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

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The currents study evaluated the morphology (apperture, size, perine structures and surface ornamentation) of treated spores of 45 selected fern species from the Western Ghats of India, using Scanning Electron Microspcopy (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 ornementaion 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 Ghats[1]. 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 speed[2]. 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 taxa[3]. There have been several Palynology studies of pteridophytes that examined the characteristics of spore samples using basic

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microscopic staining techniques[2, 4-9] to the modern use of scanning electron microscope (SEM)[10-16]. Palynology data depositories have been very useful for taxonomic purposes in ferns and have been previously used for identifying palynological fern sediments[17], interspecies discrimination[18-19], relatedness and phylogeny of the fern species[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[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 samples were considered in the study as Morajkar[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.[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 pteridohyte species, multiple spore samples were examined from two different herbarium accession. The spore appertures, size, perine structures and surface variations were recorded as per Tschudy[27].

RESULTS AND DISCUSSION

All 45 selected fern spore samples were successfully treated and spore morphology was examined in details (**Table1**, **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[11], Zenkteler[2], and Shaikh & Madhav[28].

The SEM results divide the spores of 45 fern species into 2 aperture types, trilete type and monolete type. 26 species of fern spores were found to be trilete, 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 apperture 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[7] and Vijayakanth et al.[29] It was also observed that the fern ultrastructure, apperture 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 gammate and psilate, with nine and eight fern species having the respective spore ornamentation. Among all the spores examined, *Pteris camerooniana* was the only fern with clavate type of perine ornamentation. Echinate type of perine ornamentation was seen in only two fern species namely *Bolbitis semicordata* and *Tectaria coadunate. B. semicordata* is known to be endemic to the Western Ghats. Additionally, seven species namely *S. tenera*, *Osmunda huegeliana*, *Pteris quadriaurita*, *Bolbitis subcrenatoides*, *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 gammate while *T. polymorpha* and *O. huegeliana* had baculate type of perine ornamentation. Other endemic fern species namely *B. subcrenatoides*, *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		
				Aperture Type	Shape	Perispore Surface
1.	*Acrositucum 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.	*Aleuritopteris 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	Baculate
7.	*Araiostegia pulchra* (D. Don) Copel.	21 KNP	48×36	Monolete	Globose	Verrucate
8.	*Asplenium yoshinagae makino* Subsp. *Indicum* (Sledge) Frazer-Jenk.	18 KNP	50×40	Monolete	Ellipsoidal	Rugulate
9.	*Blechnum orientale* L.	11 KNP	30×28	Monolete	Spheroidal	Psilate
10.	*Bolbitis semicordata* (Baker) Ching	37 KNP	40×50	Monolete	Globose	Echinate
11.	*Bolbitis subcrenatoides* Fraser-Jenk.	29 KNP	30×35	Monolete	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 nilgirensis* Holttum	13 KNP	50×45	Trilete	Globose	Psilate
15.	*Cyclosorus (Christella) dentata* (Forssk.) Brownsey& Jermy	30 KNP	45×30	Monolete	Ellipsoidal	Reticulate
16.	*Cyclosorus (Christella) parasitica* (L.) H. Lev.	14 KNP	50×30	Monolete	Ellipsoidal	Striate
17.	*Dicranpteris linearis* (Born. f.) Underwood	31 KNP	55×50	Trilete	Spheroidal	Psilate
18.	*Diplazium esculentum* (Retz.) Sw.	53 KNP	30×20	Monolete	Ellipsoidal	Reticulate
19.	*Drynaria quercifolia* (L.) J. Sm.	48 KNP	65×40	Monolete	Ellipsoidal	Scabrate
20.	*Lepisorous nudus* (Hook.) Ching	43 KNP	50×25	Monolete	Ellipsoidal	Gemmate
21.	*Dindsaea heterophylla* Dryand.	33 KNP	35×33	Trilete	Globose	Gemmate
22.	*Lindsaea ensifolia* Sw.	16 KNP	40×36	Trilete	Globose	Psilate
23.	*Lycopodiella cernua* (L.) Pic. Ser.	07 KNP	54×40	Monolete	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	Monolete	Spheroidal	Faveolate
27.	*Microlepia speluncae* (L.) T. Moore	17 KNP	25×30	Trilete	Tertrahedral	Pisilate
28.	*Microsorum membranaceum* (Don) Ching	34 KNP	60×40	Monolete	Ellipsoidal	Faveolate
29.	*Microsorum punctatum* (L.) Copeland	44 KNP	40×30	Monolete	Ellipsoidal	Gammate
30.	*Nephrolepis hirsutula* (G. Frost.) C. Presl	36 KNP	28×17	Monolete	Ellipsoidal	Verrucate
31.	*Odontosoria chinensis* (L.) J. Smith	41 KNP	32×40	Monolete	Ellipsoidal	Gammate
32.	*Osmunda huegeliana* C. Presl	08 KNP	60×60	Trilete	Spheroidal	Baculate
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 biaurita* L.	38 KNP	60×45	Trilete	Tetrahedral	Gemmate
37.	*Pteris camerooniana* Kuhn	40 KNP	54×55	Trilete	Tetrahedral	Clavate
38.	*Pteris confusa* T.G. Walker	39 KNP	40×35	Trilete	Tetrahedral	Striate
39.	*Pteris quadriaurita* Retz.	20 KNP	50×35	Trilete	Tetrahedral	Gemmate
40.	*Pteris vittata* L.	45 KNP	45×35	Trilete	Tetrahedral	Rugulate
41.	*Selaginella tenera* (Hook. &Grev.) Spring	22 KNP	33×30	Trilete	Globose	Winged / Gemmate
42.	*Sellaginella delicatula* (Desv.) Alston	42 KNP	40×32	Trilete	Globose	Winged / Psilate
43.	*Tectaria coadunata* (J. Sm.) C. Chr.	24 KNP	30×25	Monolete	Ellipsoidal	Echinate
44.	*Tectaria polymorpha* (Wall. ex Hook.) Copel.	09 KNP	65×40	Monolete	Ellipsoidal	Baculate
45.	*Thelypteris (metathelypteris) flaccida* (Bl.) Ching	52 KNP	54x45	Monolete	Ellipsoidal	Scabrate

had winged perispore. Earlier study such by Zhou et al.[30] has also reported winged perispores in *Sellaginella* species. The perispore surfaces observed in the current study were mostly in agreement with earlier studies[2,7,17,29], this suggests that perispore morphology is generally consistent

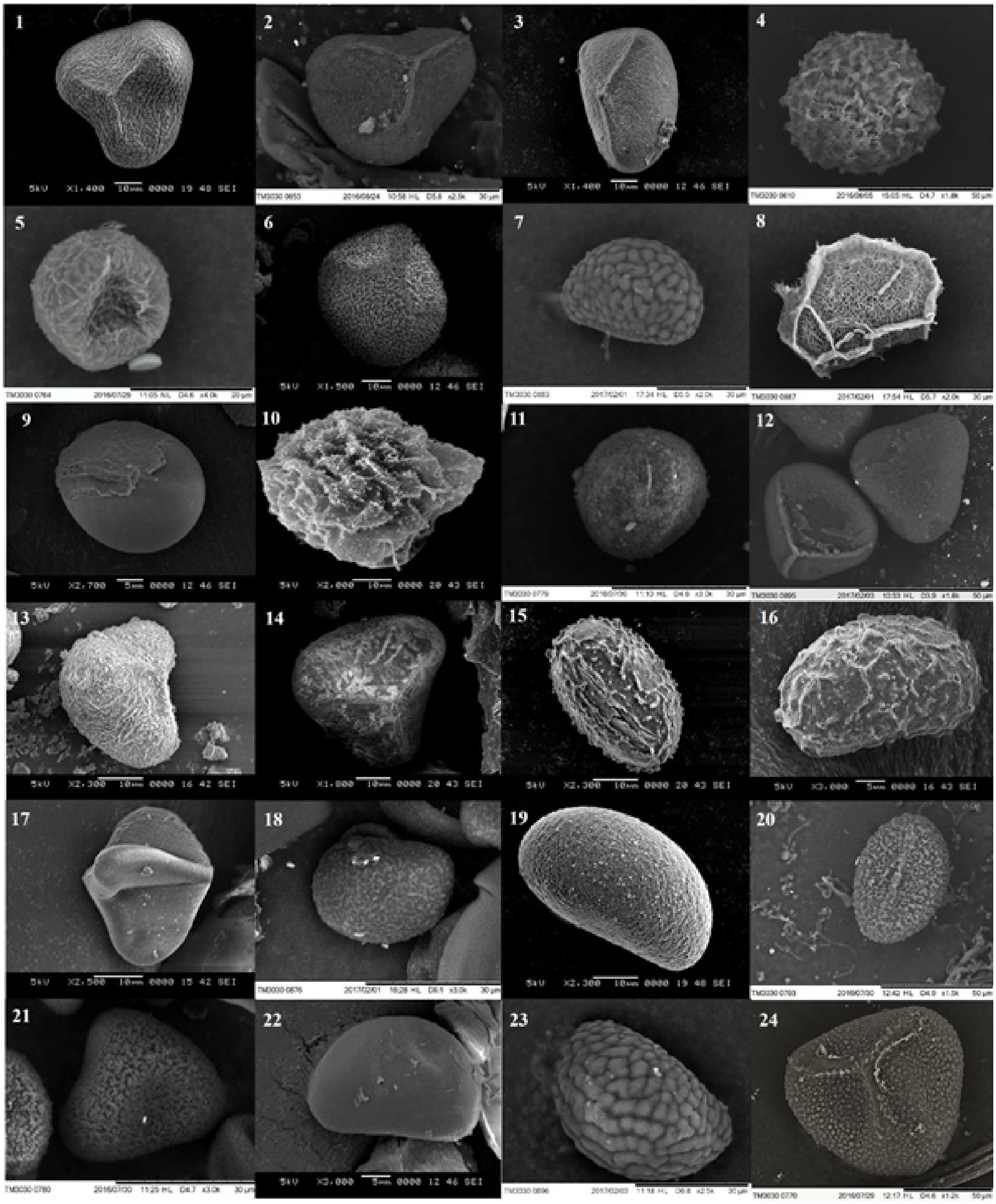

Plate 1. 1. *A. aureum*, 2. *A. philippense*, 3. *A. raddianum*, 4. *A. anceps*, 5. *A helferiana*, 6. *A. tripinnata*, 7. *A. pulchra*, 8. *A. yoshinagae*, 9. *B. orientale*, 10. *B. semicordata*, 11. *B. subcrenatoides*, 12. *C. tenuifolia*, 13. *C. gigantean*, 14. *C. nilgirensis*, 15. *C. dentate*, 16. *C. parasitica*, 17. *D. linearis*, 18. *D. esculentum*, 19. *D. quercifolia*, 20. *L. nudus*, 21. *L. heterophylla*, 22. *L. ensifolia*, 23. *L. cernua*, 24. *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[23] in his study of perispore structures in Dryopteridaceae.

The spore structure given by Zenkteler[2], describes the spore of *P. aquilinum* as tetrahedral and trilete with verrucae and baculate structures

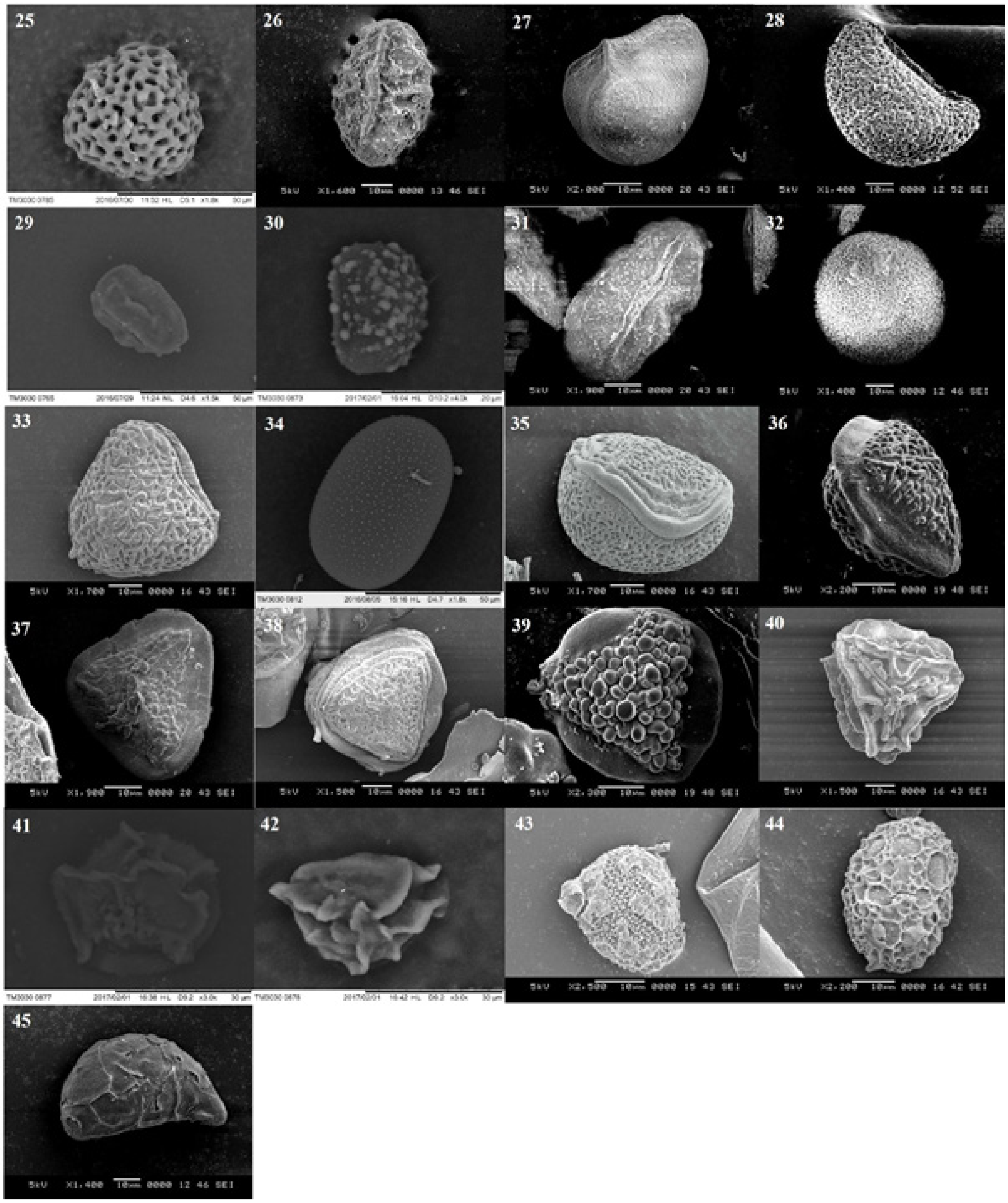

Plate 2. 25. *L. microphyllum*, 26. *M. torrensiana*, 27. *M. speluncae*, 28. *M. membranaceum*, 29. *M. punctatum*, 30 *N. hirsutula*, 31. *O. chinensis*, 32. *O. huegeliana*, 33. *P. calomelanos*, 34. *P. aquilinum*, 35. *P. argyraea*, 36. *P. biaurita*, 37. *P. camerooniana*, 38. *P. confuse*, 39. *P. quadriaurita*, 40. *P. vittata*, 41. *S. tenera*, 42. *S. delicatula*, 43. *T. coadunate*, 44. *T. polymorpha*, 45. *T. flaccida.* **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 gammate perine structure is observed in the current study. It was noted by Zenkteler[2] that *P. aquilinum* had the potential to release large numbers of spores which are known to be toxic and carcinogenic[31-32] 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 Ghats[7], The Western Ghats[34-36] and other parts of India[37], there are also claims that *P. aquilinum* is not to be found in the Indian subcontinent[38]. 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 distintictively 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 alow palynological features to have an evolutionary value in determining relationships between groups above the genus level. But the study on spore morphology and charaters 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 Huang[24] 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 charateristics 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 ultarastructure of the spores will suppliment other morphological characters and advance molecular tools, to enable precise and standard taxanomic identification and differentiation in other fern species in question, such as *P. aquilinum* and *P. revolutum* debate.

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Conflict of Interest

No potential conflict of interest is reported by the authors.

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Statement of Informed Consent

All the authors involved in the manuscript assure their consent for the publication of the manuscript.

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