



COMPARATIVE ANATOMY AND SPORE MORPHOLOGY OF *PSILOTUM NUDUM* (PSILOACEAE) OF TWO DIFFERENT HABITATS IN ASSAM, INDIA

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Psilotum nudum a fern allies of the family Psilotaceae is found in two different habitats i.e. epiphyte and lithophyte. The present communication provided a comparative analysis of the anatomy and palynology between epiphytic and lithophytic habitats of *P. nudum* from Assam, India. Free hand transverse sections were made, following double staining technique, and permanent slides were prepared then examined under the microscopes. Scanning Electronic Microscopy (SEM) of the spores was conducted. The result shows similarity in the arrangement of cells in different vegetative parts in both habitats except minor differences in sizes. The presence of secondary metabolite i.e. phlobaphene in aerial stem is almost consistent, but the differences noted in its occurrence between the epiphytic and lithophytic rhizoids may reflect adaptive anatomical and chemical nature of the species. The spores are kidney shaped and similar in both habitats except minor differences in size. The study is expected to be useful to interpret the adaptive responses of epiphytic and lithophytic forms of *P. nudum* to different microhabitats.

Keywords: Assam, Fern allies, Habitats, Microscopy, Psilotaceae.

Introduction

The family Psilotaceae Kanitz is represented by only two genera¹ viz., *Psilotum* Swartz. and *Tmesipteris* Swartz. So far, there are two species known under *Psilotum* viz., *P. flaccidum* Hook. & Grev. and *P. nudum* (L.) Pal. Beauv²⁻⁴. Earlier, *P. complanatum* Sw. and *P. nudum* are considered as Near Threatened species in India⁵. However, a study⁶ recognized only two species globally viz. *P. nudum*, *P. flaccidum* Wall. ex Hook. & Grev. with a hybrid $P \times complanatum$. *Psilotum nudum* is widely distributed species⁶ and its preferable habitat are epiphytic or on rocks crevices⁷. During a study in Assam, India,

this species was also observed in two different habitats, one as lithophyte (Fig. 1 A-C) or in rocks crevices in association with *Drynaria quercifolia* (L.) J.Sm. in Dima Hasao district (collection no. *H. Roy 122*) and another as epiphyte (Fig. 1 D-F) on host plant *Ficus religiosa* L. trunk in association with *Dischidia bengalensis* Colebr. in Sibsagar district (collection no. *H. Roy 142*). The anatomy and spore morphology of this primitive species was studied by many authors across globally and reported significant differences^{6, 8-10}. However, there is no report to compare the anatomy and spore morphology of *P. nudum* occurring in different habitats from

Table 1: Comparative analysis of anatomy and spore morphology of both habitats (lithophyte and epiphyte).

Parts	Region	Lithophytic	Epiphytic
Stem	Outline	Round-triangular, lobed (Fig. 2 A-B)	Rectangular or irregular square (Fig. 2 F-H)
	Epidermis	1-layered, cells ovate, 21.6–39×37.8–50.4 μm , cuticulate	1-layered, cells round to ovate, 22–32×38.9–41.5 μm , cuticulate
	Stomata	Scattered, 37–45.4×39.3–34.5 μm	Scattered, ca. 46×43 μm
	Cortex	Outer (chlorophyllous), middle (sclerenchymatous) and inner (parenchymatous) regions	Same
	Outer cortex	2–4 layered, cells thick-walled, round-polygonal, 24–44×18.1–44.5 μm	2–3 layered, cells thick walled, hexangular-round, 30–74×27–64 μm
	Middle cortex	3–4 layered	3–4 layered
	Inner cortex	Cells thin walled, hexangular, 32–74×35–66 μm	Cells thin walled, round-hexangular, 58–90×32–70 μm
	Stele (protostele)	389–402×157–261.2 μm	ca. 388×380 μm
	Metaxylem	15–25×13–25 μm , hexangular	18–27×17–35 μm , hexangular
	Protoxylem	11–14×10–12 μm	16–19×10–11 μm
	Phloem	14–25×13–19 μm	15–22×12–19 μm
	Phlobaphene	Epidermis, stomata (Fig. 2 C), cortex (Fig. 2 B), and stele (Fig. 2 D)	Epidermis, hypodermis, cortex and stele
	Rhizoids	Colour	Dark brown-black
Outline		Spherical (Fig. 2 E)	Spherical (Fig. 2 I)
Epidermis		1-layered, cells ellipsoid, ovate, round, thin walled, 43.8–80.9×24.4–69 μm	1-layered, cells round, ellipsoid, ovate, thin walled, 29.2–41.8×23.3–50.3 μm
Cortex		Multi-layered, cells thick walled, ovate, round, ellipsoid, polygonal, 50.7–100.3×61.8–97.5 μm	Multi-layered, cells thin walled, rectangular to angular, 40–91×27–72 μm
Endodermis		1-layered	1-layered
Stele (protostele)		ca. 172.2×146.1 μm	ca. 206×228 μm
Metaxylems (diameter)		26–28 μm	16–25 μm
Phloem		18–20×12–15 μm	10–13×9.8–14 μm
Phlobaphene		Inner cortex (Fig. 2 E)	Inner cortex region, metaxylem and phloem
Spores	Type	Homosporous (Fig. 2 J), isopolar, elliptic, kidney shaped, one side constricted and grooved, monolete, bilaterally symmetrical	Same (Fig. 2 K)
	Shape class	Prolate (1.65)	Same
	Perispore	Densely folded without reticulation,	Same

	surface ornamentation verrucate	
Largest spore	62.12×34.95 μm	66.37×33.42 μm
Smallest spore	57.77×34.29 μm	47.19× 27.91 μm
Polar axis	60.11 ± 2.1	56.52 ± 9.4
Equatorial diameter	34.45 ± 0.52	32.35 ± 4.74



Figure 1: *P. nudum*: A-C. Habit and sporangia of lithophytic plants. D-F. Habit and sporangia of epiphytic plant.

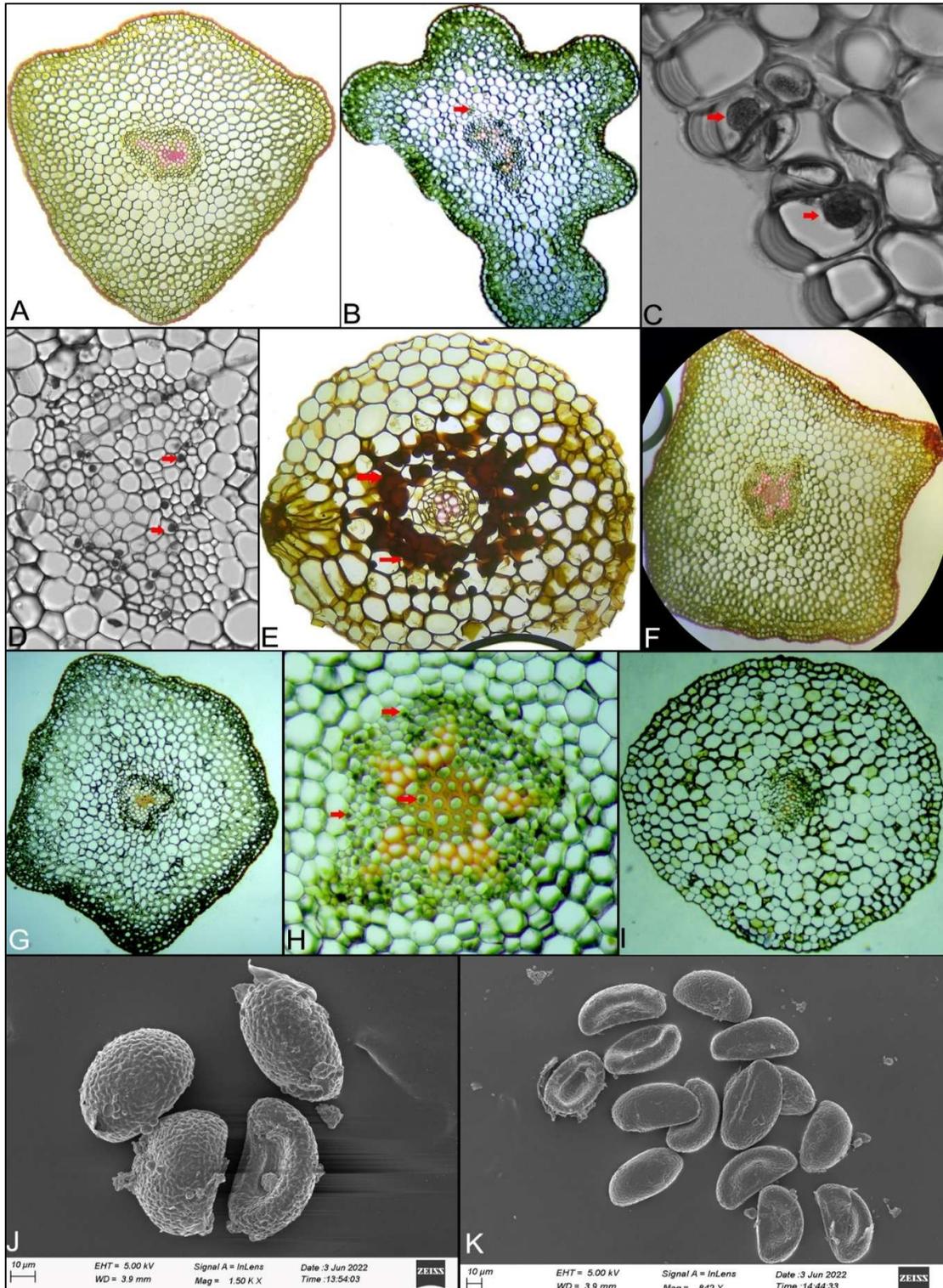


Figure 2: Lithophytic plants: A-B. Sections of aerial stem. C. Epidermis and stomata. D. Stele. E. Section of rhizoids. **Epiphytic plants:** F-G: Sections of aerial stem. H. Stele. I. Section of rhizoids. J. Spores of lithophytes. K. Spores of epiphytes. (arrow showing phlobaphene).

Assam, and the present study is an attempt to fill this gap.

Material and Methods

For the study, plant samples were collected during their reproductive stages. Vouchers representing both habitats are housed at Gauhati University Herbarium (GUBH). Fresh specimens were preserved in formalin for anatomical studies. The microscopic preparations of aerial stem and rhizoids of each collection were selected for free hand transverse sections (T.S.). Then the sections were double stained using safranin and fast green by passing through different alcoholic grades¹¹ and permanent slides were prepared by mounting the specimen in DPX. All anatomical slides are examined, measured and photographed under a phase contrast and fluorescence microscopes. The spores were analyzed under Scanning Electron Microscope (SEM).

Results and Discussion

The comparative anatomy and palynology of both habitats are provided in table 1. Earlier stem anatomy of the epiphytic form of the species was conducted from West Bengal¹⁰. Results of present study reveals similarities in aerial stem shape with the one reported from West Bengal¹⁰, but differs in protosteles shape. However, other differences have also been noted in the present study. This comparative study recorded differences in cell sizes, e.g. the cell of outer cortex is slightly larger in epiphytic stem, the epidermal cells of lithophytic rhizoids is comparatively larger, steles larger in epiphytic form etc. In previous literature, the aerial stem was recorded with large numbers of stomata mainly confined in grooves, and with actinostele and rhizoids with protosteles¹⁰; whereas, here, scattered stomata recorded throughout the stem of both habitats and protosteles found in aerial stem and rhizoids. One of the significant observations is the occurrences of phlobaphenes in stem and rhizoids. However, its deposition in aerial stem and

rhizoids was found comparatively more in lithophytic than the epiphytic plants. The variation in its occurrence in epiphytic versus lithophytic rhizoids suggests distinct adaptive anatomical and chemical strategies in response to different environmental conditions. Phlobaphenes are secondary metabolites and chemically an insoluble phenolic compound and its presence in maize varieties shows high resistance against fungal infection which also reduces the level of mycotoxin contamination¹². However, colonization of mycorrhizal fungi in cortex of the rhizome of *P. nudum* was also observed¹³. It indicates that, the cortical cells of the rhizome are prone to the fungal colonization; but it is interesting to note that, phlobaphenes are densely deposited in the inner cortical cells of studied samples (Fig. 2E) and did not observe any fungal colony. Deposition of phlobaphenes in aerial stem of *P. nudum* was not recorded earlier^{6,8,10,14}. The anatomy of aerial stem reveals all three distinct zones of the cortex i.e. outer chlorophyllous layers followed by sclerenchymatous layers, then inner parenchymatous layers which are found similar to other reports⁸. The spores of both habitats are kidney shaped and almost similar to each other regarding type, shape class and perispore etc. except minor size differences i.e. larger size in lithophytic form.

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