

# *Murdannia vaginata* var. *glabrisepala* (Commelinaceae): a new report for India inferred from morphological and molecular data

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**Abstract:** *Murdannia vaginata* (L.) G.Brückn. var. *glabrisepala* Faden was originally described from Sri Lanka. Its occurrence in India is indicated in the protologue, but not firmly established. During a recent field survey in Kerala, India, this variety was collected, compared with its morphologically allied congener *Murdannia vaginata* (L.) G. Brückn. var. *vaginata*, and reported here as a new record for India. A detailed description, photo plates, information on flowering and fruiting, habitat, distribution, SEM data on seeds and conservation status are provided. A combined morphology and molecular-based study using the *rps16* intron, *rbcL*, and nuclear ribosomal internal transcribed spacer sequences confirmed its distinctiveness from the type variety.

**Keywords:** Chloroplast markers, Commelinales, Kerala, nrITS, *rbcL*, *rps16*.

## Introduction

*Murdannia* Royle is the fourth largest genus of Commelinaceae (subfamily Commelinoideae), with about 60 species worldwide (Pellegrini *et al.*, 2016; POWO, 2023). It is also one of the six genera in the family that has native species in both the Old World and the New World (Faden, 1998), with its centre of diversity in India (Ancy & Nampy, 2015) with about 32 species.

During fieldwork in Kakkayam, Kozhikode district, in northern Kerala, as part of the ongoing taxonomic and molecular studies of *Murdannia* in India, we came across a population of *Murdannia*, morphologically akin to *Murdannia vaginata* (L.) G.Brückn. After a critical examination of the specimen with relevant literature, including type specimens (Faden, 2000, 2001; Ancy &

Nampy, 2015), it was identified as *M. vaginata* (L.) G.Brückn. var. *glabrisepala* Faden, a variety originally described from Sri Lanka. Even though, its occurrence in India is indicated in the protologue, it has not been firmly established with voucher materials. Sequence data from the *rps16* intron, and *rbcL* gene and nuclear ribosomal internal transcribed spacer (nrITS) DNA regions confirmed its distinctiveness from the type variety and phylogenetic relationships. A detailed description, photo plates and information based on SEM of seeds are also provided.

## Materials and Methods

**Sample collection and identification:** Field surveys were conducted in different parts of India for collecting fresh samples of the ingroup and outgroup taxa during 2018–2022 (except *Commelina benghalensis* L.) (Table 1). Photographs of *Murdannia vaginata* var. *glabrisepala* Faden were taken with a Stemi 508 stereomicroscope (Zeiss, Oberkochen, Germany) outfitted with an Axiocam 105 colour camera (Zeiss). Specimens were processed following Bridson and Forman (1991) and deposited in the Calicut University herbarium (CALI). The identity of the varieties was confirmed by consulting types, protologues and online herbarium digital sources (AAU, B, BM, C, E, F, G, GH, K, L, MO, NSW, P, PDA, TI, and US). A detailed description of *Murdannia vaginata* var. *glabrisepala* was prepared by examining freshly collected specimens. For SEM analysis, dried seeds were directly fixed to aluminium stubs and coated with 10–15 nm of gold with an SC7620 mini sputter coater (Emitech Quorum, PA, USA). The samples were examined with a Gemini 300 field emission scanning electron microscope (Zeiss) and images captured.

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*Ingroup and outgroup sampling:* Silica-dried leaf samples were collected in the field from two different populations for *Murdannia vaginata* (L.) G.Brückn. var. *vaginata* and from one population for var. *glabrisepala*. One sample from each of 10 *Murdannia* species [*M. blumei* (Hassk.) Brenan, *M. dimorpha* (Dalzell) G.Brückn., *M. edulis* (Stokes) Faden, *M. japonica* (Thunb.) Faden, *M. loriformis* (Hassk.) R.S.Rao & Kammathy, *M. nudiflora* (L.) Brenan, *M. semiteres* (Dalzell) Santapau, *M. simplex* (Vahl) Brenan, *M. spirata* (L.) G.Brückn. and *M. versicolor* (Dalzell) G.Brückn.], representing all sections in the genus, were added to complete the ingroup for the molecular analysis, totalling 14 samples of 11 out of c. 32 species occurring in India. According to Lee *et al.* (2022), *Floscopa* Lour. is the nearest genus to *Murdannia* and together with two further samples of tribe Commelineae (*Commelina benghalensis* L. and *Rhopalephora scaberrima* (Blume) Faden) were chosen as outgroups, the last to root the trees. Sequences of all taxa (except *Commelina benghalensis*) were newly generated as per procedure detailed below. The GenBank accession numbers used in this study are listed in Appendix S1 (as supporting information).

*DNA extraction, PCR amplification and sequencing:* Total DNA was extracted from silica-dried leaf tissue according to the modified CTAB method of Doyle and Doyle (1987). The nrITS region (c. 737 bp) was amplified using the primers ITS 5P and ITS 8P (Möller & Cronk, 1997), the *rps16* intron (c. 1010 bp) with *rpsF* and *rpsR2* (Oxelmann *et al.*, 1997) and *rbcL* gene (c. 629 bp) by *rbcLF* and *rbcLR* (Terachi *et al.*, 1987). PCR amplification was performed in 25 µl reactions in a mixture of 15.5 µl of distilled water, 2.5 µl of 10X buffer with MgCl<sub>2</sub> (25 mM), 2 µl of dNTP mix, 1.25 µl of DMSO, 0.25 µl of Taq polymerase with TaKaRa Taq polymerase (TaKaRa Bio Inc.), 1.25 µl of each forward and reverse primer and 1 µl of genomic DNA. The PCR profile for nrITS included an initial denaturation for 5 min at 96°C followed by 35 cycles of denaturation at 96°C for 45 s, annealing at 55.8°C for 45 s, and extension at 72°C for 1 min, followed by 10 min of final extension at 72°C. For *rps16*, the PCR profile included an initial denaturation for 4 min at 94°C followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 58.5°C for 30 s, and extension at 72°C for 1 min, followed by 4 min of final extension at 72°C. For *rbcL*, the PCR profile included an initial denaturation for 5

min at 95°C followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min, followed by 5 min of final extension at 72°C. Amplified PCR products were purified using Exosap-IT PCR Product cleanup reagent (GE Healthcare, Cleveland, USA) and the purified PCR fragments were Sanger cycle sequenced using the same forward and reverse primers used for PCR amplification using the Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, USA), following the manufacturer's protocol. The forward and reverse electropherograms were carefully checked, and sequences assembled using Sequencher v.4.14 (<https://sequencher.software.informer.com/4.1/>).

### Phylogenetic analysis

The DNA sequences obtained were subjected to a search in Basic Local Alignment Search Tool (BLAST) against the GenBank nucleotide database to test for contamination and to confirm the targeted marker. Alignments were made using MUSCLE embedded in Mega v.11 (Tamura *et al.*, 2021). The nrITS, *rps16* intron and *rbcL* gene sequences were combined and analysed. The combinability of the three datasets was determined by the incongruence length difference (ILD) test of Farris *et al.* (1995) implemented in PAUP v.4.0a (Swofford, 2003) as Partition homogeneity test using heuristic search, on 100 replicates of repartitioning with TBR (Tree Branching Reconnection) as branch swapping algorithm and MulTrees on. All characters were given equal weight, and gaps were treated as missing data. The concatenated sequences of nrITS and chloroplast *rbcL* gene and *rps16* intron alignments were subjected to Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. The ML analysis was performed in RAxML v.8.2.12 (Stamatakis, 2014), implemented in raxmlGUI v.2 (Edler *et al.*, 2021), with a selection of ML + rapid bootstrapping and support assessment using 1000 rapid bootstraps. For the BI analysis, models and parameters priors were obtained independently for nrITS, *rps16*, *rbcL* and were GTR+I+G4, TPM3uf+I, and TrN+I, suggested by Akaike Information Criterion (AIC) estimated using jModeltest (Santorum *et al.*, 2014) embedded in RAxML GUI software. Two independent Markov Chain Monte Carlo (MCMC) runs with four chains each were performed in MrBayes v.3.2.7

Table 1. Material of *Murdannia* and outgroups used in the present study, with voucher information

Sl. No.	Species	Country	Locality	Collection	Deposited in
1	<i>Murdannia blumei</i> (Hassk.) Brenan	INDIA	North Lakhimpur, Assam	Sreekutty TK. & Santhosh Nampy 167953	CALI
2	<i>M. dimorpha</i> (Dalzell) G.Brückn.	INDIA	Madayippara, Kannur, Kerala	Sreekutty TK. & Santhosh Nampy 158924	CALI
3	<i>M. edulis</i> (Stokes) Faden	INDIA	Kuruva island, Waynad, Kerala	Sreekutty TK. & Santhosh Nampy 158911	CALI
4	<i>M. japonica</i> (Thunb.) Faden	INDIA	Malappuram, Kerala	Sreekutty TK. & Santhosh Nampy 158915	CALI
5	<i>M. loriformis</i> (Hassk.) R.S.Rao & Kammathy	INDIA	Sholayar, Trissur, Kerala	Sreekutty TK. & Santhosh Nampy 158989	CALI
6	<i>M. nudiflora</i> (L.) Brenan	INDIA	Maharashtra	Sreekutty TK. & Santhosh Nampy 158941	CALI
7	<i>M. semiteres</i> (Dalzell) Santapau	INDIA	Madayippara, Kannur, Kerala	Sreekutty TK. & Santhosh Nampy 158927	CALI
8	<i>M. simplex</i> (Vahl) Brenan	INDIA	Panchalimedu, Idukki, Kerala	Sreekutty TK. & Santhosh Nampy 167969	CALI
9	<i>M. spirata</i> (L.) G.Brückn.	INDIA	Maharashtra	Sreekutty TK. & Santhosh Nampy 158930	CALI
10	<i>M. vaginata</i> (L.) G.Brückn. var. <i>vaginata</i>	INDIA	CU Botanical garden, Malappuram, Kerala	Sreekutty TK. & Santhosh Nampy 158921	CALI
11	<i>M. vaginata</i> (L.) G.Brückn. var. <i>vaginata</i>	INDIA	Kakkayam, Kozhikode, Kerala	Sreekutty TK. & Santhosh Nampy 167998	CALI
12	<i>M. vaginata</i> (L.) G.Brückn. var. <i>glabrisepala</i> Faden	INDIA	Kakkayam, Kozhikode, Kerala	Sreekutty TK. & Santhosh Nampy 167995	CALI
13	<i>M. vaginata</i> (L.) G.Brückn. var. <i>glabrisepala</i> Faden	INDIA	Kakkayam, Kozhikode, Kerala	Sreekutty TK. & Santhosh Nampy 168000	CALI
14	<i>M. versicolor</i> (Dalzell) G.Brückn.	INDIA	Karol gave, Maharashtra	Sreekutty TK. & Santhosh Nampy 158962	CALI
15	<i>Floscopa scandens</i> Lour.	INDIA	Idukki, Kerala	Sreekutty TK. & Santhosh Nampy 190511	CALI
16	<i>Rhopalephora scaberrima</i> (Blume) Faden	INDIA	Malappuram, Kerala	Sreekutty TK. & Santhosh Nampy 190513	CALI
17	<i>Commelina benghalensis</i> L.	CHINA	Unknown	Unknown	Unknown

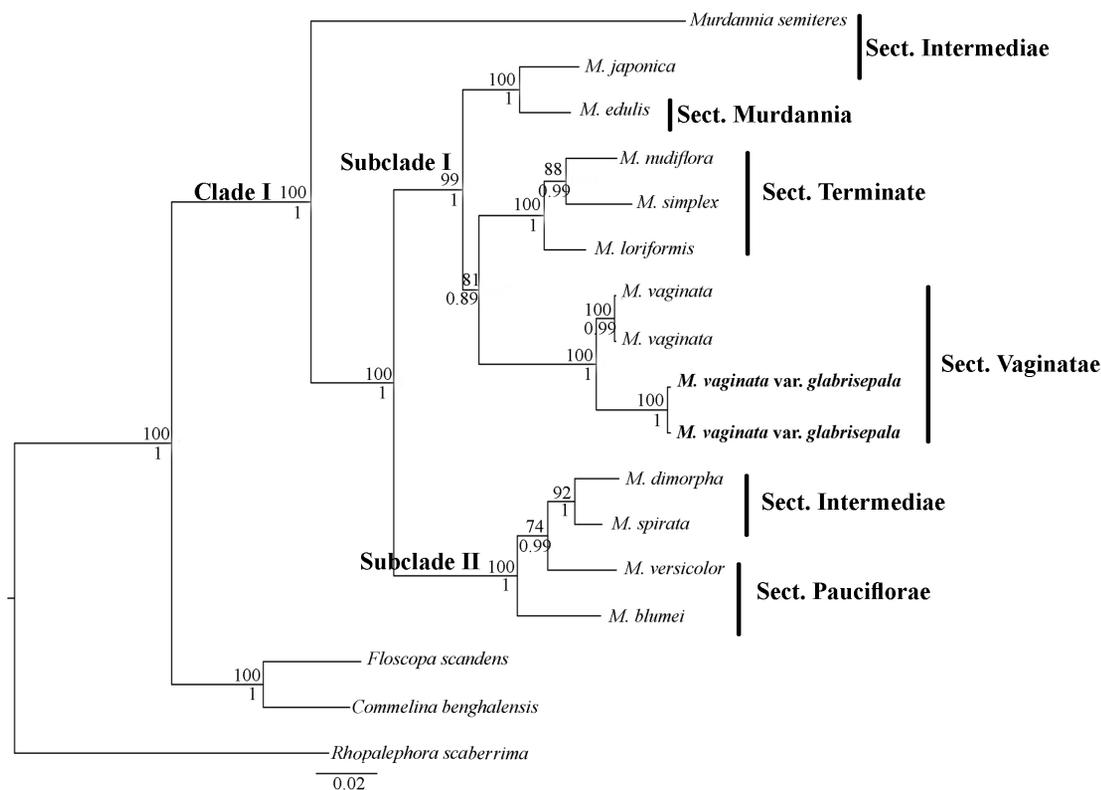


Fig. 1. *Maximum Likelihood* (ML) phylogram based on combined nrITS, *rps16* intron and *rbcL* gene regions, of 14 *Murdannia* Royle and three outgroup samples, rooted on *Rhopalephora scaberrima* (Blume) Faden. ML bootstrap percentages higher than 50% and Bayesian Inference posterior probabilities higher than 0.50 are shown above and below branches, respectively.

(Ronquist *et al.*, 2019) for 1,000,000 generations, sampled every 1000 generations. The output parameters were checked in Tracer v.1.6 (Rambaut *et al.*, 2014) to assess convergence. The first 25% of the sampled trees were discarded as burn-in and the rest were used to calculate the posterior probability. The MrBayes output was also checked to ensure that the standard deviation of split frequencies was below 0.01. The trees obtained from ML and Bayesian analyses were exported and viewed in FigTree v.1.4.3. (Rambaut, 2017).

## Results

### Sequence data and phylogenetic tree

The combined matrix of nuclear ribosomal ITS and chloroplast *rps16* and *rbcL* sequences had a length of 2377 characters. Of these, 1736 were constant, 281 parsimony uninformative variable characters and 360 parsimony informative characters. The partition homogeneity test indicated a high level of congruence between the three datasets ( $P = 0.9$ ) and the data was deemed combinable. The ML and BI tree topology retrieved after analysing the

combined nrITS+*rps16*+*rbcL* regions have the same tree topology (Fig. 4). The samples of *Murdannia* were monophyletic (Clade I: PP = 1.00, BS = 100). In this clade, *M. semiteres* was the first diverging lineage with maximum branch support (PP = 1.00, BS = 100) and the remaining species were separated into two subclades (Subclade I and Subclade II). Subclade I included *M. japonica*, *M. edulis*, *M. nudiflora*, *M. simplex*, *M. loriformis*, *M. vaginata* var. *vaginata* and *M. vaginata* var. *glabrisepala*. Subclade II included *M. spirata*, *M. dimorpha*, *M. versicolor* and *M. blumei*. The *Murdannia vaginata* samples formed a single clade (PP = 1.00, BS = 100) as sister to the clade with *M. nudiflora*, *M. loriformis* and *M. simplex* with medium support (PP = 0.89, BS = 81). The two samples of the *Murdannia vaginata* varieties each formed sister pairs with maximum and near maximum support (PP = 0.99–1, BS = 100), and formed sister clades with maximum support (PP = 1.00, BS = 100) (Fig. 1). The two varieties were separated by 7 and 10, 4 and 2, and 10 and 5 transitions and transversions in the aligned sequences of *rbcL*, *rps16*, and nrITS, respectively.

## Seed micromorphology

*Murdannia vaginata* (L.) G.Brückn. var. *vaginata*

Seeds, 1 per locule, 1.7–2.4 × 1.2–1.7 mm, broadly elliptic, cleft towards the embryotega, dorsiventrally compressed; testa light brown, scrobiculate with shallow furrows between the ridges, cells slightly tuberculate, outer cell layer partially sloughing off, not leaving a finely raised reticulum, hilum linear, longer than ½ the length of the seed, on a slightly raised ridge; embryotega dorsal, inconspicuous. (Fig. 2 a-d).

*Murdannia vaginata* (L.) G.Brückn. var. *glabrisepala*  
Faden

Seeds, 1 per locule, 2.2–2.6 × 1.7–1.9 mm, broadly elliptic, cleft towards the embryotega, dorsiventrally compressed; testa dark grey to black; scrobiculate with furrows between the ridges, cells alveolate to tuberculate, outer cell layer sloughing off, leaving a finely raised reticulum; hilum linear, c. ½ the length of the seed, on a raised ridge; embryotega dorsal, inconspicuous. (Fig. 2 e-h).

## Taxonomic Treatment

***Murdannia vaginata*** (L.) G.Brückn. var. ***glabrisepala*** Faden, Novon 11: 26. 2001, in Dassan., Rev. Handb. Fl. Ceylon 14: 162. 2000. *Type*: SRI LANKA, **Western Province**, Colombo district, Muthuraja Wela, Nugape, at the junction of road to Bopitiya and Kandana, N 7° 03', E 79° 51' 40", sea level, sedge marsh, 30.11.1976. R.B. Faden & A.J. Faden 76/419 (holo US barcode 00160794 digital image!).

Fig. 3

Annual herbs 5–30 cm tall. Stems erect to ascending, glabrous. Roots fibrous, from the base and rarely from the lower nodes touching the soil, to 1 mm thick. Leaves all cauline, distichous to spirally arranged; sheaths 0.5–1.5 cm long, green, maroon towards the base, glabrous with a line of cilia along the fused edges and mouth, clasping the stem; lamina linear-lanceolate, 3–7 × 0.3–0.6 cm, base truncate, entire, apex acute to acuminate, glabrous. Inflorescences terminal and axillary, 1–2 fascicles of 2–6 one-flowered cincinni enclosed in a prominently ribbed, persistent bract; bracts lanceolate, 1–1.5 × 0.1–0.2 cm, base rounded, entire, apex narrowly acute, glabrous; peduncles 5–15 cm long, narrow, erect, glabrous; cincinni 2-noded, 0.9–1.3 cm long, basal segment glabrous, middle segment minutely pubescent towards apex,

distal segment the pedicel. Flowers bisexual or staminate; pedicels 4–5 mm long, pubescent, erect in fruit. Sepals 3, lance-ovate, 4–5 × 1.2–1.5 mm at anthesis, 5–6 × 1.5 mm in fruit, free, base slightly rounded, margins entire, apex acute, glabrous on both surfaces, hyaline, pale green, tinted maroon at apex. Petals 3, c. 3 × 2 mm, free, middle petal rhombic and lateral petals elliptic, base acute to obtuse, margins entire, apex acute to obtuse, glabrous on both sides, lavender. Stamens 2, held closely parallel in the centre of the flower in bisexual flower, or symmetrical and divergent in the male flower, 2–2.2 mm long, antesealous; filaments free, violet, densely hairy towards base; anthers dorsifixed, anther sacs elliptic, bluish-brown, longitudinally dehiscent; pollen ellipsoid, yellow. Staminodes 4, 1 antesealous, 3 antepetalous, 1.8–2 mm long; filaments free, glabrous; antherodes hastate, yellow. Ovary 0.8–1 × 0.6–0.8 mm, widely elliptic, green; style c. 1.5 mm long, lavender-white, tip slightly curving to one side; stigma white or pale yellow, papillate. Capsules globose to sub-globose, 2.5–3 × 2.5–3 mm, persistent calyx exceeding the length and completely covering the capsule. Seeds 1 per locule, widely elliptic, 2.2–2.6 × 1.7–1.9 mm, cleft towards the embryotega; testa dark brown, scrobiculate with furrows between the ridges, cells alveolate to tuberculate, outer cell layer sloughing off, leaving a finely raised reticulum; hilum linear, c. ½ the length of the seed on a raised ridge; embryotega dorsal, inconspicuous.

*Flowering & fruiting*: October to December.

*Habitat*: The species grows in exposed, moist, sandy areas along with *Murdannia vaginata* var. *vaginata* (Commelinaceae), *Utricularia caerulea* L., *U. uliginosa* Vahl (Lentibulariaceae), *Eriocaulon* sp. (Eriocaulaceae) and some grasses (Fig. 4a).

*Distribution*: India and Sri Lanka (Fig. 4b). According to Faden (2001), *M. vaginata* var. *glabrisepala* is also found in India. However, the author provided no voucher specimen to support this statement. We have consulted specimens of *Murdannia* in different herbaria (see materials and methods) but could not find any specimens of *M. vaginata* var. *glabrisepala* from India. Ancy and Nampy (2015) and Nandikar and Gurav (2015) also did not mention its occurrence in India. Hence the present report forms an extended distribution of this taxon in India.

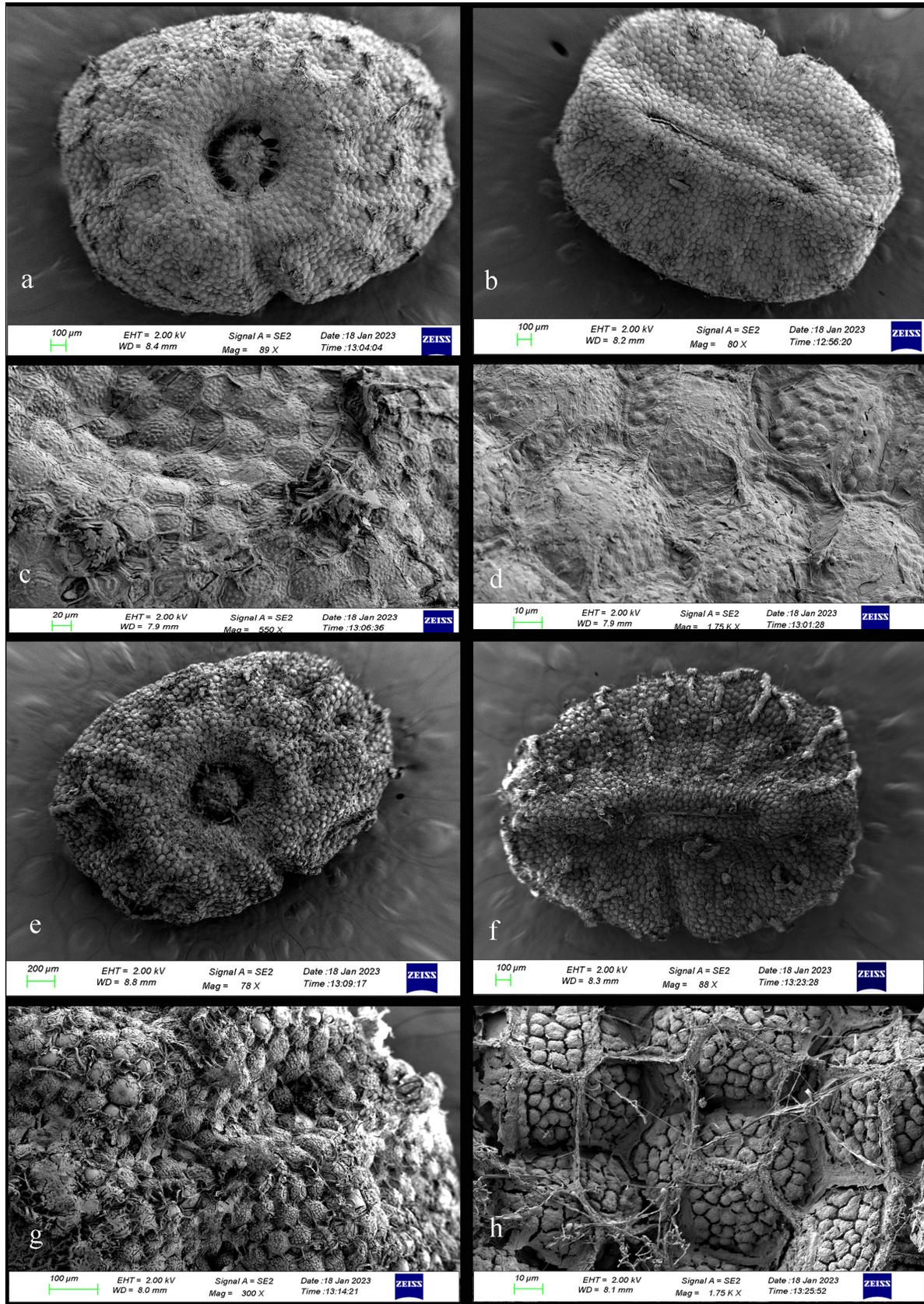


Fig. 2. SEM images of seeds: a–d. *Murdannia vaginata* (L.) G.Brückn. var. *vaginata*: a. dorsal view; b. ventral view; c & d. portions enlarged; e–h. *M. vaginata* (L.) G.Brückn. var. *glabrisepala* Faden: e. dorsal view; f. ventral view; g & h. portions enlarged.



Fig. 3. *Murdannia vaginata* (L.) G.Brückn. var. *glabrisepala* Faden: a. Habit; b. Flower; c. Petals; d. Sepal; e. Ribbed sheath; f. Stamens; g. Staminodes; h. Gynoecium; i. Capsules; j. Seed–dorsal view; k. Seed–ventral view (from Sreekutty T.K. & Santhosh Nampy 168000; photos by Sreekutty T.K.).

*Specimens examined*: INDIA, Kerala, Kozhikode district, Kakkayam, way to Urakkuzhi waterfalls, N 11°32'12.9", E 75°55'01.403"; 656 m, 06.10.2022, Sreekutty, Harishma, Krishnapriya & Santhosh Nampy, 167995 (CALI); *Ibid.*, 23.10.2022, Sreekutty TK. & Santhosh Nampy 167999, 168000 (CALI).

*Conservation status*: This variety is so far known only from four locations, one in South India (Kakkayam in Kozhikode district) and three (Karuvakkeni in Batticaloa, Galle and Pedcambra) in Sri Lanka (Faden, 2001). The Extent of Occurrence (EOO) is c. 85,500 km<sup>2</sup>, and the Area of Occupancy is 16 km<sup>2</sup>. The present locality in India is on the outskirts of the Malabar Wildlife Sanctuary, and we observed about 250 mature plants there. The habitat is under pressure from tourism and the grazing of animals, which may cause a decline in habitat quality and the number of mature individuals. Since, no information is available on the population size and threat status of this taxon for Sri Lanka, the assessment is done for India only. Because of the restricted distribution and known threat to the habitat in India, the variety is assessed here as Critically Endangered (CR), B2ab (iii,v), according to IUCN Red LIST Categories and Criteria (IUCN, 2012, 2022).

*Notes*: *Murdannia vaginata* is quite distinct among all other species of *Murdannia* in having a highly reduced inflorescence with fascicles of 1-flowered cincinni with two nodes and a persistent bract on the lower node enclosed in a prominently ribbed, glabrous, bladeless sheath, pubescent pedicels, and one seeded capsule locules. *Murdannia vaginata* var. *glabrisepala* differs from var. *vaginata* by its glabrous sepals, a persistent calyx that completely covers and exceeds the capsule, seeds with dark grey-black testa, scrobiculate with furrows between the ridges, cells of the testa alveolate to tuberculate, and outer cell layer sloughing off and leaving a finely raised reticulum, and hilum c. ½ the length of the seed, on a raised ridge. But in *M. vaginata* var. *vaginata*, the seeds are light brown, scrobiculate with shallow furrows between the ridges, cells slightly tuberculate, outer cell layer partially sloughing off, not leaving a finely raised reticulum, and hilum longer than ½ the length of the seed, on a slightly raised ridge (Fig. 3). Faden (2001) considered *M. vaginata* var. *glabrisepala* to be a perennial with relatively thick roots and smooth

to alveolate-reticulate seeds. Even though the roots are little bit thick in the present collection, the plants are annual and the seeds are scrobiculate with furrows between the ridges, with the cells of the testa being alveolate to tuberculate. According to Faden (2001), var. *vaginata* has only bisexual flowers and and stamens held closely parallel in the center of the flower while var. *glabrisepala* has andromonoecious flowers exhibiting enatiostyly. He further indicated that “enatiostyly [is] present or absent, according to the population”. However, we observed that, both varieties gathered from the same population have andromonoecious flowers and both grow and bloom together in the present location, and no intermediates were found.

## Discussion

A combined analysis of both morphological and molecular data was used for the first time to investigate the distinctiveness of *M. vaginata* var. *glabrisepala* from the type variety using multiple samples per taxon. The sister relationships of the samples of each variety and the great genetic distance between the varieties strongly indicates their separate taxon status.

According to Faden (2001), var. *glabrisepala* differs from var. *vaginata* by having thick roots, glabrous sepals, scrobiculate seeds having furrows between the ridges, cells alveolate to tuberculate, outer cell layer sloughing off, leaving a finely raised reticulum; hilum linear, c. ½ the length of the seed, on a raised ridge. The infrageneric classification in *Murdannia* was attempted by Brückner (1930), Hong (1974) and Faden (1980) based on the morphology of the inflorescence, recognizing five sections: sect. *Pauciflorae* G.Brückn. (few-flowered terminal and axillary cymes), sect. *Intermediae* G.Brückn. (terminal and axillary many-flowered cymes), sect. *Terminatae* G.Brückn. (terminal flowered cymes), sect. *Murdannia* and sect. *Vaginatae* Faden.

The present phylogenetic study, though only including 11 out of c. 60 species and one variety, included species from all these sections. Irrespective of the low number of species included, some comments on the sections can be made: In the resulting tree, sect. *Intermediae* was found not monophyletic, with *M. japonica* forming a sister pair with *M. edulis* of sect. *Murdannia* in Subclade I, with maximum support. The other

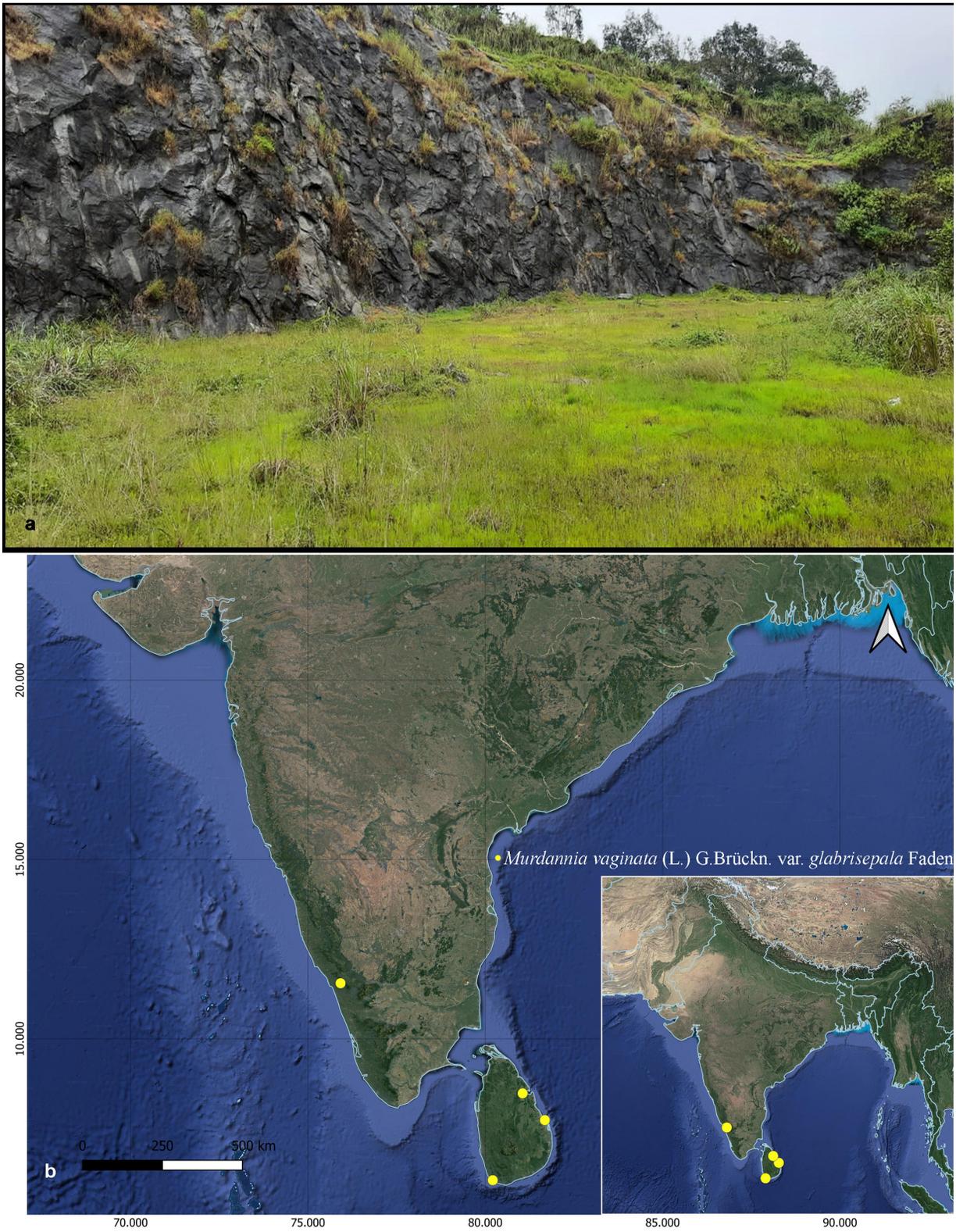


Fig. 4. *Murdannia vaginata* (L.) G.Brückn. var. *glabrisepala* Faden: a. Habitat; b. Distribution map, drawn with QGIS ver. 3.28.7 (QGIS, 2023).

members of sect. *Intermediae*, were very distant from these, *M. semiteres* on the first diverging lineage within the samples included, and the sister pair *M. dimorpha* and *M. spirata*, deeply nested in Subclade II. Whether this changes with the inclusion of additional samples needs to be tested. The other sections with more than one sample included were monophyletic, with sect. *Vaginatae* sister to sect. *Terminatae*. In sect. *Vaginatae*, *M. vaginata* var. *glabrisepala* appeared sister to *M. vaginata* var. *vaginata* with strong support values. The morphological analysis also revealed the distinctness of var. *glabrisepala* when compared to var. *vaginata*.

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#### SUPPORTING INFORMATION

Additional supporting Information may be found in the online version of this article at the publisher's web-site:  
Appendix S1: GenBank accession numbers.