



RESEARCH ARTICLE

Comparative spore morphology of ten species of the genus *Ophioglossum* L. from Kerala, India

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Abstract

The eusporangiate fern *Ophioglossum* brings some uncertainty among taxonomists while identifying the species. Therefore, a combined approach by considering the spore morphology and other morphological characters is more authentic for delimiting species of this genus. This study documented and compared the spore morphology of *O. costatum*, *O. gramineum*, *O. lusitanicum*, *O. lusoaffricanum*, *O. parvifolium*, *O. pendulum*, *O. petiolatum*, *O. raphaelianum*, *O. reticulatum*, and *O. rubellum* collected from Kerala, India by Scanning Electron Microscopy (SEM) analysis. SEM images of the spores for each species were provided, the P/E ratio was calculated, and the size classes of the spores were determined here. The occurrence of *O. lusitanicum* in India was confirmed, and *O. madhusoodhananii* was synonymised under *O. costatum*.

Keywords

Ophioglossum; spore; trilete; SEM; fern; India

Introduction

The snake tongue or Adder's tongue fern, *Ophioglossum*, is a eusporangiate fern that belongs to the family Ophioglossaceae. Taxonomists have worked on *Ophioglossum* in India (1-11) since its discovery by Bouhin (12) and validation of its generic status by Linnaeus (13) in his "Species Plantarum". Previously, there were a total of ten *Ophioglossum* species reports in Kerala, of which nine *Ophioglossum* species, viz., *O. costatum* R. Br., *O. gramineum* Willd., *O. gomezianum* Welw. Ex A. Braun, *O. lusoaffricanum* Welw. ex. Prantl., *O. parvifolium* Grev. & Hook., *O. pendulum* L., *O. petiolatum* Hook., *O. reticulatum* L. and *O. rubellum* Welw. Ex A. Braun was recorded by Fraser Jenkins *et al.* (14, 15), and *O. raphaelianum* Anto, Afs. Khan, F. Francis & I. Antony was reported by Anto *et al.* (16). However, morphological variations due to the polyploidy and highest chromosome number found in some species (17) and the simple morphology of the genus make species identification more problematic (18-22). *Ophioglossum* is a complex genus with bewildering morphological characters. Recently, many new species have been reported from India without considering the morphotypes of this genus. So Fraser-Jenkins *et al.* (15) synonymised different species of *Ophioglossum* in their work. So, considering the spore characters and the rhizome morphology, tropophylls and fertile segments are more reliable in the case of *Ophioglossum* (23, 24). Moreover, the number of taxonomists considering the spore characters for species identification has increased with time (25-32). In this study, the spore morphology of ten *Ophioglossum* species was compared

and plated using their light microscopy and scanning electron microscopy images, which will be helpful for the exact identification of each species.

Materials and Methods

Fresh *Ophioglossum* plants were collected from different areas of Kerala state. Field photographs were taken, and all the characters that could be documented from the field were noted in the field book. The plants were then taken into the laboratory for further examination. The sporangia were covered with an envelope from the field to preserve the spore morphology. The morphological characters of the external plant parts were recorded. The light microscopic structures of the spores were observed under a Binocular BIOMED Research Microscope using 40 X, and their photographs were taken. For SEM (Scanning Electron Microscope) analysis, the spores were directly dusted over carbon tape-coated stubs and sputter-coated with gold and examined using the JOEL, JSM-6390LV Scanning Electron Microscope. Chemical treatments were avoided since they dissolve the fine ornamentation and are hence not recommended as a standard technique for the family Ophioglossaceae (33, 34). The spore characters were recorded. Polar length (P) and equatorial length (E) were measured from four spore samples of each species, and the P/E ratio was calculated, and the size of the spores was determined accordingly (35).

Results

Ophioglossum costatum R. Br. Prod. Fl. Nov. Holl. 163.1810. (Fig. 1: 1A-1D)

Ophioglossum pedunculosum Desv. In Ges. Naturf. Freunde Berlin Mag. Neuesten Entdeck. Gesamten Naturk. 5:306. 1811, *Ophioglossum fibrosum* Schumach. In Beskr. Guin. Pl.: 452. 1827, *Ophioglossum wightii* Grev. & Hook. In Bot. Misc. 3: 218. 1833, *Ophioglossum vulgatum* var. *costatum* (R.B.) Hook.f. in Fl. Tasman. 2: 153. 1858, *Ophioglossum brevipes* Bedd., in Ferns. Southern India 23. t. 72. 1863, *Ophioglossum aphrodisiacum* Welw. ex Hook. In Hooker & Baker, Syn. Fil.: 446. 1868, *Ophioglossum bulbosum* Bedd. In Suppl. Ferns S. Ind.: 28. 1876, Bedd., Handb. 465. t. 289. 1883, Suppl. 109. 1892, *Ophioglossum vulgatum* var. *pedunculosum* (Desv.) Domin in Biblioth. Bot. 20: 221. 1913, *Ophioglossum felixii* Tardieu in Notul. Syst. (Paris) 13: 169 1948 publ. 1949, *Ophioglossum hitkishorei* M.Patel & M.N.Reddy in Bot. Lett. 166: 426. 2019, *Goswamia hitkishorei* (M.Patel & M.N.Reddy) Li Bing Zhang & Liang Zhang in Molec. Phylogen. Evol. 173-107512: 23. 2022, *Goswamia costata* (R.Br.) Li Bing Zhang & Liang Zhang in Molec. Phylogen. Evol. 173-107512: 23. 2022

Spores are globose and trilete; the distal face sizes up to 30.28 x 31.8-40.66 x 41.44µm; In micro ornamentation, the spores are foveolate, granulose, reticulate, muri anastomosing; lumina tetragonal-pentagonal-circular, narrow-wide; proximal face concave with a deep proximal cavity having shallow lumina; triradiate leasural arms unequal, one arm double the length of the rest of the two

arms, more or less straight, less wavy, extending up to the rim; Polar axis 16.35-18.42 µm, Equatorial axis (proximal face) 25.19-37.02 µm mean P/E ratio is 0.58±0.04 µm, large spores (Fig 3: 1A-1D).

Specimens examined : India: South India: Kerala: Ernakulam Dist: Malayattoor: 10°21'60.32"N, 76°50'87.46"E. Kanur Dist: Madayippara 12°02'68.86"N, 75°26'30.12"E. Palakkad Dist.: Kallekad 10°47'2"N and 76°36'2"E, Thrissur Dist.: Cheruchakkichola 10°42'05.26"N, 76°11'59.07"E, Killannur. 10°61'56.38"N, 76°24'97.55"E, Kayampooovam 10°41'10.16"N, 76°23'07.50"E: Afsana Khan, Anto P.V. 20, 22, 18, 16, 24, 17 (STC!)

Ophioglossum gramineum Willd. Abhandl. Kurf.-Mainz. Akad. Wiss. Erf. 2(6): 18, t. 1, f. 1. 1802. (Fig. 1: 2A-2D)

Ophioglossum vulgatum var. *gramineum* (Willd.) Hook.f. in Fl. Nov.-Zel. 2: 30.1854, *Ophioglossum dietrichiae* Prantl in Ber. D tsch. Bot. Ges. 1: 352. 1883, *Ophioglossum lanceolatum* Prantl in Ber. Deutsch. Bot. Ges. 1: 352. 1883, *Ophioglossum vulgatum* var. *lanceolatum* Luerss. In J. Mus. Godeffroy 3: 247. 1875, *Ophioglossum moluccanum* f. *inconspicuum* Rac. In Natuurk. Tijdschr. Ned.-Indië 59. 237. 1900, *Ophioglossum prantlii* C.Chr. In Index Filic.: 471. 1906, *Ophioglossum inconspicuum* (Rac.) Alderw. In Bull. Dép. Agric. Indes Néerl. 21: 9. 1908, *Ophioglossum inconspicuum* f. *majus* Alderw. In Bull. Dép. Agric. Indes Néerl. 21: 9.1908, *Ophioglossum gregarium* Christ in Nova Guinea 8: 164. 1909, *Ophioglossum vulgatum* var. *prantlii* (C.Chr.) W.R.B.Oliv. In Trans. & Proc. New Zealand Inst. 49: 126. 1917, *Ophioglossum gramineum* var. *majus* (Alderw.) Wieff. In Blumea 12: 324. 1964, *Ophioglossum gracile* Pocock ex J.E.Burrows in Bothalia 25: 61. 1995

Spores are globose and trilete; the distal face sizes up to 36.59 x 35.42 µm; granulose, retate-reticulate; lumina are deep, conical-circular; muri reticulate with pointed triangular edges; proximal cavity narrow, with shallow lumina on its face; triradiate leasural arms are short, less wavy, restricted only to the half portion of the proximal cavity, only one among the three leasural arms is extended to the rim and jointed to the edge, rest of the two arms are ends within the cavity. Polar axis 17.28-20µm, Equatorial axis (proximal face) 25.36-33.3µm, mean P/E ratio is 0.59±0.03 µm, large spores (Fig. 3: 2A-2D)

Specimens examined : India: South India: Kerala: Kasaragod District: Posadigumpe: 12°40'33.55"N, 75°01'03.85"E., Palakkad District: Kallekad 10°47'2"N, 76°36'2"E, Thrissur District: Killannur. 10°61'56.38"N, 76°24'97.55"E. Peruvanmala 10°62'25.27"N, 76°13'46.49"E., Afsana Khan, Anto P.V. 23, 19, 20, (STC!)

Ophioglossum lusitanicum L., Sp. Pl. 2: 1063. 1753. (Fig. 1: 3A-3D)

Ophioglossum loureiroanum C.Presl in Suppl. Tent. Pterid.: 55. 1845, *Ophioglossum vulgatum* var. *lusitanicum* (L.) Hook. & Arn. In Brit. Fl., ed. 8, éd. 8: 593. 1855, *Ophioglossum vulgatum* subsp. *lusitanicum* (L.) Hook.f. in Student. Fl. Brit. Isl.: 469. 1870, *Ophioglossum braunii* Prantl in Ber. Deutsch. Bot. Ges. 1:351. 1883, *Ophioglossum vulgatum* subsp. *lusitanicum* Bonnier & Layens in Tabl. Syn. Pl. Vasc.

France: 381. 1894, *Ophioglossum lusitanicum* var. *latifolium* Rouy in G. Rouy & J. Foucaud, Fl. France. 14: 460. 1913, *Ophioglossum lusitanicum* var. *longepedunculatum* Rouy in G. Rouy & J. Foucaud, Fl. France 14: 460. 1913, *Ophioglossum vulgatum* var. *macrophyllum* Rouy in G. Rouy & J. Foucaud, Fl. France 14: 458. 1913.

Spores globose-ellipsoid, trilete, dimorphic; distal face sizes up to $34.69 \times 29.21 \mu\text{m}$; patellate, sporoderm is smooth due to the extensive deposition of perine layer over it, lumina shallow, irregular in shape; muri reticulate, smooth and flattened; the proximal face is dimorphic with and without a proximal cavity; the triradiate leasural arms almost equal, more straight, less wavy, extending to the rim and jointed to the edges. Polar axis $14.36\text{--}17.21 \mu\text{m}$, Equatorial axis (proximal face) $22.23\text{--}30.36 \mu\text{m}$, mean P/E ratio is $0.58 \pm 0.03 \mu\text{m}$, large-sized spores (Fig. 3: 3A-3D).

Specimens examined : India: South India: Kerala: Kasaragod District: Posadigumpe $12^{\circ}40'33.55''\text{N}$, $75^{\circ}01'03.85''\text{E}$., Thrissur District: Killannur. $10^{\circ}61'56.38''\text{N}$, $76^{\circ}24'97.55''\text{E}$, Peruvanmala. $10^{\circ}62'25.27''\text{N}$, $76^{\circ}13'46.49''\text{E}$., Afsana Khan, Anto P.V. 31, 7, 6 (STC!)

Note : There was an opinion that the Indian *O. lusitanicum* corresponds to the African *O. lusoaffricanum* (Fraser Jenkins *et al.* 2021). But *O. lusitanicum* (Type: France: Brest, Deschamps, (Epitype, MNHN, digital image!)) and *O. lusoaffricanum* (Type: Angola, apaungo Andongo, Welwitsch 34, K, digital image!) collected from different localities of Kerala during this study is different in the morphological characters of vegetative parts and in the exine ornamentation. The dorsal face of *O. lusitanicum* is smooth, whereas in *O. lusoaffricanum*, spores are verrucate. The spores of *O. lusitanicum* are trilete and dimorphic with or without a clear deep proximal cavity, whereas *O. lusoaffricanum* spores are alete or trilete with a proximal cavity.

Ophioglossum lusoaffricanum Welw. ex Prantl., Ber. Deutsch. Bot. Ges. 1: 351. 1883. (Fig. 1: 4A-4D)

Spores under Light microscope (LM) are large, verrucate, dimorphic with alete and trilete spores, globose; In SEM, the dorsal surface non astamosing, areoles are absent, ventral surface alete or trilete. Trilete spores have arms extending up to rim, unequal, one arm double the size of rest of the two arms, the deep proximal cavity have small lumina. Polar axis $21.49\text{--}32.54 \mu\text{m}$, Equatorial axis (proximal face) $42.28\text{--}66.73 \mu\text{m}$, mean P/E ratio is $0.52 \pm 0.02 \mu\text{m}$, large spores (Fig. 3: 4A-4D)

Specimens examined : Kerala, Thrissur Distr. Killannur, $10^{\circ}37'7.5828''\text{N}$, $76^{\circ}15'11.9628''\text{E}$., Afsana Khan, Anto P.V.15, (STC!)

Ophioglossum parvifolium Grev. & Hook., Bot. Misc. 3: 218. 1833. (Fig. 1:5A-5D)

Ophioglossum macrorrhizum Kunze in Analecta Pteridogr.: 2. 1837, *Ophioglossum schmidii* Kunze in Linnaea 24: 246. 1851, *Ophioglossum vulgatum* var. *macrorrhizum* (Kunze) Luerss. In J. Mus. Godeffroy 3: 242. 1875, *Ophioglossum luerssenii* Prantl in Ber. Deutsch. Bot. Ges. 1:352. 1883, *Ophioglossum tenerum* Mett. ex Prantl in Ber. Deutsch. Bot. Ges. 1:352. 1883, *Ophioglossum moluccanum* f. *pumilum*

Rac. In Natuurk. Tijdschr. Ned.-Indië 59. 237. 1900, *Ophioglossum pumilum* (Rac.) Alderw. In Malayan Ferns: 774. 1909, *Ophioglossum schlechteri* Brause in Bot. Jahrb. Syst. 49: 58. 1912, *Ophioglossum vulgatum* var. *luerssenii* (Prantl) Domin in Biblioth. Bot. 20: 222. 1915, *Ophioglossum nudicaule* var. *macrorrhizum* (Kunze) R. T. Clausen in Mem. Torrey Bot. Club 19(2): 150. 1938, *Ophioglossum nudicaule* var. *tenerum* (Mett. ex Prantl) R. T. Clausen in Mem. Torrey Bot. Club 19(2): 146. 1938.

Spores globose and trilete; distal face sizes up to $33.67 \times 30.69 \mu\text{m}$; granulose, reticulate- patellate, perine layer is present; lumina are shallow, more or less circular, muri reticulate; triradiations less wavy, extending up to the rim; one leasural arm is double the length of rest of the two arms, Polar axis is $14.11\text{--}16.7 \mu\text{m}$, the Equatorial axis $21\text{--}33.01 \mu\text{m}$, mean P/E ratio is $0.56 \pm 0.05 \mu\text{m}$, large spores (Fig. 3: 5A-5D).

Specimens examined : India: South India: Kerala: Thrissur District: Cheruchakkichola $10^{\circ}42'13.82''\text{N}$, $76^{\circ}12'12.39''\text{E}$., Peruvanmala. $10^{\circ}62'25.27''\text{N}$, $76^{\circ}13'46.49''\text{E}$., Afsana Khan, Anto P.V. 8, 5 (STC!)

Ophioglossum pendulum L. Sp. Pl., ed. 2. 2: 1518. 1763. (Fig. 2: 1A-1D)

Ophioderma pendulum L. C. Presl in Suppl. Tent. Pterid.: 56. 1845, *Ophioglossum furcatum* J. Sm. In Ferns Brit. For.: 272. 1866, nom. nud., *Ophioglossum moultonii* Copel. In J. Straits Branch Roy. Asiat. Soc. 63: 72. 1912.

Spores are globose and trilete and comparatively more enormous than the spores of other *Ophioglossum* species included in this study; the distal face sizes up to $51.83 \times 52.26 \mu\text{m}$; perine layer deposition towards the centre; lumina narrow, more or less conical, muri reticulate, very thin and narrow without sharply pointing edges, somewhat wavy and smooth in appearance; the proximal cavity is shallow, triradiate leasural arms extending to the margins. Polar axis $25\text{--}28.02 \mu\text{m}$, Equatorial axis $39.7\text{--}43.69 \mu\text{m}$, mean P/E ratio is $0.62 \pm 0.02 \mu\text{m}$, large spores (Fig. 3: 6A-6D).

Specimens examined : 12832 (KFRI!)

Ophioglossum petiolatum Hook., Exot. Fl. 1(4): t.56. 1823. (Fig. 2: 2A-2D)

Ophioglossum moluccanum Schltld. In Adumbr. Pl.: 9. 1825, *Ophioglossum elongatum* A. Cunn. In Compan. Bot. Mag. 2: 361. 1837, *Ophioglossum moluccanum* f. *Complicatum* Miq. In Ann. Mus. Bot. Lugduno-Batavi 4: 290. 1870, *Ophioglossum vulgatum* var. *Australasiaticum* Luerss. In J. Mus. Godeffroy 3: 246. 1875, *Ophioglossum litorale* Makino in J. Jap. Bot. 6: 27. 1929, *Ophioglossum floridanum* E.P.St.John in Amer. Fern J. 26: 53. 1936, *Ophioglossum floridanum* f. *Favosum* E.P.St.John in Amer. Fern J. 26: 54. 1936, *Ophioglossum floridanum* f. *Reticulosum* E.P.St.John in Amer. Fern J. 26: 55. 1936, *Ophioglossum reticulatum* f. *Complicatum* (Miq.) Wieff. In Blumea 12: 330. 1964.

Spores are trilete and globose, rugate, granulose; distal face sizes up to $30.68 \times 33.75 \mu\text{m}$ in SEM.; lumina as minute depressions, more or less rounded; muri as discontinuous ridges with thick and thin exine regions, thick

ridges have granulose deposition over it, perine layer absent; the triradiated leasural arms are almost equal, wavy, jointed, up to the rim of the deep proximal cavity, granulose. Polar axis 17.21-18.09 μm , Equatorial axis 33.01-34.07 μm , mean P/E ratio is $0.52 \pm 0.00 \mu\text{m}$, large spores (Fig. 3: 7A - 7D).

Specimens examined : India: South India: Kerala: Idukki District: Munnar 10° 4' 5.988" N, 77° 4' 0.984" E, Stephen Sequeira, Afsana Khan, Anto P.V.14 (STC!)

Ophioglossum raphaelianum Anto, Afs.Khan, F.Francis & I.Antony, Int. J. Advanced Res. 4(5): 1269, f.1-2. 2016. (Fig. 2: 3A-3D)

Ophioglossum malviae M. Patel & M. N. Reddy in Sci. Rep.8 (art. 5911): 1. 2018, *Goswamiana malviae* (M. Patel & M. N. Reddy) Li Bing Zhang & Liang Zhang in Molec. Phylogen. Evol. 173-107512: 24. 2022, *Goswamiana raphaeliana* (Anto, Afs. Khan, F. Francis & I. Antony) Li Bing Zhang & Liang Zhang in Molec. Phylogen. Evol. 173-107512: 24. 2022.

Spores are trilete and globose-ellipsoid, foveolate; distal face sizes up to $26.24 \times 20.44 \mu\text{m}$ in SEM.; lumina shallowly depressed, more or less square shaped; muri reticulate, narrow without sharp pointed edges, perine layer present; the triradiated leasural arms are more or less wavy, jointed, up to the centre of the proximal cavity and are pointing towards the wall in between trilobed proximal end; the distal face is granulose, Polar axis 14.27-18.96 μm , Equatorial axis 30.53-37.4 μm , mean P/E ratio is $0.53 \pm 0.04 \mu\text{m}$, large spores (Fig. 3: 8A-8D).

Specimens examined : India: South India: Kerala: Thrissur District: Mangadu, Kottappuram. 10°68'90.41"N, 76° 19'53.83"E. 2 (STC!), Peruvanmala. 10°62'25.27"N, 76° 13'46.49"E.Afsana Khan, Anto P.V 112 (CAL!)

Note : *O. raphaelianum* is distinguished by olivaceous or bluish green-coloured tropophylls, whereas *O. rubellum* is distinguished by its red or copper-coloured tinge. The globose-ellipsoid rhizome of *O. raphaelianum* is distinguishable from the orbicular-fusiform rhizome of *O. rubellum*. *O. raphaelianum* has elliptic-orbicular tropophylls, whereas *O. rubellum* has spatulate-obovate, ovate, or suborbicular tropophylls. However, *O. raphaelianum* has a 1-3 cm long fertile segment with 6-7 alternately arranged sporangia per spike, whereas *O. rubellum* has a 1.5-7 cm long fertile segment with 6-10 oppositely arranged sporangia. The spore characters are also distinct for the two species, and hence the species status of *O. raphaelianum* is reinstated here. The spores of *O. raphaelianum* are foveolate-granulose with more or less square-shaped shallow lumina and narrow muri, whereas *O. rubellum* has lophate spores with an outer exospore wall raised to form ridges. Detailed examination of the fresh specimens as well as the type specimens of *O. rubellum* (Type: Africa; Angola, Welwitsch 33 (Isotype, BM, digital image!)), and *O. raphaelianum* (Type: India; Kerala, Anto P.V.112 (holotype, CAL!)) were carried out. Based on the morphological characters of vegetative parts and spore ornamentation of both species, the species status of *O. raphaelianum* is reinstated here.

***Ophioglossum reticulatum* L.**, Sp. Pl. 2: 1063. 1753. (Fig. 2: 4A-4D).

Ophioglossum ovatum Bory in Voy. iles Afrique 2: 206. 1804, *Ophioglossum cordifolium* Roxb. In Numer. List: n. 47; Roxb. Calc. Journ. 1829, *Ophioglossum peruvianum* C. Presl in Suppl. Tent. Pterid.: 52. 1845, *Ophioglossum cognatum* C. Presl in Suppl. Tent. Pterid.: 53. 1845. *Ophioglossum cumingianum* C. Presl in Suppl. Tent. Pterid.: 52. 1845. *Ophioglossum timorense* Miq. In Ann. Mus. Bot. Lugduno-Batavi 4: 93. 1868, *Ophioglossum moluccanum* f. *Dilatatum* Miq. In Ann. Mus. Bot. Lugduno-Batavi 4: 92. 1868, *Ophioglossum vulgatum* var. *Minutum* F.M.Bailey in Bot. Bull. Dept. Agric. Queensland 5: 27. 1892, *Ophioglossum reticulatum* var. *Acutius* Christ in Denkschr. Kaiserl. Akad. Wiss., Wien. Math.-Naturwiss. Kl. 79(1): 56. 1908, *Ophioglossum reticulatum* var. *Polyangium* Christ in Denkschr. Kaiserl. Akad. Wiss., Wien. Math.-Naturwiss. Kl. 79(1): 56. 1908, *Ophioglossum usterianum* Christ in A.Uster, Fl. Umgebung São Paulo: 137. 1911, *Ophioglossum raciborskii* Alderw. In Bull. Jard. Bot. Buitenzorg, sér. 2, 28: 35. 1918, *Ophioglossum pedunculatum* Desv. & Nakai in Bot. Mag. (Tokyo) 40: 373. 1926, *Ophioglossum ramosii* Copel. In Philipp. J. Sci. 56: 97. 1935, *Ophioglossum austroasiaticum* Nishida in J. Jap. Bot. 34: 46. 1959, *Ophioglossum reticulatum* f. *Dilatatum* (Miq.) Wieff. In Blumea 12: 329. 1964, *Ophioglossum holm-nielsenii* B.ollg. In Fl. Ecuador 66: 16. 2001, *Ophioglossum aletum* M.Patel, M.N.Reddy & H.K.Goswami in Indian Fern J. 35: 323. 2018, *Ophioglossum chaloneri* H. K. Goswami, M. Patel & K. K. Nag in Phytotaxa 468: 103. 2020.

Spores are globose-ellipsoid, trilete, reticulate, distal face sizes upto $32.95 \mu\text{m} \times 27.50 \mu\text{m}$; lumina shallowly reticulate, perine layer is present, muri narrow, without pointed edges; triradiate leasural arms are wavy, more or less equal, jointed, up to the rim. Polar axis 15.55-23.72 μm , Equatorial axis 28.2-40.82 μm , mean P/E ratio $0.55 \pm 0.01 \mu\text{m}$, large spores (Fig. 3: 9A- 9D).

Specimens examined : India: South India: Kerala: Idukki District: Munnar 10°04'07.10" N, 77°03'58.74" E. Stephen Sequeira, Afsana Khan, Anto P.V, Kozhikode District: Farook College 11°11'57.44"N, 75°51'27.12"E., Malappuram District: Nilambur 11°16'13.61"N, 76°12'26.46"E., Afsana Khan, Anto P.V. 21, 23, 12 (STC!)

Ophioglossum rubellum Welw. ex A.Braun., Filic. Afr. [Kuhn] 179. 1868. (Fig. 2: 5A-5D).

O. oleosum Khand. In Indian Fern J. 4: 102. 1987.

Spores under Light microscope (LM) are trilete or alete and globose, lophate or smooth. In SEM, spore sizes up to $22 \times 33 - 25.69 \times 22.29 \mu\text{m}$, the dorsal surface is convex in shape, muri are lophate with outer exospore wall raised to form ridges, muri surrounds the lumina; lumina are polygonal with three- five angles, some spores have very

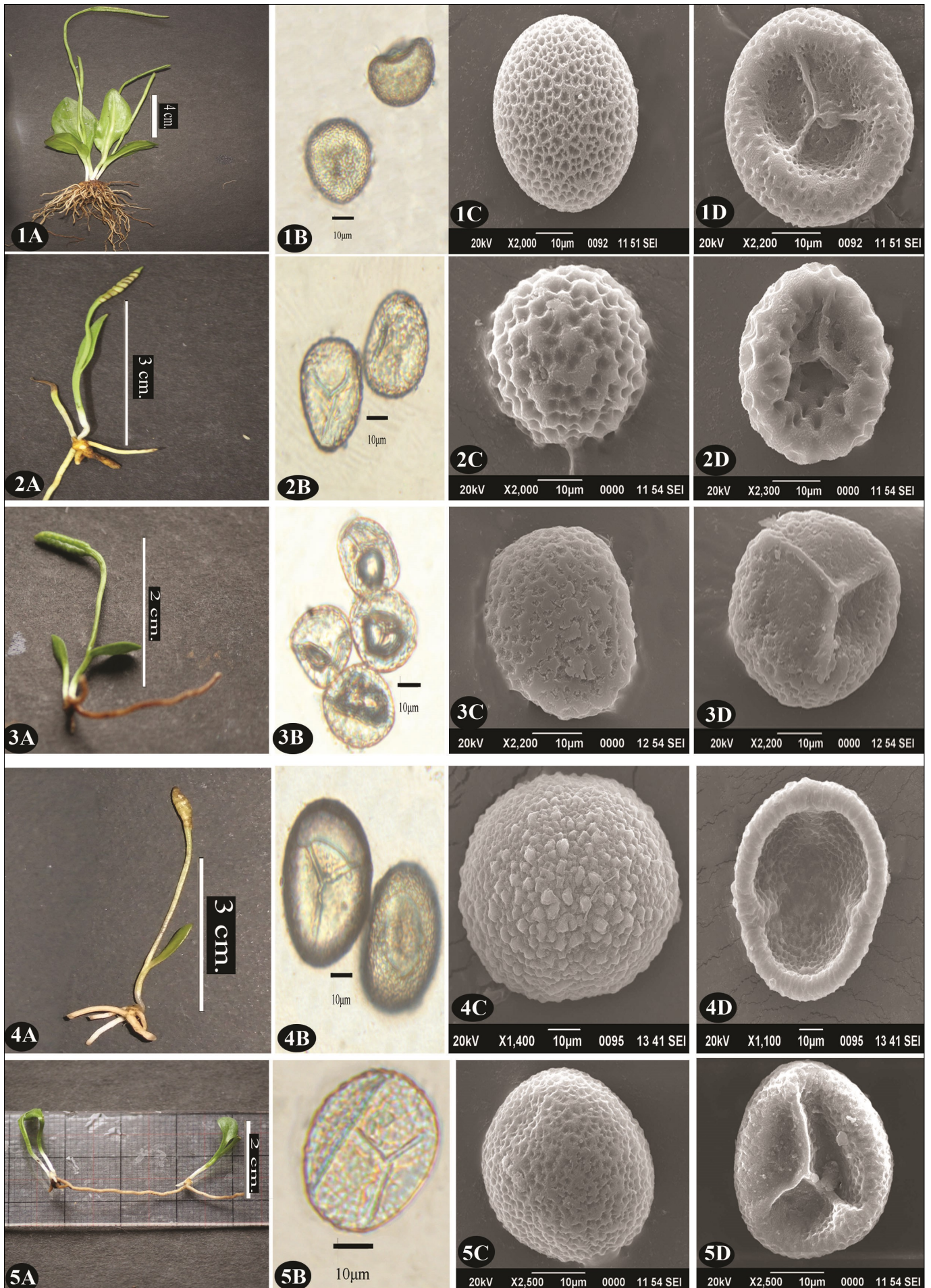


Fig1. Entire plant, Spore surface under LM., Distal spore surface under SEM, Proximal spore surface under SEM of different *Ophioglossum* species. **1A-1D.** *O. costatum*, **2A-2D.** *O. gramineum*, **3A-3D.** *O. lusitanicum*, **4A-4D.** *O. lusoaffricanum*, **5A-5D.** *O. parvifolium*

smooth and plain surface in the complete absence of lumi-

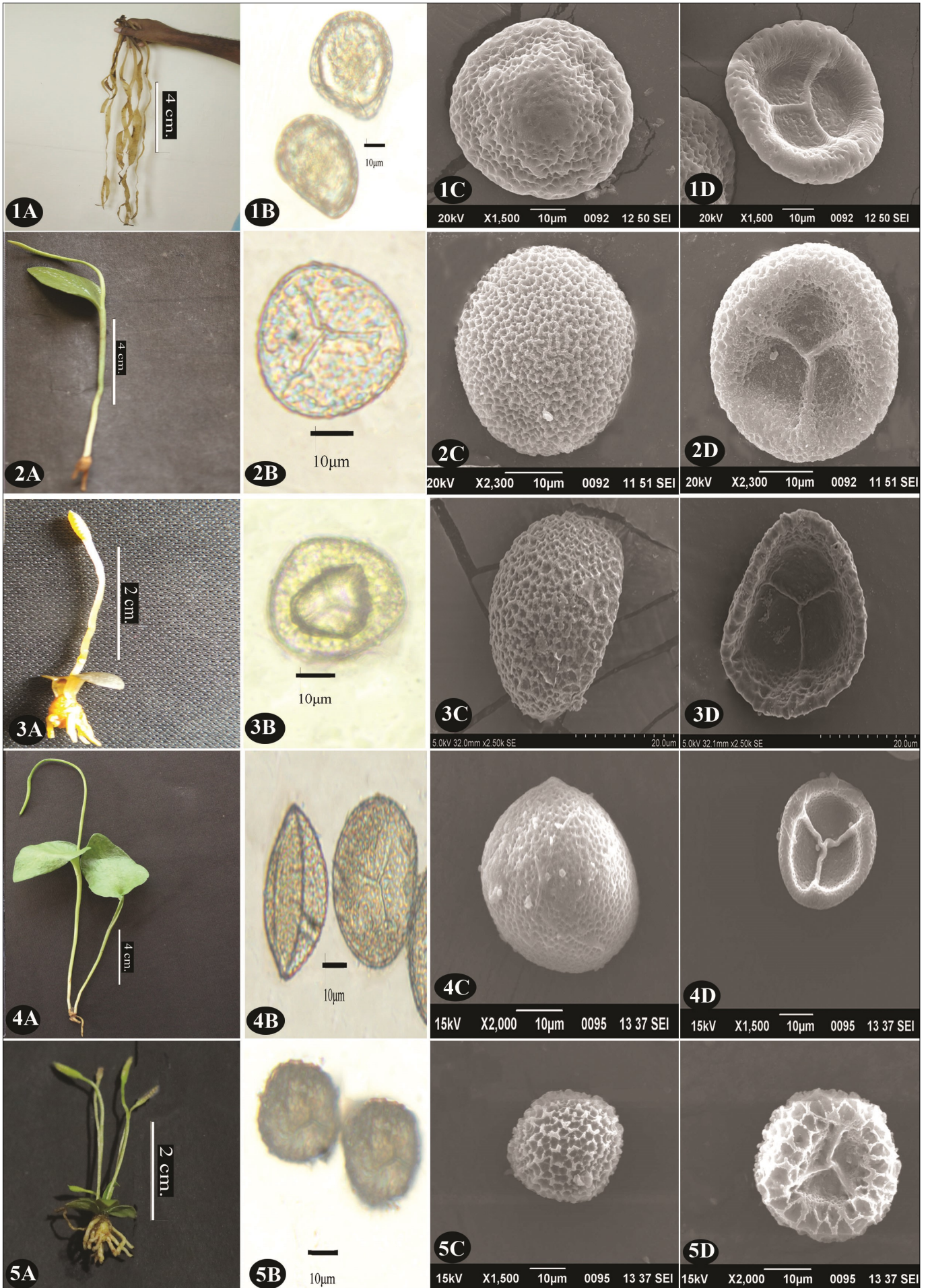


Fig 2. Entire plant, Spore surface under L.M., Distal spore surface under SEM, Proximal spore surface under SEM of different *Ophioglossum* species. **1A-1D.** *O. pendulum*, **2A-2D.** *O. petiolatum*, **3A-3D** *O. raphaelianum*, **4A-4D.** *O. reticulatum*, **5A-5D.** *O. rubellum*

na and muri; in trilete spores, the leasalar arms more wavy and jointed, almost equal, ends at middle of the proximal

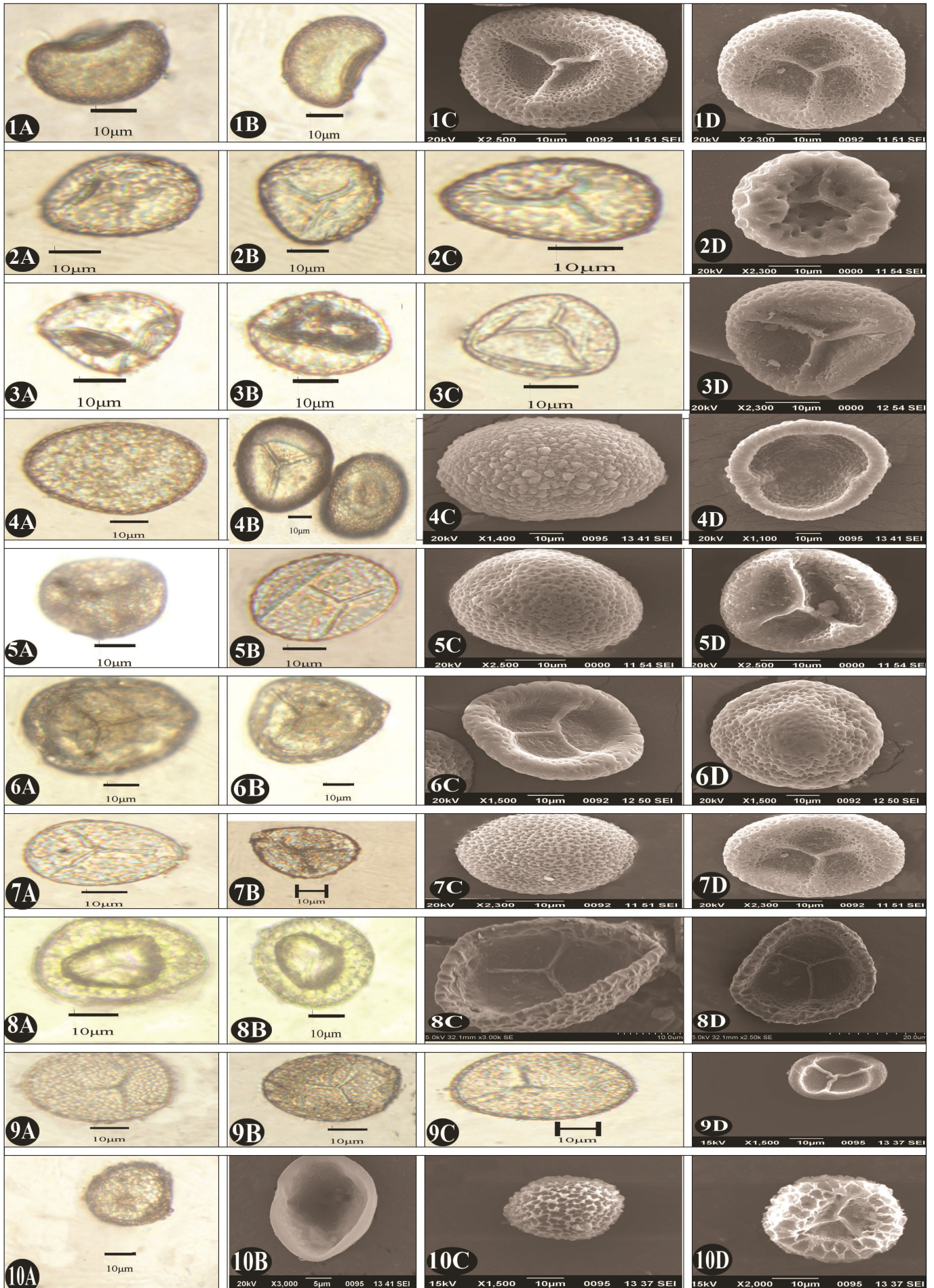


Fig 3. Spores used for P/E ratio calculation. **1A-1D.** *Ophioglossum costatum*, **2A-2D.** *O. gramineum*, **3A-3D.** *O. lusitanicum*, **4A-4D.** *O. lusoaficanum*, **5A-5D.** *O. parvifolium*, **6A-6D.** *O. pendulum*, **7A-7D.** *O. petiolatum*, **8A-8D.** *O. raphaelianum*, **9A-9D.** *O. reticulatum*, **10A-10D.** *O. rubellum*

cavity; ventral surface is concave shaped, and having an ornamentation similar to that of distal side, with de-

Table 1. Comparison of the spore characters of *Ophioglossum costatum*, *O. gramineum*, *O. lusitanicum*, *O. lusoaffricanum*, *O. parvifolium*, *O. pendulum*, *O. raphaelianum*, *O. reticulatum* and *O. rubellum*

Name of the taxa	Polar length (P) (µm)		Equatorial length (E)(µm)		P/E ratio (µm)		Size class
	Min.- Max.	Mean± SE.	Min.- Max.	Mean± SE.	Min.- Max.	Mean±SE.	
<i>O. costatum</i>	16.35-18.42	17.78±0.48	25.19-37.02	30.62±2.05	0.53-0.72	0.58±0.04	large
<i>O. gramineum</i>	17.28-20	18.27±0.60	25.36-33.3	31.01±1.8	0.53-0.68	0.59±0.03	Large
<i>O. lusitanicum</i>	14.36-17.21	15.57±0.70	22.33-30.36-	27.1±1.79	0.49-0.65	0.58±0.03	Large
<i>O. lusoaffricanum</i>	21.49-32.54	26.03±2.39	42.28-66.73	50.19±5.58	0.48-0.58	0.52±0.02	Large
<i>O. parvifolium</i>	14.11-16.7	15.51±0.46	21-33.01	28.11±2.63	0.47-0.67-	0.56±0.05	Large
<i>O. pendulum</i>	25-28.02	26.02±0.69	39.7-43.69	41.94±0.78	0.57-0.67	0.62±0.02	Large
<i>O. petiolatum</i>	17.21-18.09	17.55±0.21	33.01-34.07	33.60±0.25	0.50- 0.54	0.52±0.00	Large
<i>O. raphaelianum</i>	14.27-18.96	17.15±1.03	30.53-37.4	32.57±1.6	0.38-0.61	0.53±0.04	Large
<i>O. reticulatum</i>	15.55-23.72	20.09±1.94	28.2-40.82	36.19±2.8	0.49-0.58	0.55±0.01	Large
<i>O. rubellum</i>	8.76-14.1	11.46±1.09	22.15-28.89	24.5±1.5	0.36-0.51	0.46± 0.03	Medium

pressed lumina and lophate muri. Polar axis 8.76-14.1µm, Equatorial axis 22.15-28.89µm, mean P/E ratio 0.46±0.03µm, medium sized spores (Fig. 3: 10A- 10D).

Note: By the detailed examination of vegetative as well as spore morphology and comparison with the type specimens, *O. Indicum* B. L. Yadav & H. K. Goswami in Bull. Natl. Mus. Nat. Sci., Tokyo, B. 36: 155 (2010) (Type:Rajasthan, B. L. Yadav 3011, (isotype, CAL, digital image!))

Collected from Kerala was found to be distinct form *O. rubellum* (reinstatement in press) and hence the names *O. indicum* and *Goswamia indica* (B. L. Yadav & H. K. Goswami) Li Bing Zhang & Liang Zhang are not considered as the synonym of *O. rubellum* in this study.

Specimens examined : Kottayam Distr.: Elampally 9° 35' 13.902" N, 76° 42' 46.4688" E, Thrissur Dist.: Mangadu 10° 41' 21.876" N, 76° 11' 43.368" E, Afsana Khan, Anto P.V, 24, 9, (STC!), Pazhiyottumuri 10° 41' 9.978" N, 76° 8' 40.776" E, Vimal K. R, Afsana Khan, Anto P.V, 12, (STC!)

Discussion

Taxonomists used the exine patterns as the most significant features while differentiating species in the *Ophioglossum* genus (36-39). The spore characters were treated as valid for identifying some species (40). They are more consistent, less easily affected by environmental factors and are more reliable; and hence, a thorough examination of the wall layers is necessary, along with the morphological study of each plant part (41, 42, 43, 44, 45, 46). Common spores of the *Ophioglossum* genus have a reticulated pattern (47). The presence of dimorphic spores within the same sporangium was observed by (36, 48). In agreement with this, dimorphic spores are observed in *O. lusitanicum*, *O. lusoaffricanum* and *O. rubellum*. Foveolate-granulose spores are found in *O. costatum*, reticulate-retate spores are present in *O. gramineum*, patellate spores are common in *O. lusitanicum*, verrucate spores in *O. lusoaffricanum*, reticulate-patellate spores in *O. parvifolium*, reticulate and granulose spores in *O. pendulum* and *O. reticulatum*, rugate spores in *O. petiolatum*, foveolate in *O. raphaelianum* and lophate spores present in *O. rubellum*. The exine patterns of *O. parvifolium*, *O. costatum*, *O. lusitanicum* and *O.*

petiolatum in this study correlate with (19, 32, 23). Except for *O. rubellum*, all the species examined have large spores; the spores of the epiphytic *O. pendulum* are the largest, with a P/E ratio of 0.0.62±0.02. Even though *O. pendulum* is an epiphyte, its spore characters are similar to those of other terrestrial species. Trilete spores are common among all the species, but alete spores are present in *O. lusoaffricanum* and *O. rubellum*. The detailed comparison of spore characters between the ten collected species is given in Table 1.

The morphological variations in the vegetative characters and spore characters are common within the same species of genus *Ophioglossum* (14, 15). Spores of *O. rubellum* collected from Thrissur and Palakkad districts have similar spore ornamentation. But the ornamentation slightly varies in the specimens collected from Kottayam District. Variations are also observed in the cytotypes of *O. costatum*. The morphological characters of *O. madhusoodhananii* Sojan, V.S.A. Kumar, Sindhu Arya, V. Suresh, L. Leeja & Alen Alex (49) are similar to the characters of *O. costatum*. Mahabale (2) collected and described several specimens from India with cormatous rhizomes, bifurcated spikes, lanceolate-ovate tropophylls. He identified these specimens as *O. Fibrosum* Schumach. *O. fibrosum* was synonymised as *O. costatum* by Roskov *et al.* (50). The reticulate, granulose, and foveolate spores are common in all the cytotypes of *O. costatum*. The luminal width may vary within the spores of the same species. So, the present study considered *O. madhusoodhananii* as a cytotype of *O. costatum*.

Conclusion

The species identification by considering the morphology of the external plant parts along with the spore morphology sounds more authentic in the genus *Ophioglossum*. The spores of the ten *Ophioglossum* species collected from Kerala, Southern India, viz., *O. costatum*, *O. gramineum*, *O. lusitanicum*, *O. lusoaffricanum*, *O. parvifolium*, *O. pendulum*, *O. petiolatum*, *O. raphaelianum*, *O. reticulatum*, and *O. rubellum* possess unique and distinct characters for each species. The presence of *O. lusitanicum* in India was con-

firmed, the species status of *O. raphaelianum* was reinstated and *O. madhusoo-dhananii* was synonymised with *O. costatum*.

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Authors contributions

AK carried out the spore morphology studies, participated in the plant collection and identification and drafted the manuscript. APV participated in collection, identification and drafting of the manuscript. IA participated in the coordination of the work and correction of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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