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Additional Notes on Spore Morphology of Two *Huperzia* (Lycopodiaceae) Species and Systematic Significance

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ABSTRACT

An additional note on spore morphology of two important species of tassel ferns viz. *Huperzia phlegmaria* (L.) Rothm. and *Huperzia squarrosa* (G. Forst.) Rothm. from Manipur (North East India) are presented and compared with the existing literatures. Some key spore parameters are newly highlighted through Scanning Electron Microscopic studies and presented elaborately in the present study. Spore in both the species are remarkably similar to one another and rather indistinguishable at first glance. They are triangular to sub-triangular in outline, trilete in nature with sides straight to somewhat concave margins. Distal spore surface shows foveolate ornamentation while proximal surfaces are unornamented in both the species. The two species differ in having more number of pits or depressions separated from one another between (0.5-1) μm with broader ridges of (5-7) μm diameter in *Huperzia phlegmaria* while the pits are sparsely distributed in between (0.5-3) μm with narrower ridges (>3 μm diameter) in *Huperzia squarrosa*. The undulations on distal surface in between the pits are more prominent in later species. In contradiction to earlier view of categorizing the two species under Phlegmaria (*H. phlegmaria*) and Selago (*H. squarrosa*) type, the present findings supported that both spores are assumed to be closely related to one another with common phylogeny and can be categorized as same spore type. The study also depicts the immense significance of sporal parameters in accurate circumscription of taxonomic status.

Key words: *Huperzia phlegmaria*, *Huperzia squarrosa*, spore morphology, additional notes, systematic significance

INTRODUCTION

Spore morphology serves as a vital source of systematic character to circumscribe sub generic groups or hypothetical relationships among species within a genus. It not only forms a unique entity with regard to their structure and function, but also serves as taxonomical markers in deducing phylogenetic relationship amongst species (Verma, 2000; Nath and Sharma, 2006). Though fern spores were known as early as sixth century, the pioneer of modern Pteridology and criteria in taxonomy and classification of ferns were introduced by Fee in mid nineteenth century. Sachs for the first time classified pteridophytes into homosporous and heterosporous condition (Devi, 1977). Holttum (1947), Tryon and Tryon (1982), Srivastava *et al.* (2001), Das and Dixit (2001) and Schneider and Pryer (2002) paid special attention in spore morphology.

One of the most significant works in the field of palynology was published by Erdtman (1945, 1957). In the framework of palynological study, Erdtman presented excellent illustrations of about 100 genera of pteridophytes. From here, there was no looking back and many authors have contributed much research in the field of spore morphology and its significance. The application of scanning electron microscopy to augment accurate knowledge on the ultra structure of pollens and spores for systematic and phylogenetic analysis were highlighted by many palynologists (Qureshi *et al.*, 2002a, b; Shehata, 2008; Alwadie, 2008; Fawzi *et al.*, 2010).

Huperzia Bernh. of Lycopodiaceae commonly known as tassel ferns, are epiphytes (rarely lithophytes) with pendent dichotomous stems. About 415 species are reported from the Tropics, Arctic and sub-Antarctic zones (Singh and Singh, 2010). Within the classification of Lycopodiaceae, there has been a great deal of scientific debate and until recent years, a consensus of botanical opinion on the group has not been completely resolved. Tassels have traditionally been included in the genus *Lycopodium* L., but the trend in recent treatments has been to place them in a separate genus (Wilce, 1965, 1972; Holub, 1985, 1991). Studying the long term generic problems within *Lycopodiales*, Holub (1985) reclassified 203 species of *Lycopodium* L. to *Huperzia* Bernh. The segregation of *Lycopodium* to *Huperzia* is mainly based on characteristic isodichotomous branching, growing habit, peculiar types of gametophytes and sporal morphology. The diverse opinion on different taxonomic treatments in *Lycopodium* reflects the differing perspectives with which systematics have approached. Of all the taxonomic criteria for circumscription of species level in lycopods, spore patterns often play an important role. The development of Scanning Electron Microscope (SEM) has helped in understanding the spore ornamentation and even helps to differentiate minute variation in a wider sense. Understanding the significance of spore morphology in classification of tassels, a detailed study on the development of exine ornamentation in *Huperzia phlegmaria* (L.) Rothm. and *Huperzia squarrosa* (G. Forst.) Rothm. was taken up and compared with the existing literature pertaining to *Huperzia* spores. These two tassel ferns are economically and traditionally important plants in the state Manipur (North East India). Hence, the present investigation would be worthwhile for the scientific community and also in profiling the species identity at higher level.

MATERIALS AND METHODS

Taxonomic surveys and direct field trips were conducted during March 2005 and December 2010 throughout the hilly terrain of Manipur state located in North East India. Mature sporangia of tassel ferns were collected in small paper packets and dried under natural condition. Spores were mounted and observed under Light Microscope (MIJY-Inverted) to study details like shape, size, colour and dehiscence. Size of spores was recorded by taking the mean average calculated from a minimum of 15 readings. Spore size is expressed as polar diameter \times equatorial diameter (μm). Palynological terminology by Devi (1977) and Punt *et al.* (2007) are used to describe the character of spores. Main spore revision and comparison of data with present findings is tabulated with reference to the work done by Wilce (1972). Scanning electron micrographs were taken from untreated and undamaged spores. Dried spores were fixed on brass stubs by electron-conductive point and were kept on the ion sputter and are coated with thin layer of gold vapour (300 Å layer). The specimen was then observed under the SEM. Morphological parameters are also incorporated. Voucher specimens of the plants were deposited in Herbarium, Department of Life Sciences, Manipur University (MU), India (Table 1).

Table 1: Locality and voucher specimens of tassels sampled in the present study

Species	Location	Voucher
<i>Huperzia phlegmaria</i> (L.) Rothm.	(Tamenglong) Manipur, India	Yumkham, 002139(MU)
<i>Huperzia squarrosa</i> (G. Forst.) Rothm.	(Ukhrul) Manipur, India	Yumkham, 002138(MU)

RESULTS

Spores of *Huperzia phlegmaria* and *H. squarrosa* were studied to assess their role in systematics. To supplement the taxonomic parameters, morphological descriptions are also studied critically. A key to the spore type is presented below:

- Foveolae with numerous pits, distance between (0.5-1) μm , average spore size (37 \times 35) μm *Huperzia phlegmaria*
- Foveolae with lesser pits, distance between (0.5-3) μm , average spore size (28 \times 29) μm *Huperzia squarrosa*

***Huperzia phlegmaria* (L.) Rothm. Fig. 1 (a-c), Fig. 2 (h-l):** *Huperzia phlegmaria* (L.) Rothm., Feddes Repert. Spec. Nov. Regni Veg. 54: 62. 1944.

Macromorphological characters: Plant differentiated into rhizomes and stem, shoots indeterminate (entire plant not dying after several years), rhizome short. Stem pendent, upto 80 cm long, rigid, branches dichotomous (Fig. 1a). Leaves triangular ovate, upto 1.5 \times 0.2 cm, spreading, dark green, glossy, texture sub-coriaceous, shortly petioled, distant, entire (Fig. 1c). Strobili borne at tip of branches, branch repeatedly dichotomous, narrow, flaccid, sessile, aggregated into spikes (Fig. 1b). Sporangium rounded, pear-shaped, distinguishable into stalk and capsule, pale yellow in colour, dehiscence by transverse slit at maturity (Fig. 2h).

Spore characters

Size and nature: (37 \times 35) μm , trilete, triangular, tetrahedral with angles never exceeding 130°, off-white in colour.

Distal surface: Foveolate ornamentation (pl., foveolae, adj., foveolate = feature of ornamentation consisting of more or less rounded depressions/ pits/ lumina not more than 2 μm in diameter). The distance between foveolae is greater than their breadth (Devi, 1977), diameter 0.3 μm , regular or irregular in outline, separated from one another between (0.5-1) μm , surface appear undulated around the pits due to depressions (Fig. 2j-l).

Proximal surface: Unornamented, pits absent, edges concave with prominent development of 3 laesura (pl., laesurae) in the form of ridges, never extending the margin, ridges broad, 5-7 μm across (Fig. 2i).

Ecological notes: Epiphytes found settling in dense forest, preferably sciophytes with low intensity of light, upto 3000 m altitude.

Common names: Lycopodium fern, Coarse tassel fern, Tassel fern.

Vernacular name: *Leishang-leiren* (Manipuri language).

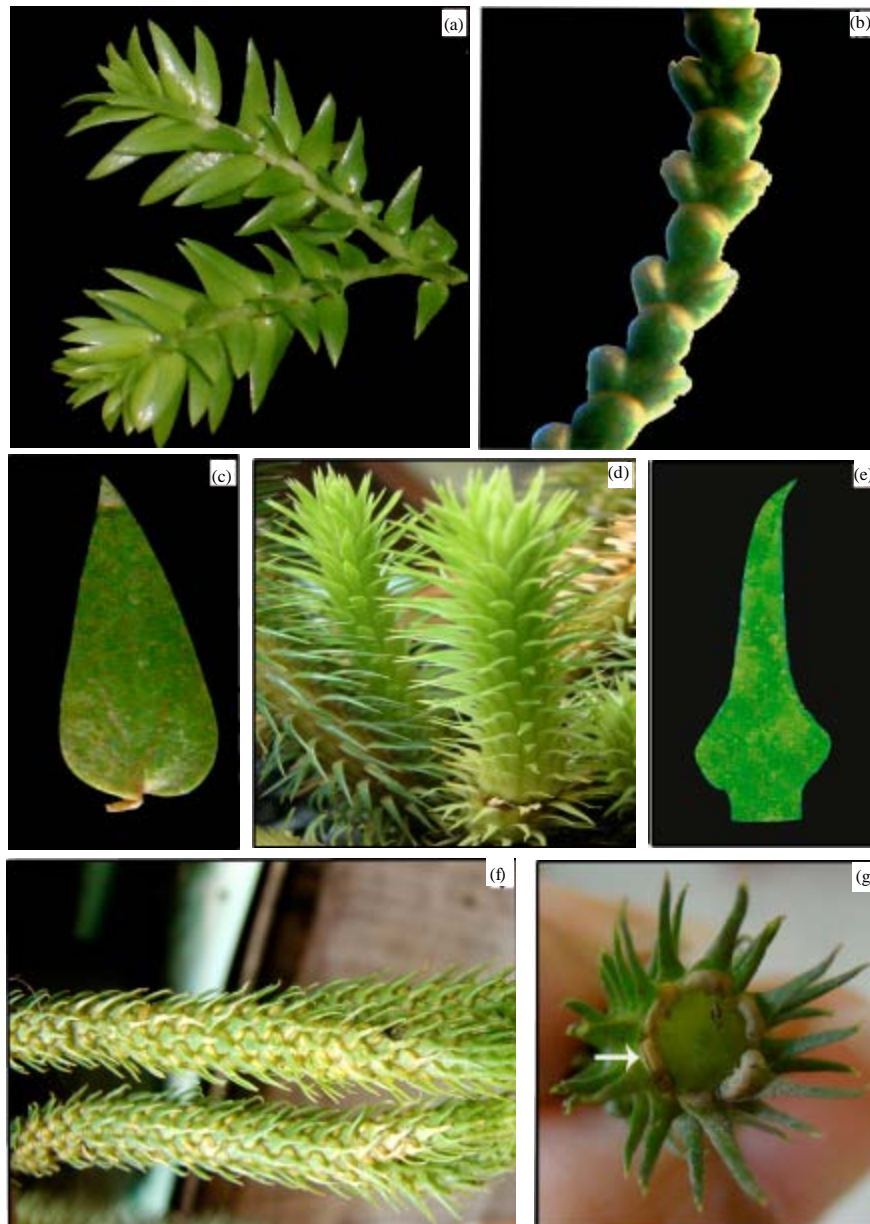


Fig. 1 (a-g): Representatives of tassel ferns found in Manipur (North East India) (a-c) *Huperzia phlegmaria* (a) dichotomous shoot (b) strobili (c) leaf (d-g) *Huperzia squarrosa* (d) young shoots (e) Leaf (f) strobili (g) orientation of sporangia on stem with transverse slit for dehiscence

Distribution: South East Asia (Bhutan, Malaysia, Myanmar, Sri Lanka, Thailand, North East and Andaman and Nicobar Islands of India), Africa in Cameroon, Equatorial Guinea and Gabon, West Tropical Africa, Western Indian Ocean including Comoros, Madagascar, China, Japan and the Islands of Taiwan.

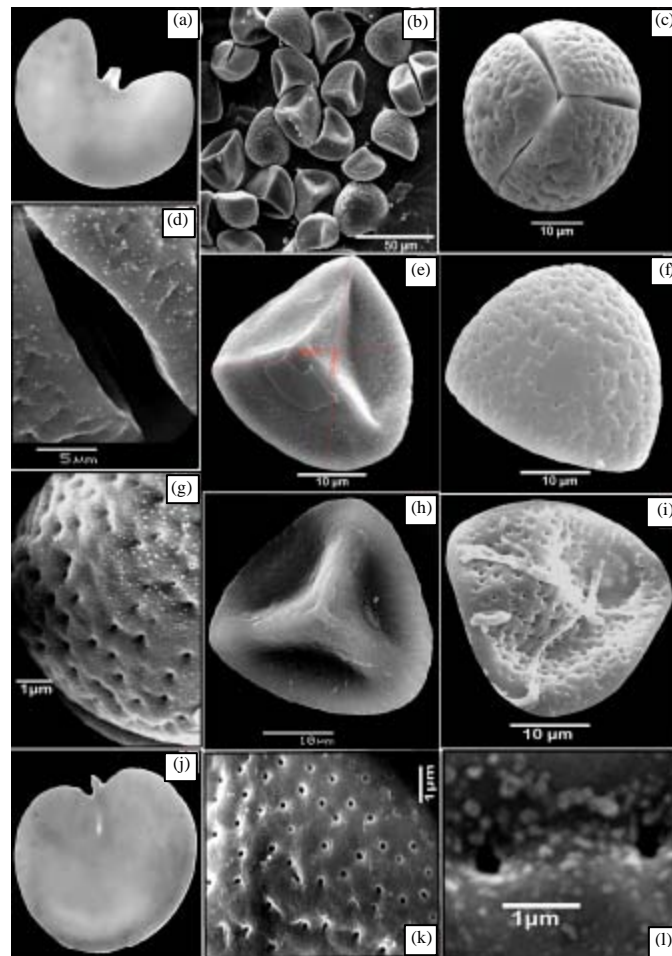


Fig. 2 (a-k): Spore micrographs taken from Light and Scanning Electron Microscopes, (a-g). *Huperzia squarrosa*, (a) kidney-shaped sporangium, 100X, (b) spores displaying both proximal and distal surfaces, (c) spores in tetrad condition, (d) section showing fusion between two spores where the margin concavity is highly reflected, (e) proximal unornamented view of spore (f) distal view of spore with foveolate ornamentation, (g) pit arrangement on distal surface. (h-l). *Huperzia phlegmaria*, (h) pear-shaped sporangium, 100X, (i) proximal view of spore with concave margins and broad ridges, (j) distal view of spore with foveolate ornamentation, (k) pit arrangement on distal position, l. Section showing distance between two pits

Specimen examined: Lohit District F. D. (N.E.F.A), INDIA, 9/12/1962, J. Joseph, 48541, ASSAM (57883); Garsing, Siang F.D. (N.E.F.A), INDIA, 22/11/1958, R. S. Rao, 17933, ASSAM (9039).

***Huperzia squarrosa* (G. Forst.) Rothm. Fig. 1 (d-g), Fig. 2 (a-g):** *Huperzia squarrosa* (G. Forst.) Rothm., Feddes Repert. Spec. Nov. Regn. Veg. 54: 62 (1944).

Macromorphological characters: Plant differentiated into rhizomes and stem, shoots indeterminate, true roots present in aerial stem portion only (Fig. 2d). Stem pendulous, upto 90 cm long, branching regularly dichotomous and isotomous. Leaves microphyllous, crowded, spirally arranged, not imbricated, monomorphic, linear, upto 1.5×0.1 cm, juvenile leaves mostly larger than mature leaves, sharply acute, entire, rigid, coriaceous, shining, veins simple with no branches or unions, extended to middle portion, sporophyll isomorphous with vegetative leaves (Fig. 1e). Strobili 10-30 cm, sessile, terminal, dichotomous (Fig. 1f). Sporangium kidney-shaped, borne individually at adaxial base of unmodified leaves, distinguishable into stalk and capsule, every sporophyll supplied from stele of stem by single trace, dehiscence of sporangium preceded by elongation of internodes of central axis, dehiscence by transverse slit, unilocular, raised on a short stalk, loosely arranged in whorl of 5 or 6 in number (Fig. 1g, 2a).

Spore characters

Size and nature: (28×29) μm , trilete, sub-triangular, tetrahedral with angles reaching upto 150°, yellow in colour, spherical in tetrad condition (Fig. 2c, d).

Distal surface: Foveolate ornamentation, more or less similar with *Huperzia phlegmaria*, pits oval-shaped, distance from one another between (0.5-3) μm , surface regularly undulated around the pits, degree of undulation more prominent than *Huperzia phlegmaria* (Fig. 2f, g).

Proximal surface: Unornamented, pits absent, edges straight to concave, 3 laesura present, less prominent ridges, never reaches margin, very narrow, less than 3 μm across, margin slightly wavy sometimes (Fig. 2e).

Ecological notes: Epiphytes or lithophytes in dense forest, prefer low intensity of light, upto 2500 m altitude.

Common name: Tassel fern, Fir mosses, Fir club mosses.

Vernacular name: Leishang-khekwaiba (Manipuri language).

Distribution: Tropical regions of India (West Bengal, Sikkim, Meghalaya, Assam, Nagaland, Manipur, Arunachal Pradesh, Kerala andaman and Nicobar Islands), Nepal, Burma, Bangladesh, Sri Lanka, Malaysia, Philippines, Polynesia, Seychelles, Mascarenes, Tahiti, peninsular Malaysia, Borneo, China, Taiwan, Queensland, Madagascar, Mauritius, Java, Thailand.

Specimens examined: Amatala, Kameng, F.D. (N.E.F.A), INDIA, 8/5/1958, G. Panigrahi, 15169, ASSAM (8972); Flora of Assam, INDIA, 26/4/1942, G.K. Deka, 21206, ASSAM (36933).

DISCUSSION

Lycopod spores are seldom studied and remain unexamined. Some of the significant contributions on the morphology of lycopod spores were contributed by Wilce (1972).

This is mainly because lycopod under tassel groups are seldom grown within human vicinity and also due to extended life cycle as much as 10 years while developing from spore to sporophyte via gametophyte. In the present investigation, an observation is made on spore ornamentation in regard to taxonomic implications. Two familiar tassel ferns from the state Manipur, North East

Table 2: Comparison of data between Wilce (1972) and the present study

Spore parameters	<i>Huperzia phlegmaria</i> (L.) Rothm.	<i>Huperzia squarrosa</i> (G. Forst.) Rothm. (as <i>Lycopodium squarrosa</i> G. Forst.)
According to Wilce (1972)		
Shape	Sub-triangular	Triangular
Margin	Straight to convex with rounded angles	Concave with truncate angles
Laesurae	Relatively prominent ridges	More or less prominent ridges
Pits	Present on distal surface only	Present on both proximal and distal surfaces
Type	Phlegmaria	Selago
According to present study		
Shape	Triangular	Sub-triangular
Margin	Concave, angles never exceeding 130°	Concavity lesser, surface much flattened, angles reaching upto 150 °
Laesurae	Very prominent, never reaches margin, broader ridges, 5-7 µm across	Less prominent, never reaches margin, narrow ridges, less than 3 µm across
Pits	Present on distal surface	Present on distal surface
Type	Spores treated under the same type/ category	

India viz. *Huperzia phlegmaria* (L.) Rothm. and *Huperzia squarrosa* (G. Forst.) Rothm. are selected for the said study. Different authors opined diverse variation on the spore type existing in lycopods. One of the most significant researches on lycopod spores was contributed by Wilce (1972). He treated *Huperzia* element of *Lycopodium* as foveolate-fossulate group and categorized into *Selago* and *Phlegmaria* type. Species under the *Selago* group includes *Lycopodium lucidulum* Michx, *L. quasipolytrichoides* Hayata, *L. selago* L., *L. serratum* Thunb. ex Murray and *L. squarrosus* G. Forst. (i.e., *Huperzia squarrosa*) added four new species in this group (*L. ceylanicum* Spring, *L. miniatum* Spring, *L. fontinaloides* Spring, *L. australianum* (Herter) Holub. Characteristic features under this group includes triangular spore outline, concave sides with truncate angles, pits on both proximal and distal surfaces and laesurae more or less prominent with ridges (Table 2). The scanning electron micrographs taken in the present study also supported the data presented by Wilce. However, on closer observation, we found the pits confined only on the distal surface while the proximal surface remains unornamented. This finding is very significant in the classification and categorization of lycopod spores. Under *phlegmaria* type, Wilce (1972) recognized only three lycopods (*L. dichotomum* (Jacq.) Trev., *L. reflexum* Lam., *L. billardieri* Spring). In both the spore types mentioned above, spore description of *Huperzia phlegmaria* (i.e. *Lycopodium phlegmaria*) was found missing. However, the name 'PHLEGMARIA' coincides with the specific name *Phlegmaria* under the genus *Huperzia* or *Lycopodium*. We also found the characters of *H. phlegmaria* almost matching with *phlegmaria* type. From the present study, it appears that *Selago* and *Phlegmaria* types are synonymous to one another except in having relatively prominent ridges, difference in the degree of concavity on the proximal surface, pit arrangement and size of the spores. Otherwise, the overall palynological data in both the species are almost identical to one another. To summarize the differences, a comparison is made between data presented by Wilce and findings accredited from the present study (Table 2).

CONCLUSION

It can be concluded that the *Selago* type in *H. squarrosa* and *Phlegmaria* type in *H. phlegmaria* categorized by Wilce (1972) are closely related to one another enacting similar patterns in spore

morphology and therefore, it is rather appropriate to place them under the same category. It would be worthwhile to acknowledge the contribution made by great scientists in the field of lycopod spores, nevertheless the findings accumulated in the present study too supplemented the existing facts. In view of the above, we also come to a point that spore morphology would be very useful in solving the pending problems of taxonomy, phylogeny and phytogeography.

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REFERENCES

- Alwadie, H.M., 2008. Pollen morphology of six aquatic angiosperms from Saudi Arabia. *Asian J. Biol. Sci.*, 1: 45-50.
- Das, S. and R.D. Dixit, 2001. Spore morphology of the Indian members of *Loxogramme* Presl. *Indian Fern J.*, 18: 78-82.
- Devi, S., 1977. Spores in Indian Ferns. *Today's and Tomorrows printers and publishers, New Delhi*, pp: 5-139.
- Erdtman, G., 1945. Pollen morphology and plant taxonomy: V. On the occurrence of tetrads and dyads. *Sven. Bot. Tidskr.*, 39: 286-297.
- Erdtman, G., 1957. Pollen and Spore Morphology/Plant Taxonomy. *Gymnospermae, Pteridophyta, Bryophyta*. Almqvist and Wiksell, Stockholm, pp: 151.
- Fawzi, N.M., A.M. Fawzy and A.A.H.A. Mohamed, 2010. Seed morphological studies on some species of *Silene* L. (Caryophyllaceae). *Int. J. Bot.*, 6: 287-292.
- Holtum, R.E., 1947. A revised classification of leptosporangiate ferns. *J. Linn. Soc. London Bot.*, 53: 123-158.
- Holub, J., 1985. Transfers of *Lycopodium* Species to *Huperzia*: With a note on generic classification in Huperziaceae. *Folia Geobot. Phytotax.*, 20: 67-80.
- Holub, J., 1991. Taxonomic changes within *Lycopodiales*. *Folia Geobot.*, 26: 81-94.
- Nath, V. and S. Sharma, 2006. Scanning electron microscopic studies on spores of some west Himalayan mosses. *Phytotaxonomy*, 6: 53-60.
- Punt, W., P.P. Hoen, S. Blackmore, S. Nilsson and A. Le Thomas, 2007. Glossary of pollen and spore terminology. *Rev. Palaeobot. Palynol.*, 143: 1-81.
- Qureshi, S.J., A.G. Awan, M.A. Khan and S. Bano, 2002a. Palynological study of the genus *Tragopogon* from Pakistan. *Asian J. Plant Sci.*, 1: 283-287.
- Qureshi, S.J., A.G. Awan, M.A. Khan and S. Bano, 2002b. Study of pollen fertility of the genus *launaea* from Pakistan. *Asian J. Plant Sci.*, 1: 73-74.
- Schneider, H. and K.M. Pryer, 2002. Structure and function of spores in the aquatic heterosporous fern family Marsileaceae. *Int. J. Plant Sci.*, 163: 485-505.
- Shehata, A.A., 2008. Pollen morphology of egyptian geraniaceae: An assessment of taxonomic value. *Int. J. Bot.*, 4: 67-76.

- Singh, H.B. and M.K. Singh, 2010. *Huperzia serrata*: A promising medicinal pteridophyte from North East India. *Nebio*, 1: 27-34.
- Srivastava, G.K., M. Srivastava and S.O. Wagai, 2001. Quillworts of Sahyadris Hills (India). *Indian Fern J.*, 18: 89-101.
- Tryon, R.M. and A.F. Tryon, 1982. *Ferns and Allied Plants with Special Reference to Tropical America*. Springer-Verlag, New York, USA.
- Verma, S.C., 2000. Evolutionary biology of homosporous ferns: An enigma. *Indian Fern J.*, 17: 36-49.
- Wilce, J.H., 1965. Section *complanata* of the genus *Lycopodium*. *Beih. Nova Hedwigia.*, 19: 1-233.
- Wilce, J.H., 1972. Lycopod spores I. General spore patterns and the generic segregates of *Lycopodium*. *Am. Fern. J.*, 62: 65-79.