Spore morphology of Selected Pteridophytes Found in the Western Ghats of India

Shaiesh Morajkar¹,², Sudha Sajeev¹ and Smitha Hegde³*

¹Department of Postgraduate Studies and Research in Biotechnology, St Aloysius College (Autonomous), Mangalore – 575 003, Karnataka, India.
²Goa State Wetland Authority, O/o Goa State Biodiversity Board, DSTE complex, Saligao-403511, Bardez, Goa, India.
³Nitte University Centre for Science Education and Research, Nitte Deemed to be University, Derelakatte, Mangalore- 575 018, Karnataka, India.

http://dx.doi.org/10.13005/bbra/2899

(Received: 15 March 2021; accepted: 29 April 2021)

The current study evaluated the morphology (aperture, size, perine structures and surface ornamentation) of treated spores of 45 selected fern species from the Western Ghats of India, using Scanning Electron Microscopy (SEM). Twenty-six species of fern spores were trilete type, while 19 of them had monolete aperture types. The size of the spore were found to be highly variable (20X20µm to 60X60µm) with an average mean spore size of 44 µmX38µm. Furthermore the spores were found to have a highly diverse perine ornamentation with 11 different types of perine structures. Gammate and psilate type of perine ornamentation, and Globose and ellipsoidal spore shape were found to be the most common within the studies fern spore samples. The variability found in the spore ultrastructure and perispore ornamentation of the selected pteridophytes species reflects the morphological differences observed in the sporophyte. The spores could be an important source of characteristics with systematic value in fern taxonomy. The spore morphology of the examined pteridophytes studied common, endemic or otherwise will find a significant role in future taxonomic surveys, and other morphology, Palynology, discrimination, and identification studies of pteridophytes in the Western Ghats.

Keywords: Endemic, Perine ornamentation, Ferns, SEM, Spore apertures.

There are approximately 1100 species of pteridophytes within 70 families and 192 genera distributed in India, with more than 349 species occurring in the Western Ghats¹. With such high diversity of pteridophyte species occurring in the biodiversity hotspot of the Western Ghats, numerous of which are endemic, rare and endangered, it is challenging to get the appropriate information required for species identification and discrimination with the desired speed². The easiest and one of the most efficient solution to this is classical taxonomic, and morphological data compendia of the extant fern diversity.

Palynology of ferns has proved to be very useful in the identification and discrimination of various fern taxa³. There have been several Palynology studies of pteridophytes that examined the characteristics of spore samples using basic
microscopic staining techniques\textsuperscript{2,4}, to the modern use of scanning electron microscope (SEM)\textsuperscript{10-16}. Palynology data depositories have been very useful for taxonomic purposes in ferns and have been previously used for identifying palynological fern sediments\textsuperscript{17}, interspecies discrimination\textsuperscript{18,19}, relatedness and phylogeny of the fern species\textsuperscript{20-24}. This study aims to investigate the spore morphology and perine ultrastructure of selected fern species found in the Western Ghats of India by using SEM. The results of the current study may provide a key for future studies in general fern morphology, palynology, fern identification and discrimination of pteridophytes in the Western Ghats.

MATERIALS AND METHODS

Forty-five different species of fern spore samples collected in June 2016, from the herbarium collection of Morajkar\textsuperscript{25}, of Kudremukh National Park (13°1' to 13°29' N latitude and 75°0' to 75°30' E longitude), Western Ghats, that were made available at St Aloysius College (Autonomous) Mangalore, Karnataka. These fern spore samples were considered in the study as Morajkar\textsuperscript{25} has reported ferns that are rare, endemic and threatened in the Western Ghats. The spore surface was studied using Scanning Electron Microscope (SEM) after treating the spores in ultrasonic wave bath (50-60Hz) and subsequent washing with ethanol in a three-step process as elaborated by Hu et al.\textsuperscript{26}. The spore structures and ornamentation were observed and photographed under a tabletop SEM. Two SEM models were utilized in the study namely, Hitachi TM 3030 and JOEL JFC-1600. For each pteridophyte species, multiple spore samples were examined from two different herbarium accession. The spore apertures, size, perine structures and surface variations were recorded as per Tschudy\textsuperscript{27}.

RESULTS AND DISCUSSION

All 45 selected fern spore samples were successfully treated and spore morphology was examined in details (Table 1, Plate 1 and Plate 2). The size of the spores were found to be highly variable and ranged from 20X20μm to 60X60μm with an average mean of 44 μmX38μm. Angiopteris helferiana and Nephrolepis hirsutula had the smallest spore, while Lygodium flexuosum and Osmunda huegeliana were found to have large spores. Similar observations of these fern spore sizes were also made by Makgomol\textsuperscript{11}, Zenkteler\textsuperscript{2}, and Shaikh & Madhav\textsuperscript{28}.

The SEM results divide the spores of 45 fern species into 2 aperture types, trilette type and monolete type. 26 species of fern spores were found to be trilette, while 19 species had monolete aperture types. The majority of the trilete spores were globose (13), followed by 11 species with tetrahedral and two with spheroidal type of spore shape. Spores with monolete aperture type were dominated by ellipsoidal shape constituting 14 species while spheroidal and globose spores were found in two and three species respectively. The apertures of most of the fern spores were found to be in accord with the studies of Vijayakanth & Sathish\textsuperscript{7} and Vijayakanth et al.\textsuperscript{29} It was also observed that the fern ultrastructure, aperture type and to much extent the shape of the spore were found to be similar within a fern genus.

The perispore forms the outer surface and often the characteristic contours of the spores. The most common ornamentation of perispore in the studied pteridophyte spore samples was found to be gammatte and psilate, with nine and eight fern species having the respective spore ornamentation. Among all the spores examined, Pteris cameronianiana was the only fern with clavate type of perine ornamentation. Echinulate type of perine ornamentation was seen in only two fern species namely Bolbitis semicordata and Tectaria quadriaurita. B. semicordata is known to be endemic to the Western Ghats. Additionally, seven species namely S. tenera, Osmunda huegeliana, Pteris quadriaurita, Bolbitis subcrenataoides, Cyathea nilgirensis, Cyclosorus parasitica, and Tectaria polymorpha are also known to be endemic to the Western Ghats. Perine structure of these species are very important for accurate identification and differentiation in future studies. As noted (Table 1), Perine structure of S. tenera and P. quadriaurita had gammatte while T. polymorpha and O. huegeliana had baculate type of perine ornamentation. Other endemic fern species namely B. subcrenataoides, C. nilgirensis, and C. parasitica had scabrate, psilate and striate type of perine ornamentation respectively. In addition to the perine surface structures fern species namely, Sellaginella tenera and Sellaginella delicatula
Table 1. Characteristic spore morphology of 45 selected pteridophytes examined in the current study

| Sr. No. | Fern species | Voucher no. | Avg. Size (µm) | Spore morphology | Perispore Surface |
|---------|--------------|-------------|----------------|------------------|------------------|
| 1       | Acrosticum Aaureum L. | 54 KNP | 40×45 | Trilete Globose | Verrucate |
| 2       | Adiantum philippense L. | 26 KNP | 50×45 | Trilete Tetrahedral | Psilate |
| 3       | Adiantum raddianum Presl | 01 KNP | 40×30 | Trilete Tetrahedral | Faveolate |
| 4       | Aleuropites anceps (Blanf.) Panigrahi | 27 KNP | 55×50 | Trilete Globose | Striate |
| 5       | Angiopteris helferiana C. Presl | 02 KNP | 20×20 | Trilete Globose | Rugulate |
| 6       | Arachniodes tripinnata (Goldm.) Sledge | 10 KNP | 35×35 | Trilete Globose | Baccate |
| 7       | Araiostegia pulchra (D. Don) Copel. | 21 KNP | 48×36 | Monolet Globose | Verrucate |
| 8       | Asplenium yoshinagae makino Subsp. Indicum (Sledge) Frazer-Jenk. | 18 KNP | 50×40 | Monolet Ellipsoidal | Rugulate |
| 9       | Blechnum orientale L. | 11 KNP | 30×28 | Monolet Spheroidal | Psilate |
| 10      | Bolbitis semicordata (Baker) Ching | 37 KNP | 40×50 | Monolet Globose | Echinate |
| 11      | Bolbitis subrenotoides Fraser-Jenk. | 29 KNP | 30×35 | Monolet Globose | Scabrate |
| 12      | Cheilanthes tenuifolia (Burn. F.) Sw. | 51 KNP | 45×45 | Trilete Globose | Psilate |
| 13      | Cyathea gigantea (Wall. ex Hook.) Holttum | 12 KNP | 35×30 | Trilete Globose | Rugulate |
| 14      | Cyathea nilgiresiensis Holttum | 13 KNP | 50×45 | Trilete Globose | Psilate |
| 15      | Cyclosorus (Christella) dentata (Forssk.) Browne-Jervy | 30 KNP | 45×30 | Monolet Ellipsoidal | Reticulate |
| 16      | Cyclosorus (Christella) parasitica (L.) H. Lev. | 14 KNP | 50×30 | Monolet Ellipsoidal | Striate |
| 17      | Dicranopteris linearis (Born. f.) Underwood | 31 KNP | 55×50 | Trilete Spheroidal | Psilate |
| 18      | Diplazium esculentum (Retz.) Sw. | 53 KNP | 30×20 | Monolet Ellipsoidal | Reticulate |
| 19      | Drynaria quercifolia (L.) J. Sm. | 48 KNP | 65×40 | Monolet Ellipsoidal | Scabrate |
| 20      | Lepisorous nudus (Hook.) Ching | 43 KNP | 50×25 | Monolet Ellipsoidal | Gemmate |
| 21      | Lindeslea ensifolia Sw. | 33 KNP | 35×33 | Trilete Globose | Gemmate |
| 22      | Lindeslea ensifolia Sw. | 16 KNP | 40×36 | Trilete Globose | Psilate |
| 23      | Lycopodiella cernua (L.) Pic. Ser. | 07 KNP | 54×40 | Monolet Ellipsoidal | Verrucate |
| 24      | Lygodium flexuosum (L.) Sw. | 04 KNP | 60×60 | Trilete Globose | Gemmate |
| 25      | Lygodium microphyllum (Cav.) R. Br. | 15 KNP | 55×40 | Trilete Globose | Reticulate |
| 26      | Macrothelypteris torrensiana (Gaudich.) Ching | 35 KNP | 45×40 | Monolet Spheroidal | Faveolate |
| 27      | Microlepia speluncae (L.) T. Moore | 17 KNP | 25×30 | Trilete Tetrahedral | Psilate |
| 28      | Microsorum membranaceum (Don) Ching | 34 KNP | 60×40 | Monolet Ellipsoidal | Faveolate |
| 29      | Microsorum punctatum (L.) Copeland | 44 KNP | 40×30 | Monolet Ellipsoidal | Gemmate |
| 30      | Nephrolepis hirsutula (G. Frost.) C. Presl | 36 KNP | 28×17 | Monolet Ellipsoidal | Verrucate |
| 31      | Odontosoria chinensis (L.) J. Smith | 41 KNP | 32×40 | Monolet Ellipsoidal | Gemmate |
| 32      | Osmunda huwejiliana C. Presl | 08 KNP | 60×60 | Trilete Sphoroidal | Baccate |
| 33      | Pityrogramma calomelanos (L.) Link | 19 KNP | 50×45 | Trilete Tetrahedral | Reticulate |
| 34      | Pteridium aquilinum (L.) Kuhn | 06 KNP | 25×30 | Trilete Tetrahedral | Gemmate |
| 35      | Pteris argyraea T. Moore | 03 KNP | 50×50 | Trilete Tetrahedral | Reticulate |
| 36      | Pteris bisaurita L. | 38 KNP | 60×45 | Trilete Tetrahedral | Gemmate |
| 37      | Pteris cameroniana Kuhn | 40 KNP | 54×55 | Trilete Tetrahedral | Clavate |
| 38      | Pteris confusa T.G. Walker | 39 KNP | 40×35 | Trilete Tetrahedral | Striate |
| 39      | Pteris quadriraurita Retz. | 20 KNP | 50×35 | Trilete Tetrahedral | Gemmate |
| 40      | Pteris viitata L. | 45 KNP | 45×35 | Trilete Tetrahedral | Rugulate |
| 41      | Selaginella tenera (Hook. &Grev.) Spring | 22 KNP | 33×30 | Trilete Globose | Winged / Gemmate |
| 42      | Sellaginella delicata (Desv.) Alston | 42 KNP | 40×32 | Trilete Globose | Winged / Psilate |
| 43      | Tectaria coadunata (J. Sm.) C. Chr. | 24 KNP | 30×25 | Monolet Ellipsoidal | Echinate |
| 44      | Tectaria polymorpha (Wall. ex Hook.) Copel. | 09 KNP | 65×40 | Monolet Ellipsoidal | Baccate |
| 45      | Thelypteris (metathelypteris) flaccida (Bl.) Ching | 52 KNP | 54×45 | Monolet Ellipsoidal | Scabrate |
had winged perispore. Earlier study such by Zhou et al.\textsuperscript{30} has also reported winged perispores in \textit{Sellaginella} species. The perispore surfaces observed in the current study were mostly in agreement with earlier studies\textsuperscript{2,7,17,29}, this suggests that perispore morphology is generally consistent

\textbf{Plate 1.} 1. \textit{A. aureum}, 2. \textit{A. philippense}, 3. \textit{A. raddianum}, 4. \textit{A. anceps}, 5. \textit{A. helferiana}, 6. \textit{A. tripinnata}, 7. \textit{A. pulchra}, 8. \textit{A. yoshinagae}, 9. \textit{B. orientale}, 10. \textit{B. semicordata}, 11. \textit{B. subcrenatooides}, 12. \textit{C. tenuifolia}, 13. \textit{C. gigantean}, 14. \textit{C. nilgirensis}, 15. \textit{C. dentate}, 16. \textit{C. parasitica}, 17. \textit{D. linearis}, 18. \textit{D. esculentum}, 19. \textit{D. quercifolia}, 20. \textit{L. nudus}, 21. \textit{L. heterophylla}, 22. \textit{L. ensifolia}, 23. \textit{L. cernua}, 24. \textit{L. flexuosum}. Note: Proximal view (1, 2, 5, 9, 11, 12, 17, 20, 24), Equatorial view (3, 7, 8, 10, 13, 14, 15, 16, 19, 22) and Distal view (4, 6, 12, 18, 21, 23).
within species. A similar observation was also made by Moran et al\textsuperscript{23} in his study of perispore structures in Dryopteridaceae.

The spore structure given by Zenkteler\textsuperscript{2}, describes the spore of \textit{P. aquilinum} as tetrahedral and trilete with verrucae and baculate structures

\textbf{Plate 2}. 25. \textit{L. microphyllum}, 26. \textit{M. torrensiana}, 27. \textit{M. speluncae}, 28. \textit{M. membranaceum}, 29. \textit{M. punctatum}, 30 \textit{N. hirsutula}, 31. \textit{O. chinensis}, 32. \textit{O. huegeliana}, 33. \textit{P. calomelanos}, 34. \textit{P. aquilinum}, 35. \textit{P. argyrea}, 36. \textit{P. biaurita}, 37. \textit{P. camerooniana}, 38. \textit{P. confuse}, 39. \textit{P. quadriaurita}, 40. \textit{P. vittata}, 41. \textit{S. tenera}, 42. \textit{S. delicatula}, 43. \textit{T. coadunate}, 44. \textit{T. polymorpha}, 45. \textit{T. flaccida}. \textbf{Note}: Proximal view (26, 31, 37, 40), Equatorial view (27, 28, 30, 34, 35, 36, 38, 42, 45) and Distal view (25, 29, 32, 33, 39, 41, 43, 44).
depicting an irregularly granulate perine structure. A similar spore structure with an uniform gammatte perine structure is observed in the current study. It was noted by Zenkteler² that *P. aquilinum* had the potential to release large numbers of spores which are known to be toxic and carcinogenic³⁴-³⁵ and are dispersed by wind. This fern also known as the bracken fern, grows like a weed that can be an immense threat to the ecosystem. There has been confusion with this fern with *P. revolutum*³³ and both have been misidentified with each other. Even though there are studies that report the distribution of this fern in various regions, such as Eastern Ghats³, The Western Ghats³⁴-³⁶ and other parts of India³⁷, there are also claims that *P. aquilinum* is not to be found in the Indian subcontinent³⁸. Hence future comprehensive taxonomic studies with a standard method, along with molecular taxonomy are required in this regard, considering the toxicity and weed ability of this fern species.

Based on the results its found that all the pteridophytes within each family have the same aperture type, with the exception of Dryopterideceae. Both the species of Tectaria were found to have monolete aperture type, while *A. tripinnata* who belongs to the same family had a trilete spore aperture. In most cases the shape of spore were same within genus, but differed distinctively when perispore surface was compared. These results significantly elaborates on the importance of spore ultrastructure and other features in identification and discrimination of pteridophytes. Contrary to this the findings of Yañez et al.,¹⁵ suggested that the spores with similar characteristics in phylogenetically unrelated families do not allow palynological features to have an evolutionary value in determining relationships between groups above the genus level. But the study on spore morphology and characters by Passarelli et al.,³⁹ suggest that perispore characters have distinct diagnostic value, since different combinations of ornamentation/structure were found to vary considerably among the pteridophytes. He also inferred that, when spore ornamentation is useful complementary feature at the specific level identification and discrimination when used in combination with other morphological traits of fern sporophytes. Additionally, the study undertaken by Chao and Huang²⁴ to investigate spore morphology evolution in *Pteris* species, revealed that spore characters, similar to leaf morphologies, reversed several times, but the combination of both characters could be useful in identification and discrimination and to establish more natural relationships within this group of fern species. Hence fern spore morphology is an important source of characteristics with systematic value in fern taxonomy.

**CONCLUSION**

The variability found in the spore ultrastructure and perispore ornamentation reflects the morphological differences observed in the sporophyte of the selected fern species. This study will be an important source of characteristics with systematic value in fern taxonomy. The spore appertures, size, perine structures and surface variations observed within the selected pteridophytes will help future taxonomic studies of ferns in th Western Ghats of India. The ultrastructure of the spores will supplement other morphological characters and advance molecular tools, to enable precise and standard taxonomic identification and differentiation in other fern species in question, such as *P. aquilinum* and *P. revolutum* debate.

**ACKNOWLEDGEMENT**

Our sincere thanks to Rev Fr Swebert D’Silva SJ, Principal St Aloysius College (Autonomous), Dr Asha Abraham, HOD, Department of Post Graduate Studies and Research in Biotechnology, St Aloysius College and Rev Fr Leo D’Souza SJ, Director, Dr Küppers Laboratory of Applied Biology, St Aloysius College for providing the research facilities.

**Conflict of Interest**

No potential conflict of interest is reported by the authors.

**Funding**

None.

**Statement of Informed Consent**

All the authors involved in the manuscript assure their consent for the publication of the manuscript.
25. Morajkar S. Biodiversity of ferns of Western Ghats using molecular markers (Doctoral dissertation thesis, Mangalore University, Mangalore, India). Shodhganga, 2018; http://hdl.handle.net/10603/249856

26. Hu X. Y., Zhai J. W., Wang F. G., Xu X. L. Simple treatment to investigate spore ornamentation of ferns for SEM observation from herbarium specimens. *Pak. J. Bot.*, 2010; 42: 2335-2338.

27. Tschudy R. H. The plant kingdom and its palynological representation. In: *Aspects of Palynology* (Tschudy RH. ed) Wiley-interscience. 1969; pp 32-34.

28. Shaikh S. D., Madhav N. A. Spore morphology of four species of pteridophytes from Northern Western Ghats of Maharashtra (India). *Indian Fern J.*, 2019; 36: 89-94.

29. Vijayakanth P, Sathish S., Palani R., Thamizharasi T., Vimala A. Palynomorphic studies on the pteridophytes of Kolli Hills, Eastern Ghats, Tamil Nadu. *Bio. Disc.*, 2017; 8(4): 752-761.

30. Zhou X., Jiang L., Zhang L., Gao X., He Z., Zhang L. Spore morphology of Selaginella (Selaginellaceae) from China and its systematic significance. *Phytotaxa*. 2015; 237(1): 1. doi: 10.11646/phytotaxa.237.1.1

31. Page CN (ed): *The Ferns of Britain and Ireland*. Cambridge: Cambridge University Press. 1997; pp 344-370.

32. Siman S. E., Povey A. C., Sheffield E. Human health risks from fern spores? - a review. *Fern Gaz.*, 1999; 15: 275-287.

33. Fraser-Jenkins C. R. (ed): Taxonomic revision of three hundred Indian sub-continental pteridophytes with a revised census list – a new picture of fern-taxonomy and nomenclature in the Indian subcontinent. Dehradun: Bishen Singh Mahendra Pal Singh Publishers, 2008.

34. Benjamin A., Manickam V. S. Medicinal pteridophytes from Western Ghats. *IJTK*, 2007; 6(4): 611-618.

35. Hegde S., Sajeev S. (eds): A collection of selected ferns of Karnataka. Karnataka: Karnataka Forest Department, Government of Karnataka, India, 2013.

36. Shaikh S. Status of some important pteridophytes from the parts of Northern Western Ghats of Maharashtra, India. *Int. Res. J. Biol. Sci.*, 2017; 6(1): 47-49.

37. Behera S. K., Khare P. B. First report of pteridophytes from Govind Wildlife Sanctuary, Uttarkashi, Uttarakhand, India. *Trop. Plant Res.*, 2014; 1(2): 37-47.

38. Ranil R. H. G., Pushpakumara G., Fraser-Jenkins C. R., Wijesundara S. Misidentification of Pteridium revolutum (Blume) Nakai as an invasive alien in Sri Lanka. In: *Invasive Alien Species in Sri Lanka – Strengthening Capacity to Control Their Introduction and Spread* (Marambe B., Silva P., Wijesundara S., Atapattu N. eds), Biodiversity Secretariat, Ministry of Environment, Colombo, Sri Lanka, 2010; pp 141-149.

39. Passarelli L. M., Galán J. M. G., Prada C., Rolleri C. H. Spore morphology and ornamentation in the genus Blechnum (Blechnaceae). *Grana*, 2010; 49(4): 243-262. doi: 10.1080/00173134.2010.524245