14. Subsidiaries 4, SI 12.11, SD 258.18 ..... *C. concanensis*
15. Leaves hirsute, epidermal cell wall undulate, SI 14.69, SD 338.18 ..... *C. anjanerica*
15. Leaves sparsely hairy, epidermal cell wall rounded, SI 12.26, SD 370.91 ..... *C. jainii*
13. Epidermal cell wall undulate, SI 21.80, SD 300.0 ..... *C. attenuata* var. *attenuata*
13. Epidermal cell wall rounded, SI 16.71, SD 390.91 ..... *C. anantii*
3. Plants succulent, leaves ovate/lanceolate/linear/minute, epidermal cell wall straight 16
3. Plants non succulent, leaves ovate/ lanceolate, epidermal cell wall otherwise 17
16. Leaves minute, hypo-stomatic, stomata paracytic, subsidiaries 2–4 distinct ..... *C. juncea*
16. Leaves conspicuous, amphi-stomatic, stomata anomocytic, subsidiaries 3–4 indistinct 18
18. Leaves ovate-lanceolate, SI 15.60, SD 127.27 ..... *C. bulbosa var. bulbosa*
18. Leaves linear, SI 13.10, SD 105.45 ..... *C. bulbosa var. lushii*
17. Leaves lanceolate, stomata paracytic 19
17. Leaves broadly ovate, stomata otherwise 20
19. Leaves minutely hairy, veins winged below, epidermal cell wall straight, SI 14.85, SD 314.55 ..... *C. media*
19. Leaves glabrous, veins not winged, epidermal cell wall rounded, SI 13.51, SD 250.91 ..... *C. odorata*
20. Plants almost glabrous, stomata para or tetracytic 21
20. Plants hairy, stomata paracytic 22
21. Stomata paracytic, subsidiaries 2 distinct, epidermal cell wall undulate ..... *C. candelabrum*
21. Stomata tetracytic, subsidiaries 4 indistinct, epidermal cell wall rounded ..... *C. elegans*
22. Epidermal cell wall rounded / straight, subsidiaries distinct 23
22. Epidermal cell wall undulate, subsidiaries distinct / indistinct 24
23. Subsidiaries 2, epidermal cell wall straight ..... *C. fantastica*
23. Subsidiaries 2–4, epidermal cell wall rounded 25
25. Plants densely hirsute, SI 11.88, SD 152.73 ..... *C. hirsuta*
25. Plants sparsely hirsute, especially on leaves, SI 14.76, SD 230.91 ..... *C. oculata*
24. Subsidiaries 2 distinct ..... *C. evansii*
24. Subsidiaries 2–3 indistinct ..... *C. vincifolia*

lower surface of leaf is an adaptation to reduce the rate of transpiration. It is interesting to note that except *C. candelabrum, C. elegans*, and *C. juncea*, all the taxa have tubers and their vegetative growth is confined to the rainy season only, but still they have hypostomatic leaves. This is an interesting fact and raises further curiosities towards the taxa. The taxa *C. candelabrum, C. elegans*, and *C. juncea* generally remain in their active growth during summer season also and therefore their hypostomatic nature is justified, but for other taxa which grow in high rainfall area and escape the dry spell, especially about 8–9 months of year except rainy season and still have the hypostomatic leaves. This fact is interesting and the hypostomatic leaves must be a qualitative character governed by genes only; because in the present study beside *C. bulbosa* three more taxa, viz., *C. candelabrum, C. elegans* and *C. juncea*, have succulent leaves but have only hypostomatic nature unlike *C. bulbosa*.

Table 4. List of characters and character state used for phylogenetic analysis.

	Character with character state
1	Tuber: Absent (0), Present (1)
2	Stem erect: Absent (0), Present (1)
3	Stem twining: Absent (0), Present (1)
4	Stem scandant: Absent (0), Present (1)
5	Stem: Non succulent (0), Succulent(1)
6	Leaves: Large (0), Minute (1)
7	Plant habit: Small (0), Robust (1)
8	Leaves ovate: Absent (0), Present (1)
9	Leaves lanceolate: Absent (0), Present (1)
10	Leaves linear: Absent (0), Present (1)
11	Leaves: Non succulent (0), Succulent (1)
12	Leaves: Non hairy (0), Hairy (1)
13	Leaf margin: Straight (0), Undulate (1)
14	Leaf veins winged on lower surface: Not winged (0), Winged (1)
15	Leaf: Amphi-stomatic (0), Hypo-stomatic (1)
16	Epidermal cell anticlinal wall, Straight: Absent (0), Present (1)
17	Epidermal cell anticlinal wall, Rounded: Absent (0), Present (1)
18	Epidermal cell anticlinal wall, Undulate: Absent (0), Present (1)
19	Stoma: Mixed types (0), Single type (1)
20	Stoma paracytic: Absent (0), Present (1)
21	Stoma anomocytic: Absent (0), Present (1)
22	Stoma tetracytic: Absent (0), Present (1)
23	Stoma isotricytic & tetracytic: Absent (0), Present (1)
24	Stoma paracytic & anomocytic: Absent (0), Present (1)
25	Subsidiary cells: Indistinct (0), Distinct (1)
26	Subsidiary cell number two: Absent (0), Present (1)
27	Subsidiary cell number four: Absent (0), Present (1)
28	Subsidiary cell number 2–3: Absent (0), Present (1)
29	Subsidiary cell number 3–4: Absent (0), Present (1)
30	Subsidiary cell number 4–5: Absent (0), Present (1)
31	Size of stoma 300–450 μ: Out of range (0), Within range (1)
32	Size of stoma 450–600 μ: Out of range (0), Within range (1)
33	Size of stoma 600–750 μ: Out of range (0), Within range (1)
34	Size of stoma 750–1100 μ: Out of range (0), Within range (1)
35	Stomatal index (SI) range value 8–11 : Out of range (0), Within range (1)
36	Stomatal index (SI) range value 11–14: Out of range (0), Within range (1)
37	Stomatal index (SI) range value 14–17: Out of range (0), Within range (1)
38	Stomatal index (SI) range value 17–22: Out of range (0), Within range (1)
39	Stomatal density (SD) range value <100: Out of range (0), Within range (1)
40	Stomatal density (SD) range value 100-200: Out of range (0), Within range (1)
41	Stomatal density (SD) range value 200-300: Out of range (0), Within range (1)
42	Stomatal density (SD) range value 300-400: Out of range (0), Within range (1)

The type of stomata is paracytic in most of the taxa (16 out of 26) under study. Rubiaceous type (paracytic) stomata are reported to be common feature of many genera of Asclepiadaceae (present Asclepiadoideae of Apocynaceae) (Metcalfe & Chalk 1950; Paliwal et al. 1980). The results of present study showed similar observations about the type of stomata in *Ceropegia*, but besides paracytic stomata, tetracytic, anomocytic and mixed stomata of isotricytic + tetracytic, & paracytic + anomocytic are also found. Thus, the variations of type of stomata occur in the genus.

The results revealed that, mean value of stomatal index for the taxa under study, i.e., experimental mean is 14.75±0.35. The highest SI is found in *C. attenuata* var. *attenuata* while the lowest value for SI is observed in *C. juncea* (8.18±0.37). The mean value for stomatal density/ mm^2 of leaf area is 252.31±8.50 with the highest value of 403.64±10.60 in *C. maharashtrensis* and the lowest 98.18±3.40 in *C. juncea.* There is no correlation observed among the taxa with respect to SI values, but for SD the taxa occurring in the dry climates and with succulent habits have relatively lower values for the parameter. The SI remain unchanged even in different seasons in soybean cultivars (Rahangdale 2003) suggesting true genetic nature of this parameter. The SD is the lowest in *C. juncea* a succulent taxon with highly reduced leaves and increasing to some extent in *C. bulbosa* var. *lushii*, *C. bulbosa* var. *bulbosa*, and *C. hirsuta*. Low SD helps these taxa to retain more water in plant body and survive during dry spell of the year. On the other hand, taxa growing under high rainfall conditions, viz., *C. attenuata, C. anantii, C. jainii, C. media, C. mahabalei, C. anjanerica*, and *C. maharashtrensis*, have high stomatal density ranging from 325 to 403 stomata/mm^2, thereby adapting to the high annual rainfall of about 2,000–4,000 mm. It is interesting to note that, *C. concanensis* found in the Konkan region, on lateritic rock plateaux with very negligible soil have intermediate value of stomatal density. This is in accordance with it's relatively broader leaves and habitat because the rain water never remain on the sloppy plateaux. Such plateaux show relatively dry conditions as compared to the little more soil rich habitats where *C. anantii* and *C. attenuata* occur.

Present study revealed that on the basis of micro-morphological characters, the taxa with overlapping vegetative characters can be easily identified. The type of stomata, number and nature of subsidiary cells and anticlinal wall pattern are important characters to differentiate between very closely allied taxa at species as well as infraspecific ranks. This has been shown in an artificial key as well as the morphological phylogeny

Table 5. Character state of taxa under study (Character no. as per Table 4).

Sno	Character No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
	Taxa																																										
1	*C. anantii*	1	1	0	0	0	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	1
2	*C. anjanerica*	1	1	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	1	1	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	1
3	*C. attenuata* var. *attenuata*	1	1	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0
4	*C. attenuata* var. *mookambikae*	1	1	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1
5	*C. bulbosa* var. *bulbosa*	1	0	1	0	1	0	1	1	0	0	1	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0
6	*C. bulbosa* var. *lushii*	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0
7	*C. candelabrum*	0	0	1	0	1	0	1	1	0	0	1	0	0	0	1	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0
8	*C. concanensis*	1	1	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0
9	*C. elegans*	0	0	1	0	1	0	1	1	0	0	1	0	0	0	1	0	1	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0
10	*C. evansii*	1	0	1	0	0	0	1	1	0	0	0	1	0	0	1	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0
11	*C. fantastica*	1	0	1	0	0	0	1	1	0	0	0	1	0	0	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0
12	*C. hirsuta*	1	0	1	0	0	0	1	1	0	0	0	1	0	0	1	0	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0
13	*C. jainii*	1	1	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	1	1	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	1
14	*C. juncea*	0	0	1	0	1	1	1	0	1	0	1	0	0	0	1	1	0	0	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0	0
15	*C. lawii*	1	1	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0
16	*C. maccannii*	1	1	0	0	0	0	1	1	0	0	0	1	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0
17	*C. mahabalei* var. *hemalatae*	1	0	0	1	0	0	1	0	0	1	0	1	1	0	1	0	1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1
18	*C. mahabalei* var. *mahabalei*	1	1	0	0	0	0	1	0	0	1	0	1	0	0	1	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1
19	*C. maharashtrensis*	1	1	0	0	0	0	1	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	1
20	*C. media*	1	0	1	0	0	0	1	0	1	0	0	1	0	1	1	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1
21	*C. oculata*	1	0	1	0	0	0	1	1	0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0
22	*C. odorata*	1	0	1	0	0	0	1	0	1	0	0	1	0	0	1	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0
23	*C. panchganiensis*	1	1	0	0	0	0	1	1	0	0	0	1	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	1	0
24	*C. rollae*	1	1	0	0	0	0	1	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0
25	*C. sahyadrica*	1	1	0	0	0	0	1	1	0	0	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0
26	*C. vincifolia*	1	0	1	0	0	0	1	1	0	0	0	1	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	1	0	0

tree derived in the present study. These results are in corroboration of the previous studies in different angiosperm taxa. Metcalfe & Chalk (1988) reported that stomatal index is independent of the changes in epidermal cell size brought by the environmental factors, and therefore, the SI is generally considered as a taxonomic character along with the others. Many of the works have considered SI as a strong taxonomic character to delimit the allied taxa of family Vitaceae (Patil & Patil 1983, 1984), in *Bauhinia* L. (Kotresha & Seetharam 1995), and *Cassia* L. species (Kotresha & Seetharam 2000). The leaf micromorphological characters are very significant to compare the fossil taxa with the allied species of extant taxa (Dilcher 1974). Doyle & Endress (2000) observed that, the morphological evidences overcome the weak molecular evidences while assigning the phylogeny of angiosperms. Kotresha & Seetharam (2000) studied epidermal micromorphology of *Cassia* L. species and delimited the species within the genus on the basis of stomatal index, size, and epidermal cell wall structure (especially the anticlinal wall pattern) and the type of stomata. Thus, stomatal characters and micro-morphology can play a very significant role in resolving the taxonomic ambiguities. Present study is also showing similar outcomes giving significant information in delimitation of *Ceropegia* species and varieties on the basis of micromorphological characters. For example, two varieties of *C. mahabalei*, viz., var. *mahabalei* and var. *hemalatae*, are different with respect to many characters including stem scandant, undulate leaf margins, 2–4 indistinct subsidiaries and rounded anticlinal walls of epidermal cells in latter, whereas var. *mahabalei* have erect stout stem, straight leaf margins, two distinct subsidiaries, and undulate epidermal anticlinal walls. These characters are other than the characters which are provided in original protologue. Variety *hemalatae* does not match in any characters with *C. oculata*. Similar is the case of varieties of *C. attenuata*, viz., var. *attenuata* and var. *mookambikae*. The later variety has paracytic stomata with two distinct subsidiaries and SI 15.05, while the variety *attenuata* has mixed stomata of isotricytic and tetracytic types with 3–4 indistinct subsidiaries stomatal index 21.80. As per the key provided in the present study variety *mookambikae* is more allied to *C. concanensis* than with *C. attenuata* var. *attenuata*.

The consensus phylogenetic tree has three clusters. The type of stomata is defining character for these three clusters and supported by the number & nature of subsidiary cells. Cluster I is having anomocytic and mixed type of stomata with indistinct variable number of (two/three/four/five) subsidiary cells. All the taxa in this cluster are erect ones. Cluster II comprises total 15 taxa having paracytic stomata. This cluster further has two sub-clusters. Sub-cluster IIa comprises eight taxa having two distinct subsidiaries in all. From the dendrogram it is observed that the taxa, *C. evansii, C. fantastica, C. oculata*, and *C. lawii* are closely related having unit distance of 0.2–0.4. Along these *C. candelabrum* is separated from others on the basis of having fascicled roots and undulate anticlinal epidermal walls. Sub-cluster IIb comprises seven taxa having indistinct and variable subsidiaries. *C. mahabalei* var. *hemalatae* is separated from others on the basis of scandant habit, undulate leaf margins and high SI value. The cluster III is an externally linked group has four taxa clustered with unit distance of 0.2–0.8. It comprised two varieties of *C. bulbosa*, *C. elegans*, and *C. juncea*. This is the group of taxa having succulent stems and leaves. Among them *C. juncea* is placed as distinct taxon as it has highly reduced leaves. Among these *C. elegans* has tetracytic stomata while others have paracytic ones and therefore, it is placed little distantly. The dendrogram depicts clear differences among related taxa on the basis of micromorphology, especially the type of stomata, anticlinal wall pattern, SI and supported by gross morphology. In the present study, the grouping of *C. maccannii*, *C. panchganiensis*, *C. sahyadrica*, and *C. rollae* in first cluster, as well as *C. anjanerica*, *C. mahabalei*, *C. jainii*, *C. media*, and *C. odorata* in second cluster is in corroboration with the molecular phylogeny done by Surveswaran et al. (2009) where these taxa were placed in two separate clusters.

It is necessary to discuss here the delimitation of some of the taxa in present study. *C. mahabalei* var. *mahabalei* and *C. mahabalei* var. *hemalatae* are distinct in gross morphology of stem, leaves, inflorescence and the corolla beak, in a similar way they also differ in having indistinct 2–4 subsidiaries, rounded anticlinal walls and higher SI in the later variety. In the dendrogram they are also placed apart from each other on the basis of above characters. Similarly, two varieties of *C. attenuata*, viz., var. *attenuata* and var. *mookambikae*, are also placed distantly in the dendrogram in two different clusters. This is because of the type of stomata, number & characters of subsidiaries, size of stomata and SI. The most confusing taxa in terms of vegetative and floral morphology are *C. rollae* and *C. lawii*, as they are morphologically very similar to each other. Results of the present study separated them in the dendrogram as well as in the identification key on the basis of type of stomata, number and feature of subsidiaries, anticlinal wall pattern, and size of stomata. Thus, their identity can be confirmed on the basis of micro-morphology even in vegetative state. Similarly,

all the above mentioned taxa are separated on the basis of micro-morphological characters in the artificial key also. Thus, micro-morphological characters when combined with the gross morphological characters give better opportunity for correct identification of the taxa. The inter-specific variations in the stomatal characters such as stomatal index and stomatal frequency in *Vitis* L., *Cissus* L., and *Leea* D. Royen ex L. were studied by Patil & Patil (1984) and the taxa within these three genera were delimited on the basis of the stomatal characters and relationship among them was also established. Stomatal size and frequency was used to delimit between 56 species of genera of family Vitaceae by Patil & Patil (1983) and opined that, the stomatal data is supporting other characters to delimit the species and also to establish phylogenetic relationship to some extent. The results of present study show a similar trend in the genus *Ceropegia*.

Therefore, on the basis of studied micromorphological characters combined with other vegetative characters 26 taxa of *Ceropegia* at species and infraspecific level are delimited and an identification key is being provided for easy identification. These characters provide a very easy way of identification even in absence of flowers of these taxa.

CONCLUSION

The micro-morphology of taxa in the genus *Ceropegia* L. is revealed and is determinative for the identification of taxa even in a vegetative state in combination with the other general morphological characters. The type of stomata, number and nature of subsidiary cells, anticlinal epidermal wall pattern and stomatal index are key characters for differentiation of the taxa. Besides paracytic stomata, anomocytic, tetracytic and mixed, isotricytic + tetracytic and paracytic + anomocytic stomata are found in the genus *Ceropegia*. These characters are crucial in correct identification of the taxa at species and infraspecific level. Occurrence of tetracytic and mixed stomata is a first report in the genus by this study. It is also confirmed that, *C. mahabalei* var. *hemalatae* and *C. attenuata* var. *mookambikae* are distinct taxa from their allied taxa, respectively.

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