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Registered Office: 3A2 Varadarajulu Nagar, FCI Road, Ganapathy, Coimbatore, Tamil Nadu 641006, India

Ph: +91 9385339863 | www.threatenedtaxa.org

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Cover: Life and death in one night - wolf hunting the hare. Mixed media—gouache, acrylics, pen & colour pencils. © Dupati Poojitha.



Morpho-anatomical characterization and conservation status of the Whisk Fern *Psilotum nudum* (L.) P.Beauv. (Polypodiopsida: Psilotaceae) from Cooch Behar District of West Bengal, India

Aninda Mandal 

Department of Botany, A.B.N. Seal College, Cooch Behar, West Bengal 736101, India.
mandal.aninda@gmail.com

Abstract: The district Cooch Behar of West Bengal, India is considered a unique zone for pteridophytes. The Whisk Fern *Psilotum nudum* (Family Psilotaceae), one of the primitive members of living vascular plants considering its age of evolution during the Devonian, is reported to occur in the Cooch Behar District. The present study has been designed to investigate the current conservation status as well as to describe the morphological and anatomical features of the epiphytic species. The present study revealed that the natural falling of old aged host trees and cleaning and white painting of host tree trunks lead to habitat destruction which may lead to extinction of the species from the studied areas. During the field visits, a new site of the occurrence (Madan Mohan Temple of Cooch Behar City) of the species has also been recorded. Plants are perennial and pendent in nature. The rhizome is achlorophyllous, freely branched, and covered with rhizoids. The aerial stem is up to 36 cm long, repeatedly dichotomously branched and chlorophyllous. Leaves are scalelike, without any midrib, lanceolate to ovate, and without ligules. Synangium are composed of three connected sporangia, bearing numerous bean-shaped spores. Stele in rhizome is haplostelic type; while, it is actinostele in the aerial stem. Morpho-anatomical description of the species has been documented for the first time from West Bengal and has enriched the botanical information of the species. Proper conservation of the taxa is utmost necessary in order to maintain their population in natural habitat.

Keywords: Anatomy, eastern indo-himalaya, ecology, epiphyte, habitat loss, living fossil, morphology, pteridophytes.

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Author details: ANINDA MANDAL is an Assistant Professor (W.B.E.S.) and Head in the Department of Botany, A.B.N. Seal College, Cooch Behar, West Bengal. He did his PhD from University of Kalyani, West Bengal. His field of interests includes pteridophytes, ecology, and ethnobotany, among others.

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INTRODUCTION

The genus *Psilotum* (belonging to the primitive family Psilotaceae), with its two Indian species, i.e., *P. nudum* (L.) P.Beauv. and *P. complantum* Sw. (Pande 2002; Chauhan et al. 2003; Muthukumar et al. 2009), is often grouped with the extinct psilophytes, the Rhyniales, and Zosterophyllales dating from the Devonian some 400 million years ago (Roux 2003). Many paleobotanists and plant morphologists considered the members under *Psilotum* as 'living fossils' or 'living rhyniophytes' (Kidston & Lang 1917; Banks 1975; Kaplan 1977; Wagner 1977; Rothwell 1999); but unfortunately, no fossils of psilophytes are known to exist. Recent morpho-anatomical studies (Bell & Hemsley 1992; Stewart & Rothwell 1993) suggest that the genus is not closely related to rhyniophytes; rather, the primitive characters present in living psilophytes represent a reduction from a more typical modern fern plant. Similarly, according to molecular data, it is evident that *Psilotum* is a fern and that psilophytes are sister to ophioglossoid ferns (Qiu & Palmer 1999; Pryer et al. 2001; Smith et al. 2006; Schneider et al. 2009).

Psilotum nudum (Greek—'psilos' means smooth or naked and Latin—'nudus' means naked, and both referring to the smooth and naked character of the stems; commonly known as whisk fern - Qiu & Palmer 1999), one of the two species in the genus *Psilotum*, is widely distributed across tropical and sub-tropical areas of both hemispheres (Zhang & Yatskiewych 2013). In India, the species is reported to be found in Andaman Islands, Nicobar Islands, Western Ghats, Eastern Ghats, central India, eastern India, northwestern India (Thothathri et al. 1970; Pande 2002; Chauhan et al. 2003; Valavan et al. 2016; Kanivalan & Rajendran 2017) with its first report in Barren Island, an isolated one in the Andaman group of islands (Prain 1893). In West Bengal, the species is reported to occur in Darjeeling (Pande 2002), Cooch Behar (Biswas 1956; Bandyopadhyay et al. 2006; Mandal 2023) and Jhargram (Sen & Bhakat 2021) districts. *P. nudum* has most recently been assessed for The IUCN Red List of Threatened Species in 2020 and is listed as 'Least Concern' (Bárrios & Copeland 2021).

The whisk fern became a popular pot plant in Japan during the Edo Period (1603–1867), leading to the selection of slightly over 100 ornamental garden varieties, most of which are illustrated in the 'Matsubaran fu' (Hoshizaki & Moran 2001; Chernova et al. 2020). The plant is also reported to be used for different medicinal purposes in India and Hawaii (Foster & Gifford 1974; Benjamin & Manickam 2007; Mannan et

al. 2008; Karthik et al 2011; Kumari et al. 2011; Revathi et al. 2013; Valavan et al. 2016, Mandal 2023).

Until now, several morpho-anatomical studies of *P. nudum* have been published from the different parts of the world (Ford 1904; Sporne 1962; Schulte et al. 1987; Nazarian et al. 2010; Vahdati et al. 2014; Rahman et al. 2015; Valavan et al. 2016); as per literature survey, detailed morpho-anatomical studies of the species from India especially from the state of West Bengal is lacking. Keeping all these in mind, the present study has been designed to investigate the present conservation status and to describe the morpho-anatomical characters of the species from the Cooch Behar District of West Bengal, India for the first time which will enrich the botanical information of the species. The current study will also report a new site of the occurrence of the species.

MATERIALS AND METHODS

The Cooch Behar District (Image 1), situated in the foothills of the eastern Indo-Himalaya, is known for its rich floristic composition. Field visits have been conducted across different seasons during January 2021 to September 2022 to observe the habitat and population growth pattern. The plant, *P. nudum* has been collected from the previously reported study area (Biswas 1956; Bandyopadhyay et al. 2006), i.e., Narendra Narayan Park (26.325 °N, 89.450 °E; elevation 14.5 m; Image 1), Cooch Behar for morphological and anatomical studies. Morphological description has also been done from the new site of occurrence of the species, i.e., Madan Mohan Temple (26.320 °N, 89.444 °E; elevation 14.5 m; Image 1), Cooch Behar based on the available plant specimens. Geographical locations of the study areas were recorded using the device GARMIN, eTrex® 22x. Anatomical studies have been performed through hand-sectioning of rhizome, aerial shoot of different orders and synangium. Slides were prepared after double staining and photographs were taken for photographic documentation. Spore morphology was examined under a light microscope (10X × 40X) and described as per Erdtman (1945, 1971). Identification of the plant specimen was made using relevant floras (Chinnock 1998; Diggs et al. 2006) and proper nomenclature was maintained following POWO (2022). Voucher specimens were deposited in the Department of Botany, A.B.N. Seal College, Cooch Behar.

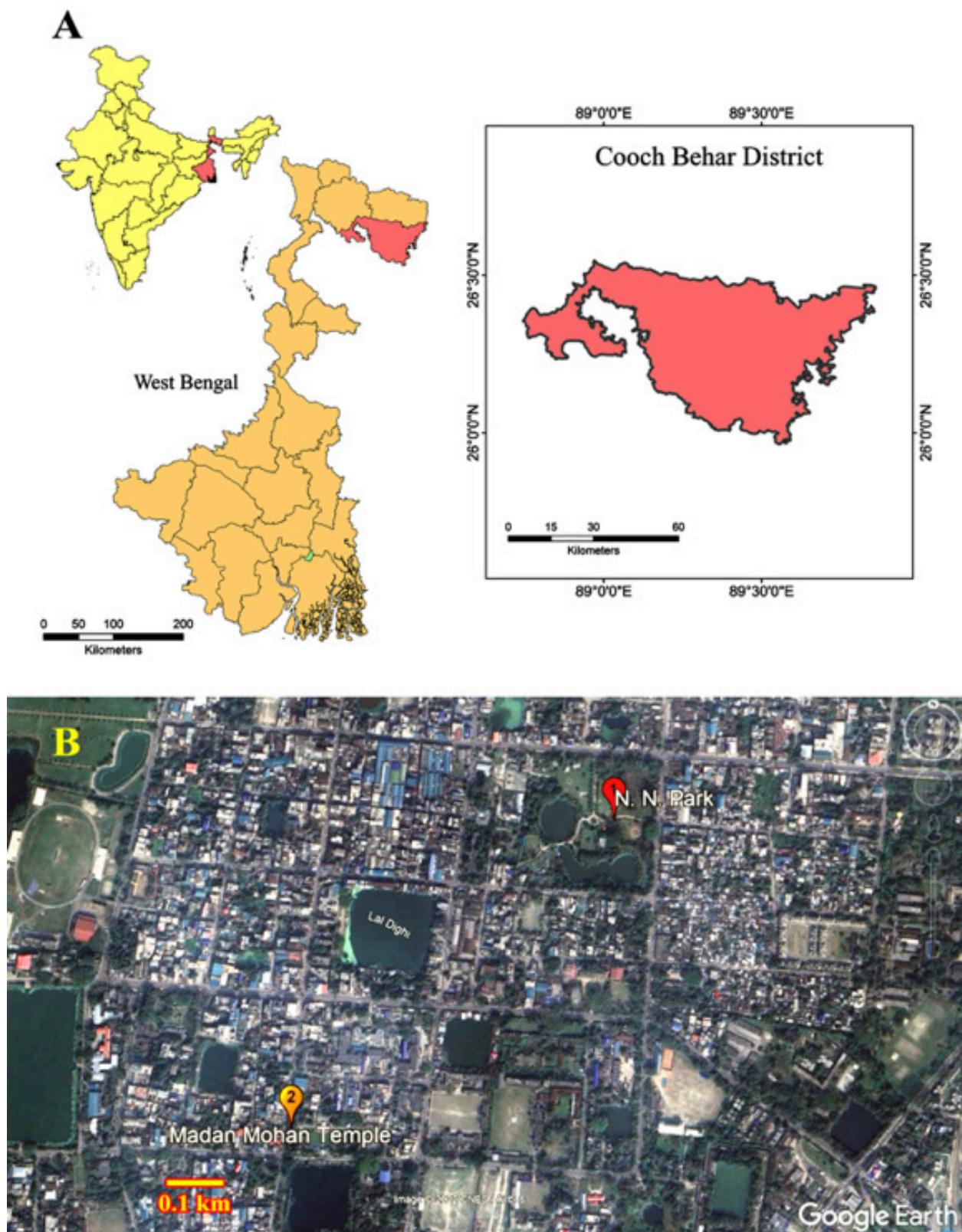


Image 1. A—Map of India and West Bengal highlighting the district Cooch Behar (red- orange color) | B—Study areas; Narendra Narayan Park (marked in red pin 1) & Madan Mohan Temple (marked in orange pin 2). Map prepared using an open source QGIS Desktop 3.22.

RESULTS AND DISCUSSION

Habitat, ecology and conservation status

The plant population of *Psilotum nudum* recorded from the new site of occurrence (Madan Mohan Temple) was found to grow on the tree trunk base (up to 1.5 m) of an old katgolap tree (*Plumeria* sp.) in association with *Drynaria quercifolia* (L.) J.Sm., *Pyrrosia lanceolata* (L.) Farw. and some species of orchids and mosses (Image 2). Approximately, 35 *Psilotum* plants were found to be grown in the tree trunk of which only a few with sporangium. The host tree is very old and eventually dying day by day as per the information provided by the gardener of the temple. Cleaning and white painting of the tree trunks of the temple before the ancient and traditional Rash Utsav is a very common practice for the beautification of the garden and may harm the concerned species and lead to loss from the site. Hence, in situ and ex situ conservation strategies should be taken to protect the species. The author made the gardener aware of the importance of the species and suggested barricading the tree and not to paint the tree trunk so that the species can grow in its natural habitat.

Biswas (1956) only reported a few plants of the species growing on *Polyalthia longifolia* along with an orchid (*Dendrobium* sp.) in the main park (now named Narendra Narayan Park) and predicted the species has been introduced from south India along with the orchid collection. Later on, a team from ENVIS, Botanical Survey of India, Kolkata visited the same park and reported that the species on the trunk of five very old trees (out of six trees) of *P. longifolia* in association with *D. quercifolia* and some orchid species (Bandyopadhyay et al. 2006). The present study revealed that approximately 15 plant species are found to be grown in the first tree; the second tree becomes dead and only the remnants of trunk remains without having *Psilotum* plant; the third tree also contains no *Psilotum* species as reported earlier; more than 50 plant species are found to be grown in the fourth tree (Image 2); there is no trace of the fifth tree and no plants are found to be grown on the tree number six, though the previous authors reported that many plants were found to be grown in fifth and sixth trees. From the current study it is clear that the plant population decreased day by day which may be due to the natural falling of host plants and or cleaning and painting of the tree trunks. The park authority made aware by the present author regarding the present status of this significant plant species in the park with a suggestion for taking necessary measures to protect this declining species.

Morphological description

Plants are perennial medium-sized herbs, that grow as an epiphyte and pendent in nature (Image 3). Rhizome is brown (achlorophyllous), cylindrical, slender, freely branched, 2.0–3.0 cm long, 1.5 mm in diameter, and covered with hair-like structures, called rhizoids. The rhizome merges above the surface with the aerial part of the plant. The aerial stem is 16–36 cm in long and 0.5–2.0 mm in diameter, repeatedly dichotomously branched above with 30–42 branches, often pentagonal towards the main stem, first and second dichotomy, quadrangular towards the third and fourth dichotomy and triangular in the most distal portions (fifth dichotomy), glabrous and green in color (chlorophyllous), with longitudinal parallel lines twisted slowly around the stem. Leaves are scale-like (Image 3), lacking a midrib, 1.5–2.0 mm long, spirally arranged on the stem, lanceolate to ovate in outline, pointed, and without any ligules. Synangia (Image 3) is 2.5–3.0 mm in diameter, orbicular, smooth, light green during young stages, become yellowish when mature and composed of three fused sporangia (Image 3) separated by three longitudinal and arched depressions, bearing numerous kidney or bean-shaped spores, a dichotomous bract is present around the synangium. Spores are homosporous, monad, bilaterally symmetrical, monolete, prolate to per-prolate in shape, large sized (polar axis: 76.68–88.52 μ m, equatorial diameter: 36.42–45.92 μ m), exine thick (3.26–4.98 μ m), exine ornamentation verrucate-tuberculate (Image 3).

Anatomical description

A model plant drawing of *P. nudum* has been displayed in the image (Image 3) to show the scheme of sampling sites for transverse sections (T.S.) of rhizome and aerial stem of different dichotomies.

Rhizome

The T.S. of the rhizome is more or less circular in shape (Image 3). Epidermis is thin, inconspicuous and single layered. Most of the epidermal cells extend into two celled absorptive rhizoids. Below the epidermal layer, there is a broad cortex of thin-walled cells. Stele is protostele (haplostele) in nature. Pith is absent and the xylem mass is usually chain-like / rod-like in outline and without any protoxylem. The xylem is surrounded by uniform layers of phloem which in turn is surrounded by the pericycle. Outside the pericycle, a distinguishable endodermal layer is present with conspicuous Caspary strips on radial walls. Vahdati et al. (2014) have also reported the presence of haplostele in the rhizome;



Image 2. Photographs of host plants bearing *Psilotum nudum*: A—Painted tree trunk of *Plumeria* sp. at Madan Mohan Temple | B—Tree trunk of *Polyalthia longifolia* at N.N. Park. © Aninda Mandal.

Nazarian et al. (2010) have reported the stellar type as actinostele.

Aerial stem

In T.S., the aerial stem appeared pentagonal towards the main stem (Image 3), first (Image 3) and second (Image 3) dichotomy, quadrangular towards the third (Image 3) and fourth (Image 3) dichotomy and triangular in the most distal portions (fifth dichotomy; Image 3). The aerial stem shows a well-marked single-layered cuticularized epidermis, and large numbers of stomata are found to be present in the grooves of the stem. The cortex is broad and is differentiated into three zones. The outermost zone is 3-layered, cells are thin-walled, vertically elongated with intercellular spaces, parenchymatous, nucleated, and contain chlorophyll. The middle cortex is 3–4 layered, and cells are thick-walled and sclerenchymatous. The inner cortex is 5–7 layered, cells are thin-walled, parenchymatous, and without any intercellular spaces. The stele is surrounded by well-marked endodermis.

Pericycle is not distinguishable. The stele in the aerial stem is of actinostele type. Xylem is star-shaped and the protoxylem lies at the tip of each ray. Presence of actinostele has also been reported earlier by different authors (Schulte et al. 1987; Khoshravesh et al. 2009; Vahdati et al. 2014); however, Gifford & Foster (1989) and Nazarian et al. (2010) interpreted the stele as siphonostele.

CONCLUSION

Psilotum nudum is one of the primitive vascular plants still living and often considered as 'living fossil'. In the present study, the species is found to grow as an epiphyte in the tree trunks of very old aged plants in the studied areas. The host trees are gradually falling down due to their very old age. Cleaning and sometimes white painting of host tree trunk for beautification of park and garden lead to habitat destruction. Continuous habitat loss may lead to extinction of the species from

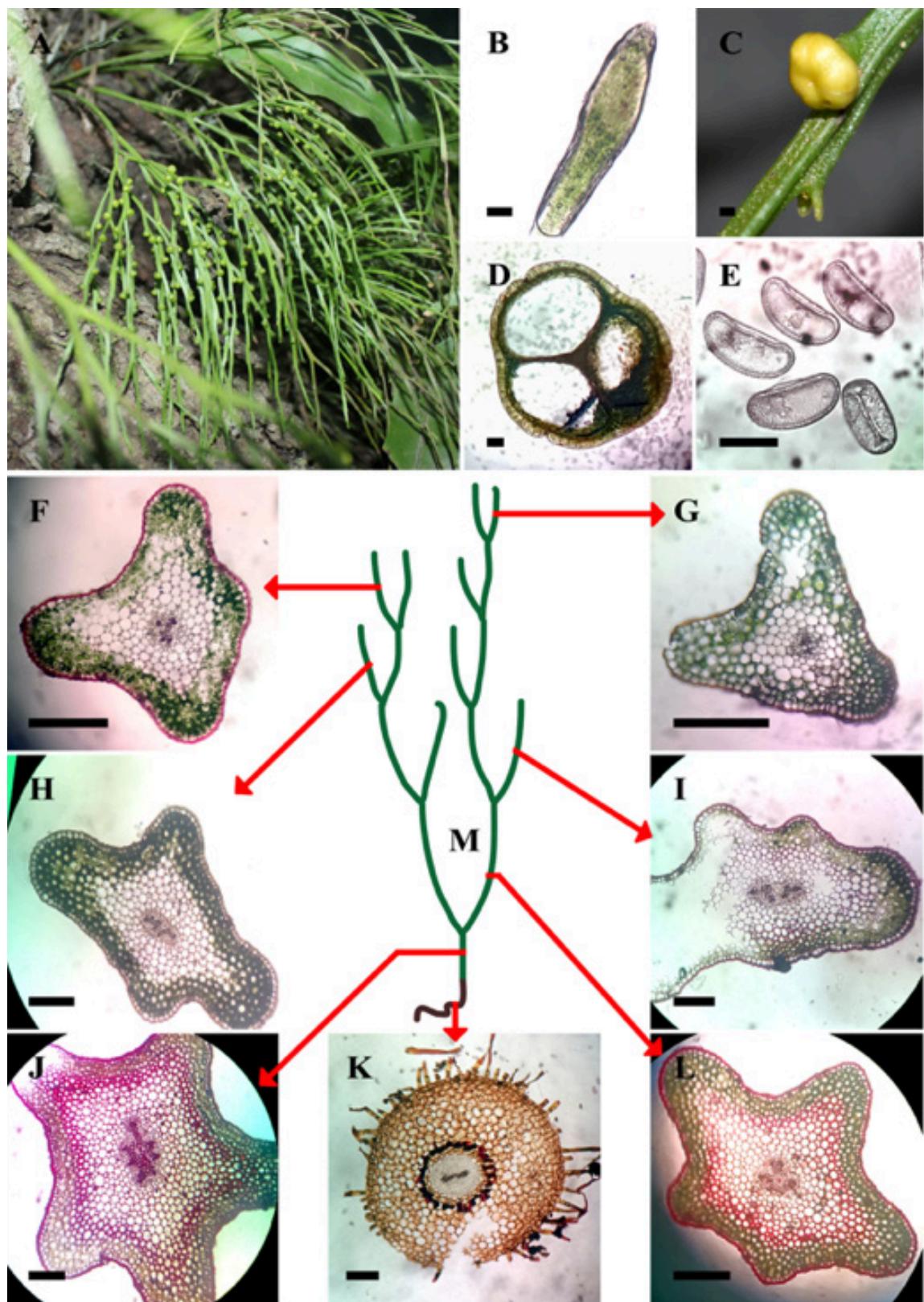


Image 3. Morpho-anatomy of *Psilotum nudum*: A—Sporophytic plant body growing on tree trunk | B—Scale leaf | C—Matured synangium | D—Transverse section of synangium with trilobed structure | E—Monolete spores | F—Fourth dichotomy | G—Fifth dichotomy | H—Third dichotomy | I—Second dichotomy | J—Transverse section of main stem | K—Transverse section of rhizome showing two-celled rhizoids | L—First dichotomy | M—Scheme of sampling sites on a typical model plant for transverse-sections of different dichotomies (Scale bar: B, D, F, G, H, I, J, K, L—0.25 mm, C—0.5 mm, E—50 μ m). © Aninda Mandal.

the studied areas. So, necessary measures should be taken immediately for in situ and ex situ conservation of the botanically interesting species. Detailed morpho-anatomical description of the species is also carried out for the first time from the district Cooch Behar of West Bengal, India which will enrich the botanical information of the species.

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