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RESEARCH ARTICLE

PALYNOLOGICAL CHARACTERIZATION OF FERNS OF ACARAI STATE PARK, SÃO FRANCISCO DO SUL, SANTA CATARINA STATE, SOUTHERN BRAZIL

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ABSTRACT

Sporopalynological descriptions are provided for fourteen species of ferns found to occur in Acarai State Park, a full-protection conservation unit in Praia Grande, São Francisco do Sul, Santa Catarina, Brazil: *Asplenium brasiliense* Sw., *Campyloneurum acrocarpon* Fée, *Cyathea phalerata* Mart, *Lindsaea lancea* (L.) Bedd, *Macrothelypteris torresiana* (Gaudich.) Ching, *Microgramma vacciniifolia* (Langsd and Fisch.). Copel, *Niphidium crassifolium* (L.) Lellinger, *Pecluma chnoophora* (Kunze) Salino and Costa Assis, *Pecluma pectinatiformis* (Lindm.) M.G. Price, *Lepidopteris pleopeltis* (Langsd. and Fisch.) de la Sota, *Rumohra adiantiformis* (G. Forst.) Ching, *Schizaea elegans* (Vahl) Sw., *Serpocaulon latipes* (Langsd. and Fisch.) A.R.Sm. and *Trichomanes cristatum* Kaulf. Botanical material was observed while walking a trail of the park during 26 biweekly expeditions from May 2017 to June 2018. Fertile botanical material was verified and photographed. The studied ferns belong to four orders, eight families and thirteen genera of the subclass Polypodiidae. Spores were prepared, analyzed, photographed and measured using light and scanning electron microscopy. Descriptions and terminology followed the literature. An identification key is provided for the species.

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INTRODUCTION

Ferns occur in a wide variety of ecosystems and exhibit considerable morphological variation, with species ranging from being very small to arborescent (Tryon and Tryon 1982; Prado and Sylvestre 2010). There are 1,361 known species of ferns in Brazil and the state of Santa Catarina is located in one of the richest and most endemic centers for the group the Atlantic Forest Domain making it the fifth richest state for ferns in Brazil (Tryon 1972; Flora do Brasil 2020 under construction). The northern coast of SC has large areas of the Atlantic Forest Domain (Capobianco 2001). The Acarai State Park, a full-protection conservation unit located on Praia Grande, municipality of São Francisco do Sul, along the northern coast of SC, is about 16 kilometers long and encompasses areas of dunes (*restinga)*. The park represents one of the last stretches of the coastal region of Santa Catarina that is free of anthropic occupation (FATMA 2009). Sporopalynological studies contribute to a better understanding of flora since they provide reference material for different areas, assist systematic studies of the botanical group and palynological studies in archaeological and Quaternary sites. Such studies are scarce in Brazil, including Santa Catarina, and there are no palynological studies of ferns for Acarai State Park. Thus, the objectives of the present work were to provide sporopalynological descriptions of the spores of ferns occurring in Acarai State Park, in order to provide useful data to further studies and to present a key for their identification.

MATERIAL AND METHODS

Study area: Acarai State Park (coordinates: 26º 17' S, 48º 33' W) is a full protection conservation unit that covers an area of approximately 6,667 hectares comprising the coast and the Tamboretes archipelago (FATMA 2009). It represents the largest continuous remnant of *restinga* in the state of Santa Catarina, and so is classified as an extremely high-priority area for biodiversity conservation (PROBIO 2003).

The park comprises the entire hydrological complex of the Acarai and Pereque rivers and the Capivaru lagoon. The climate of the region is Cfa (Köppen classification); that is, a humid zone with a predominantly humid mesothermic climate with hot summers (FATMA 2008). São Francisco do Sul, the municipality where it is located, has a mean annual temperature of 20.6 ºC and mean annual rainfall of 1,847.68 mm, with the wettest period being the first quarter (January, February and March). High average temperatures and intense rainfall create conditions for high relative air humidity (FATMA 2008). The island of São Francisco do Sul is characterized by two types of formations: scarp, which encompasses the areas of higher elevation concentrated in the western portion of the island and is part of the geomorphological environment of the Serra do Mar (mountain chain); and plain, which encompasses extensive flat areas at lower elevations and is predominant in all sectors. The distribution of soils in the region is strongly marked by the compartmentalization of the relief and topographic amplitude. The soil types of the island of São Francisco do Sul can be divided into six main orders: cambisols, argisols, spodosols, gleysols, organosols and neosols (FATMA 2008). The vegetation includes areas covered by dense rain forest and associated ecosystems, such as *restinga* and mangroves.

Sampling

Botanical specimens studied represented the orders Cyatheales (Cyatheaceae Kaulf.: *Cyathea phalerata* Mart.); Polypodiales [Aspleniaceae Newman: *Asplenium brasiliense* Sw.; Dryopteridaceae Herter: *Rumohra adiantiformis* (G. Forst.) Ching; Lindsaeaceae C. Presl: *Lindsaea lancea* (L.) Bedd.; Polypodiaceae J. Presl: *Campyloneurum acrocarpon* Fée, *Microgramma vacciniifolia* (Langsd. and Fisch.) Copel., *Niphidium crassifolium* (L.) Lellinger, *Pecluma chnoophora* (Kunze) Salino and Costa Assis, *Pecluma pectinatiformis* (Lindm.) M. G. Price, *Pleopeltis lepidopteris* (Langsd. and Fisch.) de la Sota, *Serpocaulon latipes* (Langsd. and Fisch.) A. R. Sm.; Thelypteridaceae Pic. Serm.: *Macrothelypteris torresiana* (Gaudich.) Ching]; Hymenophyllales (Hymenophyllaceae Gaudich.: *Trichomanes cristatum* Kaulf.) and Schizaeales [Schizaeaceae Kaulf.: *Schizaea elegans* (Vahl) Sw.] (PPG I 2016) (Figures 1 and 2). Twenty six biweekly expeditions were undertaken from May 2017 to June 2018, by walking along a 800-m long trail (Filgueiras *et al*., 1994), beginning in herbaceous *restinga* (HR), passing through arboreal-shrub *restinga* (ASR) and ending in transition forest (TF) where a radius of 100 meters around the end point (*Sambaqui* Casa de Pedra, a shell mound), was also observed. The studied area was selected for further archaeopalynological and paleopalynological analyses of *Sambaqui* Casa de Pedra (Bandeira *et al*., 2018). Fertile botanical herborised material, integrated into the Herbarium Joinvillea of Univille (JOI), was studied. This material is part of Vieira Junior *et al.* (2019) list. Digital images of plants in their natural environments were obtained during field work.

Palynological descriptions: For the palynological descriptions, *sori* were macerated and centrifuged, and the spores processed by acetolysis (Erdtman 1960). For each species, five permanent slides were mounted with glycerinated gelatin and sealed with paraffin or enamel. The slides were numbered and registered at LABEL Palinoteca (Bee Laboratory of Univille). The palynomorphs were photographed and measured from polar (P) and equatorial (E) views, using a light microscope (BIOVAL and Leitz LABORLUX S) equipped with a Dino-Eye Microscope Eye-Piece Camera. Observations were also made using a JEOL JSM-6701F scanning electron microscope at the State University of Santa Catarina (UDESC). For this, non-acetolised spores were spread over the surface of coverslips and numbered. Individual coverslips were then placed on double-sided carbon tape previously adhered to a support. The samples were metallized with a thin layer of gold-palladium for about three minutes and submitted to analysis and photography. For light microscopy, photomicrographs of 25 spores were made for each species. Mean values for exosporium and/or perisporium thickness, polar diameter, major and minor equatorial diameters, *laesura* length and width, and, when possible, the commissure, were calculated from measurements of the 25 spores for each species made with Dino Capture 2.0 software (trilete arms for species with triangular area). Measurements were made in micrometers (µm). The arithmetic mean (µ), standard deviation (S), mean deviation (Sµ), coefficient of variability (CV%) and 95% confidence interval (CI) were calculated for all measurements for each sample using Microsoft Excel. Descriptions and terminology followed the definitions of Tryon and Lugardon (1991) and Lellinger (2002). The size classes and the descriptions of *laesura,* sidesand apex are based on Ybert *et al.* (2012). An identification key for the species was formulated using the palynological characteristics of the palynological descriptions of the present work.

RESULTS

The spores of fourteen species of ferns, belonging to four orders, eight families and thirteen genera of the subclass Polypoiidae, which encompasses the great majority of extant fern species, were studied (PPG I 2016) (Tab.1). The photomicrographs of the studied species are on the Figures 3 to 9. The measurements and descriptions are on Tables 2 to 6.

Palynological descriptions: *Asplenium brasiliense* Sw. (Figure 3 – a-f; Table 2, 3, 6)

Examined material: BRAZIL. SANTA CATARINA: São Francisco do Sul, Acarai State Park. 28.VI.2018, N. P. Vieira Junior and G. R. Schroeder 1 (JOI). Isolated spores monolete; large in size; meridional outline ellipsoidal or reniform; amb elliptic; shape ellipsoidal; concave-convex or plane-convex in equatorial view; *laesura* straight and narrow; perisporium alate, with irregular folds forming an irregular reticle with cones and/or spicules on crest (echinate); inner perisporium with small granules; exosporium psilate.

Campyloneurum acrocarpon Fée (Figure 3 – g-k; Table 2, 3)

Examined material: BRAZIL. SANTA CATARINA: São Francisco do Sul, Acarai State Park. 20.XII.2018, N. P. Vieira Junior and G. R. Schroeder 11 (JOI). Isolated spores monolete; medium in size; meridional outline ellipsoidal or reniform; amb elliptic; shape ellipsoidal; concave-convex or plane-convex in equatorial view; *laesura* straight and narrow; perisporium psilate with the presence of globules, isolated or in masses; exosporium verrucate.

Cyathea phalerata Mart. (Figure 3 – l; Figure 4 – a-e; Table 4, 5)

Examined material: BRAZIL. SANTA CATARINA: São Francisco do Sul, Acarai State Park. 11.V.2017, N. P. Vieira Junior and G. R. Schroeder 6 (JOI).

Table 1. Taxonomic classification of the studied species based on PPG I (2016)

Subclass	Order	Suborder	Family	Subfamily	Species
Polypodiidae	Hymenophyllales		Hymenophyllaceae	Trichomanoideae	*Trichomanes cristatum* Kaulf.
	Schizaeales		Schizaeaceae		*Schizaea elegans* (Vahl) Sw.
	Cyatheales		Cyatheaceae		*Cyathea phalerata* Mart.
	Polypodiales	Lindsaeineae	Lindsaeaceae		*Lindsaea lancea* (L.) Bedd.
		Aspleniineae	Aspleniaceae		*Asplenium brasiliense* Sw.
			Thelypteridaceae	Phegopteridoideae	*Macrothelypteris torresiana* (Gaudich.) Ching
		Polypodiineae	Dryopteridaceae	Elaphoglossoideae	*Rumohraadiantiformis* (G. Forst.) Ching
			Polypodiaceae	Polypodioideae	*Campyloneurum acrocarpon* Fée
					Microgramma vacciniifolia (Langsd. & Fisch.) Copel.
					Niphidium crassifolium (L.) Lellinger
					Pecluma chnoophora (Kunze) Salino & Costa Assis
					Pecluma pectinatiformis (Lindm.) M.G. Price
					Pleopeltis lepidopteris (Langsd. & Fisch.) de la Sota
					Serpocaulon latipes (Langsd. & Fisch.) A.R.Sm.

Figure 1. a-h. Photographs of individuals of the studied plant species in their natural habitat *a. Asplenium brasiliense*; b. *Campyloneurum acrocarpon*; c. *Serpocaulon latipes*; d. *Lindsaea lancea*; e. *Macrothelypteris torresiana*; f. *Microgramma vacciniifolia*; g. *Cyathea phalerata*; h. *Niphidium crassifolium*

Isolated spores trilete; large in size, meridional outline triangular convex and ellipsoidal; amb triangular; shape tetraedric-globose; convex-hemispheric or plane-hemispheric in equatorial view; *laesuri* straight, narrow, medium size; sides slightly straight to concave; apex rounded; perisporium covered by tridimensional network of free or fused rodlets, when fused forming small spicules of various sizes; exosporium psilate.

Lindsaea lancea (L.) Bedd. (Figure 4 – f-k; Table 4, 5, 6)

Examined material: BRAZIL. SANTA CATARINA: São Francisco do Sul, Acarai State Park. 21.II.2018, N. P. Vieira Junior and G. R. Schroeder 17 (JOI). Isolated spores trilete; medium in size, meridional outline triangular convex; amb

Figure 2. a-f. Photographs of individuals of the studied plant species in their natural habitat. a. *Pecluma chnoophora*; b. *Pecluma pectinatiformis*; c. *Pleopeltis lepidopteris*; d *Rumohra adiantiformis*; e. *Trichomanes cristatum*; f. *Schizaea elegans*.

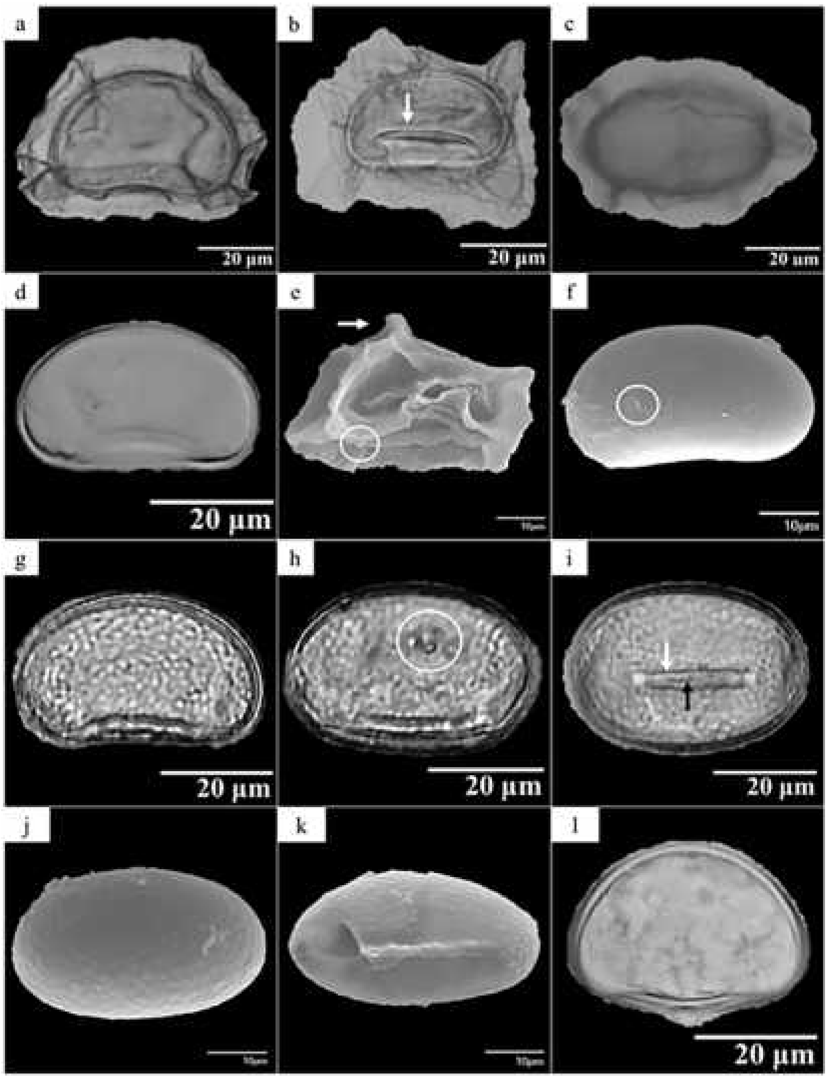

Figure 3. a-l. Photomicrographs (scale equivalent to 20 µm) and electromicrographs (scale equivalent to 10 µm) of spores. a-f. *Asplenium brasiliense*: a. Equatorial view (MO); b. Surface of alate perisporium (arrow indicates laesura) (MO); c. Proximal polar view, with perisporium (MO); d. Equatorial view, spore without perisporium (MO); e. Equatorial view (arrow indicates alate perisporium with irregular folds; circle indicates echinate crest) (MEV); f. Distal polar view (circle indicates granules) (MEV). g-k. *Campyloneurum acrocarpon*: g. Equatorial view (MO); h. Surface with globules (circle) on verrucate exosporium (MO); i. Proximal polar view (white arrow indicates labium and black arrow indicates commissure) (MO); j. Distal polar view (MEV); k. Proximal polar view (MEV). l. *Cyathea phalerata*: Equatorial view (MO).

Table 2. Measurements (µm) of the polar diameter and equatorial diameter, greater and smaller, for monolete spores (n=25; *n (Greater Equatorial Diameter)=4). Xmin – Xmax = range; µ = arithmetic mean; S_μ = standard deviation of the mean; S = standard deviation of the sample; IC = 95% confidence interval; CV = coefficient of variation

Species	Polar Diameter					Greater Equatorial Diameter					Smaller Equatorial Diameter				
	Xmin - Xmax	µ ± Sµ	S	IC 95%	CV	Xmin - Xmax	µ ± Sµ	S	IC 95%	CV	Xmin - Xmax	µ ± Sµ	S	IC 95%	CV
Asplenium brasiliense Sw.	27,96 - 45,43	37,18 ± 0,94	4,68	35,25 - 39,11	12,59	49,47 - 80,38	62,58 ± 1,70	8,52	59,06 - 66,10	13,62	21,60 - 48,23	35,26 ± 1,20	5,99	32,79 - 37,73	16,98
Campyloneurum acrocarpon Fée *	18,87 - 34,25	27,52 ± 0,70	3,50	26,08 - 28,96	12,71	35,97 - 52,15	45,14 ± 0,90	4,49	43,29 - 46,99	9,94	23,81 - 33,94	28,88 ± 2,11	4,23	22,15 - 35,61	14,64
Macrothelypteris torresiana (Gaudich.) Ching	21,86 - 30,90	26,21 ± 0,43	2,17	25,31 - 27,11	8,29	33,24 - 45,73	40,30 ± 0,67	3,33	38,92 - 41,67	8,26	18,82 - 26,90	23,59 ± 0,42	2,08	22,73 - 24,45	8,80
Microgramma vacciniifolia (Langsd. & Fisch.) Copel.	35,68 - 54,39	42,04 ± 0,90	4,51	40,18 - 43,91	10,73	56,08 - 82,19	67,29 ± 1,35	6,77	64,50 - 70,09	10,06	28,49 - 43,38	35,62 ± 0,78	3,92	34,00 - 37,24	11,02
Niphidium crassifolium (L.) Lellinger	42,74 - 63,19	54,77 ± 1,21	6,04	52,27 - 57,26	11,03	58,71 - 97,07	82,77 ± 1,91	9,56	78,83 - 86,72	11,55	33,39 - 50,33	41,97 ± 0,66	3,29	40,62 - 43,33	7,83
Pecluma chnoophora (Kunze) Salino & F. Costa Assis	20,86 - 32,65	27,51 ± 0,57	2,85	26,33 - 28,69	10,37	38,19 - 50,49	43,78 ± 0,65	3,24	42,44 - 45,12	7,40	28,63 - 41,44	33,97 ± 0,81	4,04	32,31 - 35,64	11,89
Pecluma pectinatiformis (Lindm.) M.G.Price	30,44 - 42,37	36,09 ± 0,67	3,33	34,71 - 37,46	9,24	52,20 - 73,23	63,43 ± 1,08	5,38	61,21 - 65,65	8,49	25,44 - 35,66	28,36 ± 0,48	2,38	27,38 - 29,35	8,39
Pleopeltis lepidopterist (Langsd. & Fisch.) de la Sota	40,75 - 56,63	47,73 ± 0,80	3,98	46.09 - 49,37	8,33	73,75 - 97,74	86,22 ± 1,28	6,40	83,58 - 88,87	7,43	28,53 - 50,33	37,28 ± 1,16	5,82	34,88 - 39,68	15,62
Rumohra adiantiformis (G.Forst.) Ching	19,17 - 39,70	24,66 ± 0,76	3,79	23,10 - 26,23	15,38	25,93 - 43,78	37,82 ± 0,82	4,08	36,14 - 39,51	10,79	21,92 - 26,71	23,94 ± 0,17	0,87	23,58 - 24,30	3,63
Schizaea elegans (Vahl) Sw.	21,28 - 42,00	30,67 ± 0,85	4,23	28,92 - 32,41	13,79	38,68 - 57,78	46,55 ± 0,98	4,92	44,52 - 48,58	10,57	21,51 - 34,73	26,36 ± 0,57	2,86	25,18 - 27,54	10,84
Serpocaulon latipes (Langsd. &Fisch.) A.R.Sm.	26,86 - 41,70	34,15 ± 0,81	4,04	32,49 - 35,82	11,82	46,41 - 69,48	58,77± 1,13	5,67	56,44 - 61,11	9,64	23,58 - 40,41	30,07 ± 0,82	4,12	28,37 - 31,76	13,69

Table 3. Measurements (µm) of the length and width of the laesura and thickness of the exosporium for monolete spores (n=25; *n (length and width of the laesura) = 16). Xmin – Xmax = range; µ = arithmetic mean; S_μ = standard deviation of the mean; S = standard deviation of the sample; IC = 95% confidence interval; CV = coefficient of variation

Species	Length of the Laesura					Width of the Laesura					Exosporium				
	Xmin - Xmax	µ ± Sµ	S	IC 95%	CV	Xmin - Xmax	µ ± Sµ	S	IC 95%	CV	Xmin - Xmax	µ ± Sµ	S	IC 95%	CV
Asplenium brasiliense Sw.	18,31 - 32,45	25,27 ± 0,70	3,49	23,83 - 26,71	13,81	2,51 - 5,87	4,28 ± 0,18	0,88	3,91 - 4,64	20,64	1,77 - 3,05	2,27 ± 0,06	0,30	2,14 - 2,39	13,10
Campyloneurum acrocarpon Fée*	19,35 - 34,75	27,04 ± 1,08	4,32	24,74 - 29,35	15,98	2,26 - 4,89	3,60 ± 0,21	0,83	3,16 - 4,04	22,92	2,02 - 3,17	2,67 ± 0,06	0,30	2,55 - 2,80	11,12
Macrothelypteris torresiana (Gaudich.) Ching	20,40 - 30,39	25,89 ± 0,61	3,04	24,63 - 27,15	11,75	2,98 - 7,27	4,94 ± 0,20	0,99	4,53 - 5,35	20,04	1,70 - 2,54	2,04 ± 0,04	0,20	1,96 - 2,12	9,72
Microgramma vacciniifolia (Langsd. & Fisch.) Copel.	29,31 - 44,23	35,10 ± 0,80	3,99	33,45 - 36,75	11,37	2,86 - 4,27	3,45 ± 0,07	0,35	3,30 - 3,60	10,25	2,07 - 3,55	2,56 ± 0,08	0,38	2,40 - 2,72	14,94
Niphidium crassifolium (L.) Lellinger	26,17 - 47,43	34,88 ± 0,97	4,87	32,87 - 36,88	13,95	4,79 - 6,94	6,03 ± 0,13	0,66	5,76 - 6,30	10,91	1,76 - 3,65	2,95 ± 0,10	0,48	2,76 - 3,15	16,15
Peclumachnoophora (Kunze) Salino & F. Costa Assis	24,47 - 35,60	29,31 ± 0,58	2,92	28,10 - 30,51	9,97	2,58 - 4,62	3,56 ± 0,10	0,51	3,35 - 3,77	14,31	2,20 - 3,04	2,60 ± 0,05	0,23	2,51 - 2,70	8,78
Pecluma pectinatiformis (Lindm.) M.G. Price	23,11 - 36,95	29,78 ± 0,80	4,00	28,13 - 31,43	13,43	3,02 - 5,68	3,99 ± 0,14	0,70	3,70 - 4,28	17,50	2,38 - 3,96	3,13 ± 0,09	0,43	2,95 - 3,31	13,87
Pleopeltis lepidopterist (Langsd. & Fisch.) de la Sota	31,58 - 55,46	42,91 ± 1,29	6,47	40,24 - 45,58	15,08	5,01 - 9,78	6,97 ± 0,27	1,37	6,40 - 7,53	19,64	3,03 - 5,73	4,11 ± 0,15	0,73	3,81 - 4,41	17,69
Rumohra adiantiformis (G. Forst.) Ching	19,59 - 31,26	25,06 ± 0,50	2,48	24,04 - 26,08	9,88	2,19 - 4,20	3,34 ± 0,12	0,58	3,10 - 3,57	17,24	1,59 - 2,86	2,22 ± 0,06	0,28	2,11 - 2,34	12,49
Schizaea elegans (Vahl) Sw.	22,55 - 37,86	29,82 ± 0,89	4,44	27,99 - 31,65	14,88	2,38 - 5,17	3,66 ± 0,18	0,90	3,29 - 4,03	24,61	2,39 - 3,65	3,00 ± 0,06	0,32	2,87 - 3,13	10,49
Serpocaulon latipes (Langsd. & Fisch.) A.R.Sm.	24,25 - 48,05	35,37 ± 1,17	5,87	32,94 - 37,79	16,59	3,27 - 9,92	4,74 ± 0,24	1,20	4,24 - 5,23	25,28	1,77 - 2,84	2,17 ± 0,06	0,28	2,06 - 2,29	12,94

Table 4. Measurements (µm) of the polar diameter, equatorial diameter and arms for trilete spores (n=25). Xmin – Xmax = range; µ = arithmetic mean; S_μ = standard deviation of the mean; S = standard deviation of the sample; IC = 95% confidence interval; CV = coefficient of variation

Species	Polar Diameter					Equatorial Diameter					Arms				
	X min – X max	µ ± Sµ	S	IC 95%	CV	X min – X max	µ ± Sµ	S	IC 95%	CV	X min – X max	µ ± Sµ	S	IC 95%	CV
Cyathea phalerata Mart.	40,17 - 51,46	45,91 ± 0,61	3,06	44,65 - 47,18	6,67	52,82 - 70,66	59,84 ± 0,89	4,47	57,99 - 61,69	7,47	26,19 - 36,68	31,77 ± 0,52	2,59	30,70 - 32,84	8,16
Lindsaea lancea (L.) Bedd.	21,54 - 30,07	25,61 ± 0,43	2,16	24,72 - 26,50	8,43	28,94 - 35,65	32,10 ± 0,31	1,57	31,45 - 32,74	4,88	15,71 - 19,53	17,14 ± 0,18	0,89	16,78 - 17,51	5,21
Trichomanes cristatum Kaulf.	32,33 - 40,76	36,13 ± 0,49	2,44	35,12 - 37,14	6,75	47,70 - 56,74	51,82 ± 0,43	2,16	50,93 - 52,71	4,18	22,73 - 28,41	26,18 ± 0,27	1,36	25,62 - 26,74	5,19

Table 5. Measurements (µm) of the length and width of the laesura and thickness of the exosporium for trilete spores (n=25). Xmin – Xmax = range; µ = arithmetic mean; S_μ = standard deviation of the mean; S = standard deviation of the sample; IC = 95% confidence interval; CV = coefficient of variation

Species	**Lenght of the Laesura**					**Width of the Laesura**					**Exosporium**				
	X min – X max	µ ± Sµ	S	IC 95%	CV	X min – X max	µ ± Sµ	S	IC 95%	CV	X min – X max	µ ± Sµ	S	IC 95%	CV
***Cyathea phalerata* Mart.**	14,55 - 26,90	20,44 ± 0,54	2,68	19,34 - 21,55	13,10	2,98 - 5,18	4,01 ± 0,11	0,55	3,78 - 4,23	13,71	2,07 - 3,90	2,65 ± 0,09	0,44	2,47 - 2,83	16,48
***Lindsaea lancea* (L.) Bedd.**	10,36 - 13,30	11,66 ± 0,14	0,72	11,36 - 11,96	6,18	4,03 - 5,31	4,77 ± 0,09	0,43	4,59 - 4,94	9,03	1,79 - 2,88	2,26 ± 0,05	0,27	2,14 - 2,37	12,18
***Trichomanes cristatum* Kaulf.**	20,81 - 27,77	24,05 ± 0,36	1,81	23,30 - 24,79	7,53	3,55 - 7,25	4,98 ± 0,17	0,86	4,63 - 5,34	17,35	1,12 - 2,13	1,64 ± 0,04	0,21	1,55 - 1,72	12,91

Table 6. Measurements (µm) of the thickness of the perisporium (n=25). Xmin – Xmax = range; µ = arithmetic mean; S_μ = standard deviation of the mean; S = standard deviation of the sample; IC = 95% confidence interval; CV = coefficient of variation

Species	Perisporium				
	X min – X max	µ ± Sµ	S	IC 95%	CV
Asplenium brasiliense Sw.	10,01 - 24,34	16,54 ± 0,76	3,80	14,98 - 18,11	22,97
Lindsaea lancea (L.) Bedd.	0,56 - 1,01	0,78 ± 0,03	0,13	0,73 - 0,84	16,48
Rumohra adiantiformis (G. Forst.) Ching	4,43 - 9,81	6,35 ± 0,24	1,18	5,86 - 6,84	18,67
Serpocaulon latipes (Langsd. & Fisch.) A.R. Sm.	2,77 - 12,18	5,76 ± 0,50	2,51	4,73 - 6,80	43,57
Trichomanes cristatum Kaulf.	0,63 - 1,43	1,12 ± 0,04	0,18	1,05 - 1,20	15,60

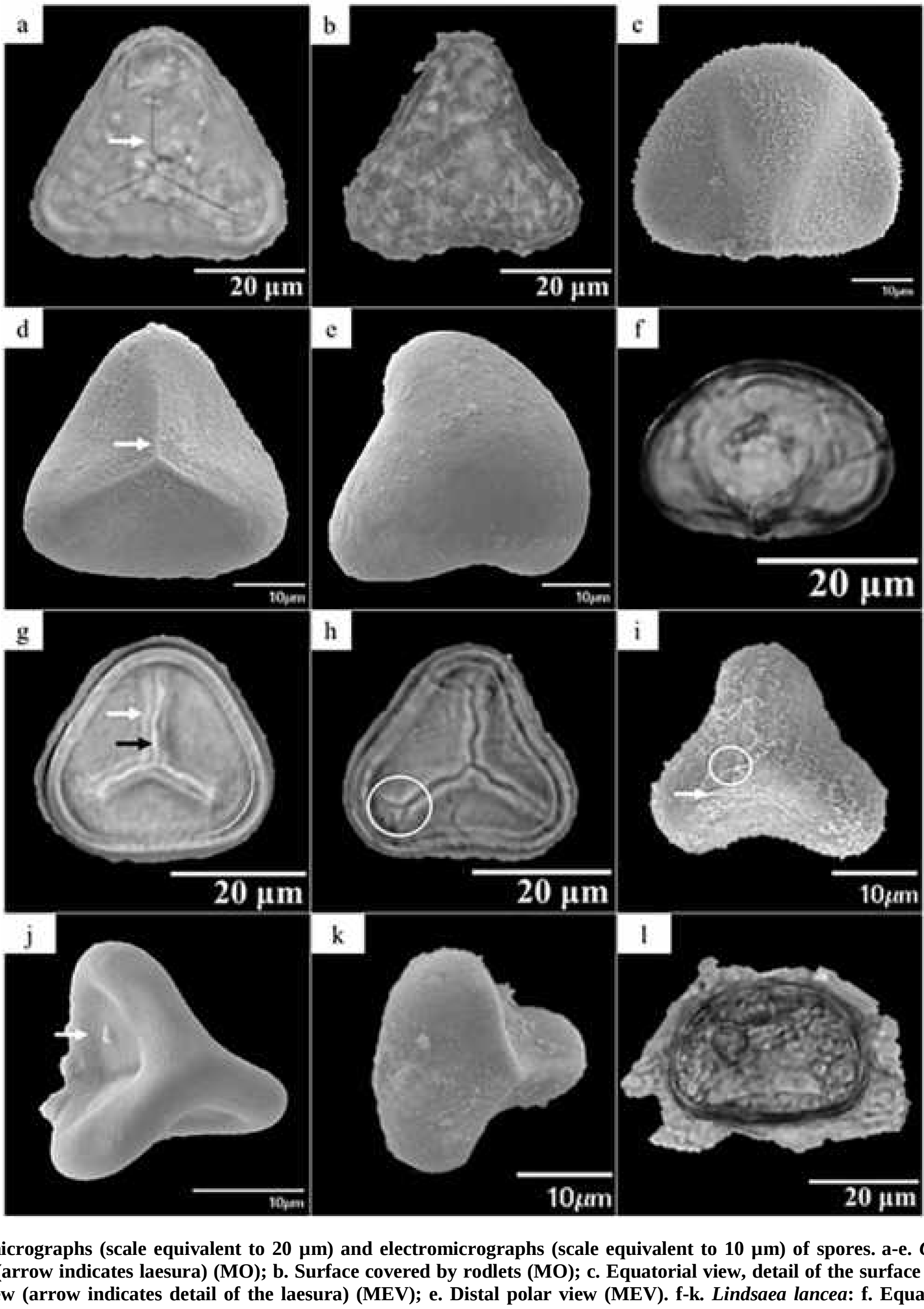

Figure 4. a-l. Photomicrographs (scale equivalent to 20 µm) and electromicrographs (scale equivalent to 10 µm) of spores. a-e. *Cyathea phalerata*: a. Proximal polar view (arrow indicates laesura) (MO); b. Surface covered by rodlets (MO); c. Equatorial view, detail of the surface with rodlets (MEV); d. Proximal polar view (arrow indicates detail of the laesura) (MEV); e. Distal polar view (MEV). f-k. *Lindsaea lancea*: f. Equatorial view (MO); g. Proximal polar view (white arrow indicates labium and black arrow indicates commissure) (MO); h. Forked laesura (circle); i. Proximal polar view (arrow indicates laesura underneath rodlets; circle indicates detail of the fusion of rodlets) (MEV); j. Surface of distal face (MEV) (arrow indicates perisporium vestige); k. Surface of proximal face (MEV). l. *Macrothelypteris torresiana*: Equatorial view with perisporium (MO)

triangular; shape tetraedric-globose; convex-hemispheric in equatorial view; *laesuri* straight to slightly ondulate, narrow, medium in size, forked on the extremities, labium thick and high; sides slightly straight to concave; apex rounded; perisporium covered by tridimensional net of free or fused rodlets, when fused forming small spicules of various sizes; exosporium psilate. Commissure (µm) = 10.72 ± 0.83.

Macrothelypteris torresiana (Gaudich.) Ching (Figure 4 – l; Figure 5 – a-f; Table 2, 3)

Examined material: BRAZIL. SANTA CATARINA: São Francisco do Sul, Acarai State Park. 25.X.2017, N. P. Vieira Junior and G. R. Schroeder 10 (JOI). Isolated spores monolete; medium in size; meridional outline ellipsoidal or reniform;

amb elliptic; shape ellipsoidal; concave-convex or plane-convex in equatorial view; *laesura* straight and narrow; perisporium perforate and reticulate with *baculi* on crest; exosporium microgranulate or gemmate, granules isolated or in masses over layer of exosporium.

Microgramma vacciniifolia (Langsd. and Fisch.) Copel. (Figure 5 – g-l; Table 2, 3)

Examined material: BRAZIL. SANTA CATARINA: São Francisco do Sul, Acarai State Park. 12.VII.2017, N. P. Vieira Junior and G. R. Schroeder 4 (JOI).

Spores monolete; large in size; meridional outline ellipsoidal or reniform; amb elliptic; shape ellipsoidal; concave-convex or plane-convex in equatorial view; *laesura* straight and narrow; perisporium microgranulate; exosporium verrucate.

Niphidium crassifolium (L.) Lellinger. (Figure 6 – a-e; Table 2, 3)

Examined material: BRAZIL. SANTA CATARINA: São Francisco do Sul, Acarai State Park. 13.IX.2017, N. P. Vieira Junior and G. R. Schroeder 9 (JOI). Spores monolet; large in size; meridional outline ellipsoidal or reniform; amb elliptic;

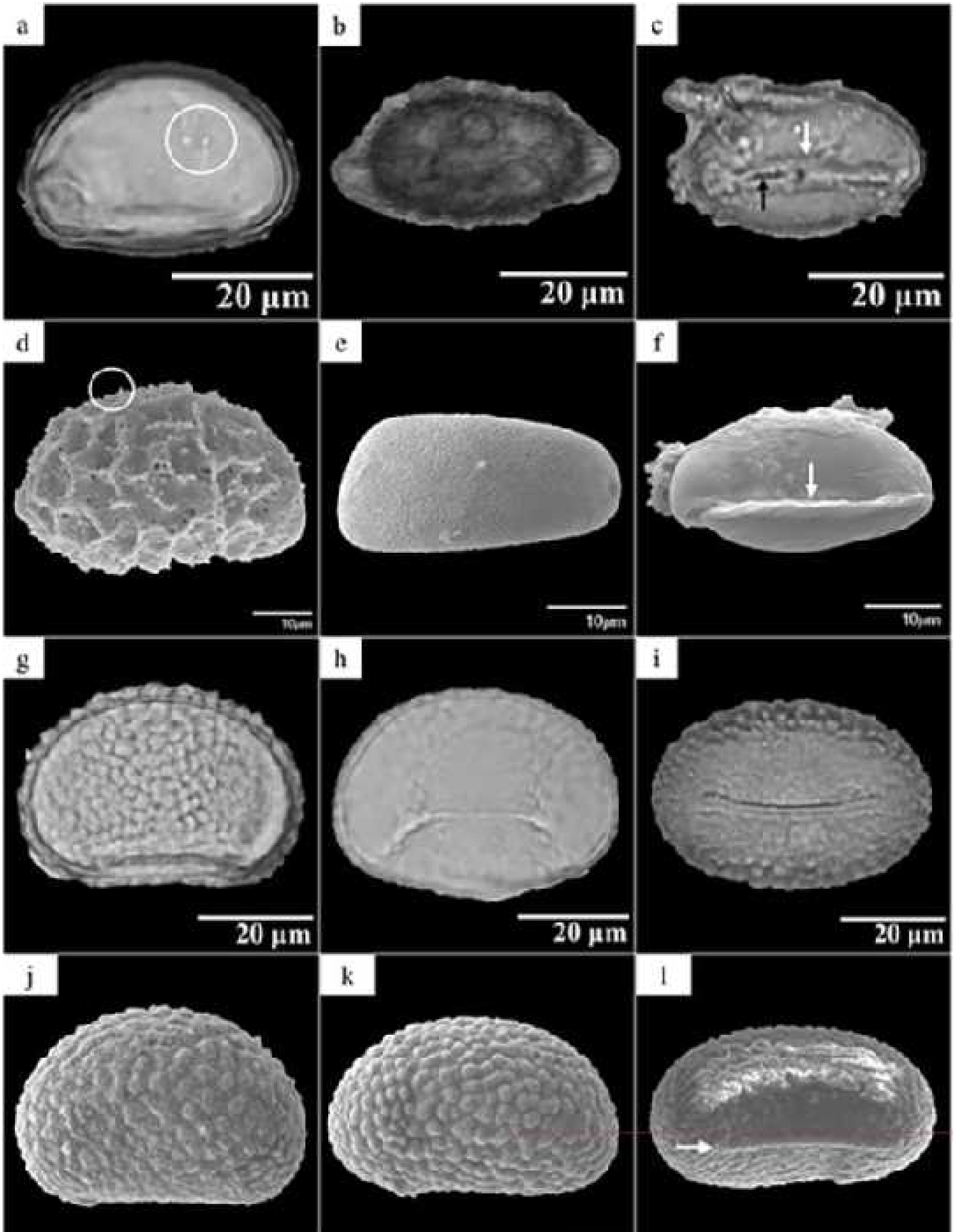

Figure 5. a-l. Photomicrographs (scale equivalent to 20 µm) and electromicrographs (scale equivalent to 10 µm) of spores. a-f. *Macrothelypteris torresiana*: a. Equatorial view without perisporium (circle indicates granules) (MO); b. Proximal polar view with perisporium (MO); c. Detail of perisporium being lost (white arrow indicates labium and black arrow indicates commissure) (MO); d. Distal polar view, perforate and reticulate surface (circle indicates baculi on crest) (MEV); e. Surface of microganulate exosporium (MEV); f. Proximal polar view, detail of perisporium being lost (arrow indicates *laesura*) (MEV). g-l. *Microgramma vacciniifolia*: g. Equatorial view (MO); h. Surface of verrucate exosporium (MO); i. Proximal polar view (MO); j. Surface of microgranulate perisporium (MEV); k. Surface of verrucate exosporium (MEV); l. Proximal polar view (arrow indicates laesura) (MEV).

shape ellipsoidal; concave-convex or plane-convex in equatorial view; *laesura* straight and narrow; outer perisporium perforate and reticulate (it disappears easily after acetolysis); inner perisporium psilate with small globules, isolated or in masses; exosporium verrucate.

Pecluma chnoophora (Kunze) Salino and Costa Assis (Figure 6 – f-i; Table 2, 3).

shape ellipsoidal; concave-convex or plane-convex in equatorial view; *laesura* straight and narrow; exosporium verrucate with larger warts on distal face.

Pecluma pectinatiformis (Lindm.) M. G. Price (Figure 6 – j-l; Figure 7 – a-b; Table 2, 3).

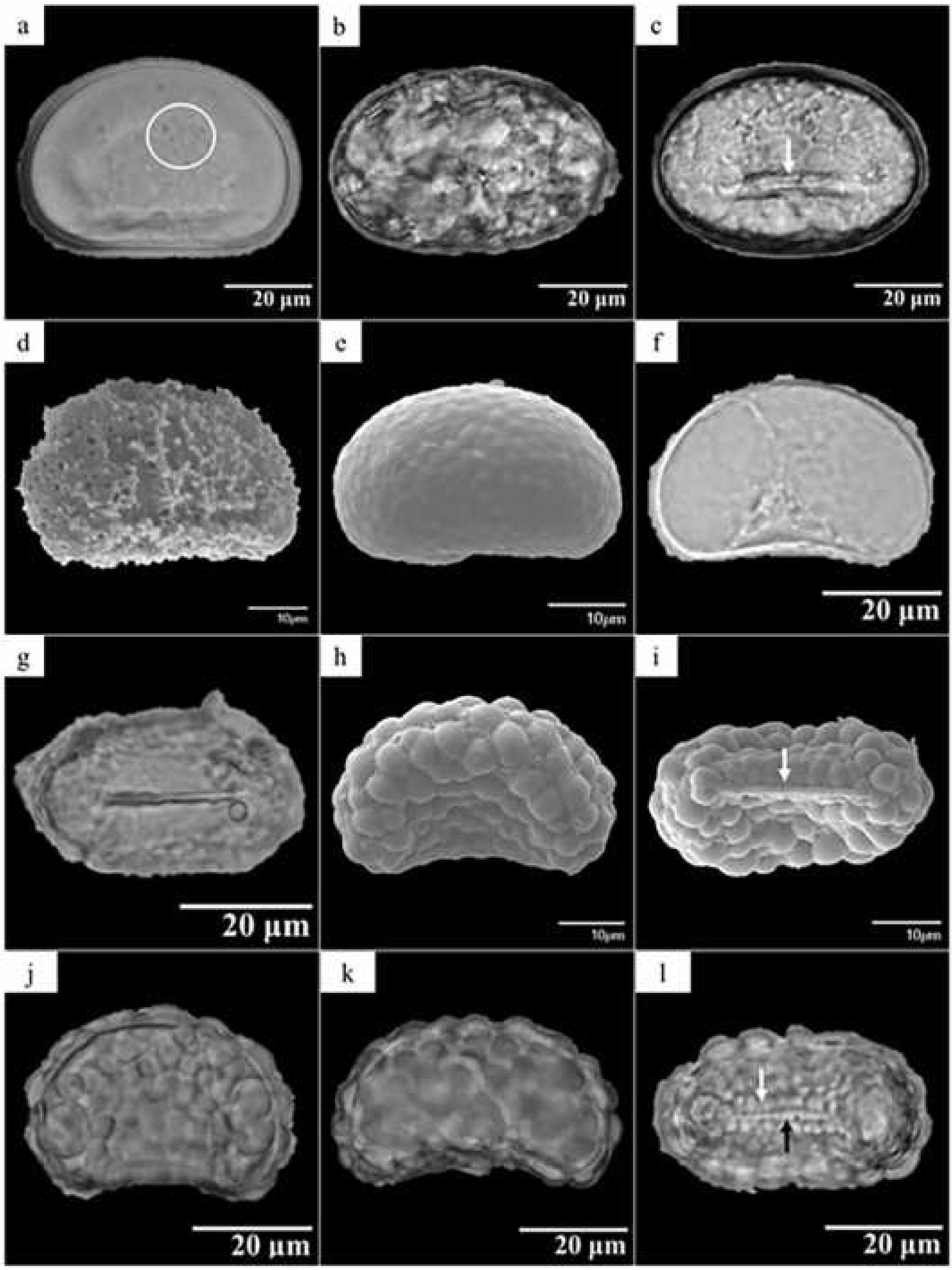

Figure 6. a-l. Photomicrographs (scale equivalent to 20 µm) and electromicrographs (scale equivalent to 10 µm) of spores.a-e. *Niphidium crassifolium*: a. Equatorial view, surface of inner perisporium (circle indicates globules) (MO); b. Surface of outer perisporium (MO); c. Proximal polar view (arrow indicates laesura) (MO); d. Equatorial view, surface of outer perisporium (perforate and reticulate) (MEV); e. Distal polar view, laevigate inner perisporium over verrucate exosporium (MEV). f-i. *Pecluma chnoophora*: f.Equatorial view, verrucate exosporium; g. Proximal polar view (MO); h. Equatorial view, verrucate exosporium (MEV); i. Proximal polar view (arrow indicates details of the *laesura*) (MEV). j-l. *Pecluma pectinatiformis*: j. Equatorial view (MO); k. Verrucate surface (exosporium) (MO); l. Distal polar view (white arrow indicates ornate labium, black arrow indicates commissure) (MO)

Examined material: BRAZIL. SANTA CATARINA: São Francisco do Sul, Acarai State Park. 28. VI. 2017, N. P. Vieira Junior and G. R. Schroeder 2 (JOI). Spores monolete; medium in size; meridional outline ellipsoidal or reniform; amb elliptic;

Examined material: BRAZIL. SANTA CATARINA: São Francisco do Sul, Acarai State Park. 02.VIII.2017, N. P. Vieira Junior and G. R. Schroeder 5 (JOI). Spores monolete; large in size; meridional outline ellipsoidal or reniform; amb elliptic;

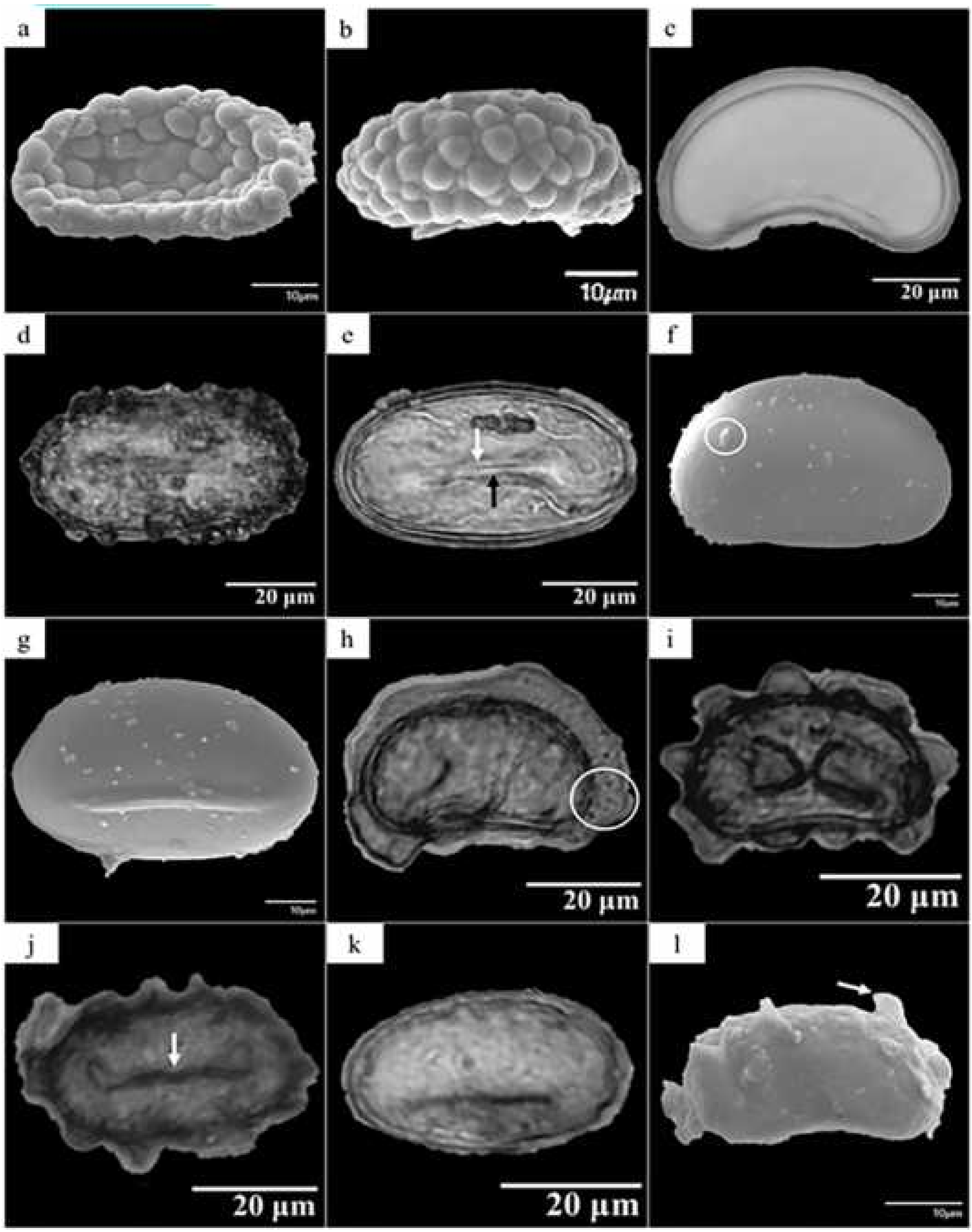

Figure 7. a-l. Photomicrographs (scale equivalent to 20 µm) and electromicrographs (scale equivalent to 10 µm) of spores. a-b. *Pecluma pectinatiformis*: a. Verrucate surface (MEV); b. Distal polar view (MEV). c-g. *Pleopeltis lepidopteris*: c. Equatorial view (MO); d. Surface of laevigate perisporium with globules isolated or in masses (MO); e. Proximal polar view (white arrow indicates labium, black arrow indicates commissure) (MO); f. Equatorial view (circle indicates globules in masses on laevigate perisporium) (MEV); g. Proximal polar view (MEV). h-l. *Rumohra adiantiformis*: h. Equatorial view (circle indicates granules) (MO); i. Alate surface (MO); j. Laesura (arrow) (MO); k. Laevigate surface (MO); l. Equatorial view (arrow indicates projections and folds of the perisporium that make the spore alate) (MEV)

shape ellipsoidal; concave-convex or plane-convex in equatorial view; *laesura* straight and narrow, labium thick ornate with small warts; exosporium verrucate. Greater separation between warts compared to *P. chnoophora*.

Pleopeltis lepidopteris (Langsd. and Fisch.) de la Sota (Figure 7 – c-g; Table 2, 3).

Examined material: BRAZIL. SANTA CATARINA: São Francisco do Sul, Acarai State Park. 31.VIII.2017, N. P. Vieira Junior and G. R. Schroeder 8 (JOI). Spores monolete; large in size; meridional outline ellipsoidal or reniform; amb elliptic; shape ellipsoidal; concave-convex or plane-convex in equatorial view; *laesura* straight and narrow; perisporium psilate with small globules, isolated or in masses, with or without warts; exosporium verrucate.

Rumohra adiantiformis (G.Forst.) Ching (Figure 7 – h-l; Figure 8 – a; Table 2, 3, 6)

Examined material: BRAZIL. SANTA CATARINA: São Francisco do Sul, Acarai State Park. 20.XII.2017, N. P. Vieira Junior and G. R. Schroeder 13 (JOI). Spores monolete; medium in size; meridional outline ellipsoidal or reniform; amb elliptic; shape ellipsoidal; concave-convex or plane-convex in equatorial view; *laesura* straight and narrow; outer perisporium with thick folds and irregular projections making the spore alate; inner perisporium slightly ondulate, with or without granules similar to *papillae* in both perisporia; exosporium psilate, nearly plane, with ripples.

Schizaea elegans (Vahl) Sw. (Figure 8 – b-g; Table 2, 3)

Examined material: BRAZIL. SANTA CATARINA: São Francisco do Sul, Acarai State Park. 22.I.2018, N. P. Vieira Junior and G. R. Schroeder 14 (JOI). Spores monolete; medium in size; meridional outline ellipsoidal or reniform; amb elliptic; shape ellipsoidal; concave-convex or plane-convex in equatorial view; *laesura* straight and narrow; perisporium papillae with small spheres of different sizes; exosporium verrucate-tuberculate.

Serpocaulon latipes (Langsd. and Fisch.) A. R. Sm. (Figure 8 – h-l; Table 2, 3, 6)

Examined material: BRAZIL. SANTA CATARINA: São Francisco do Sul, Acarai State Park. 28.VI.2017, N. P. Vieira Junior and G. R. Schroeder 3 (JOI). Spores monolete; large in size; meridional outline ellipsoidal or reniform; amb elliptic; shape ellipsoidal; concave-convex or plane-convex in equatorial view; laesura straight and narrow; perisporium with irregular folds forming an irregular reticle; exosporium verrucate.

Trichomanes cristatum Kaulf. (Figure 9 – a-f; Table 4, 5, 6)

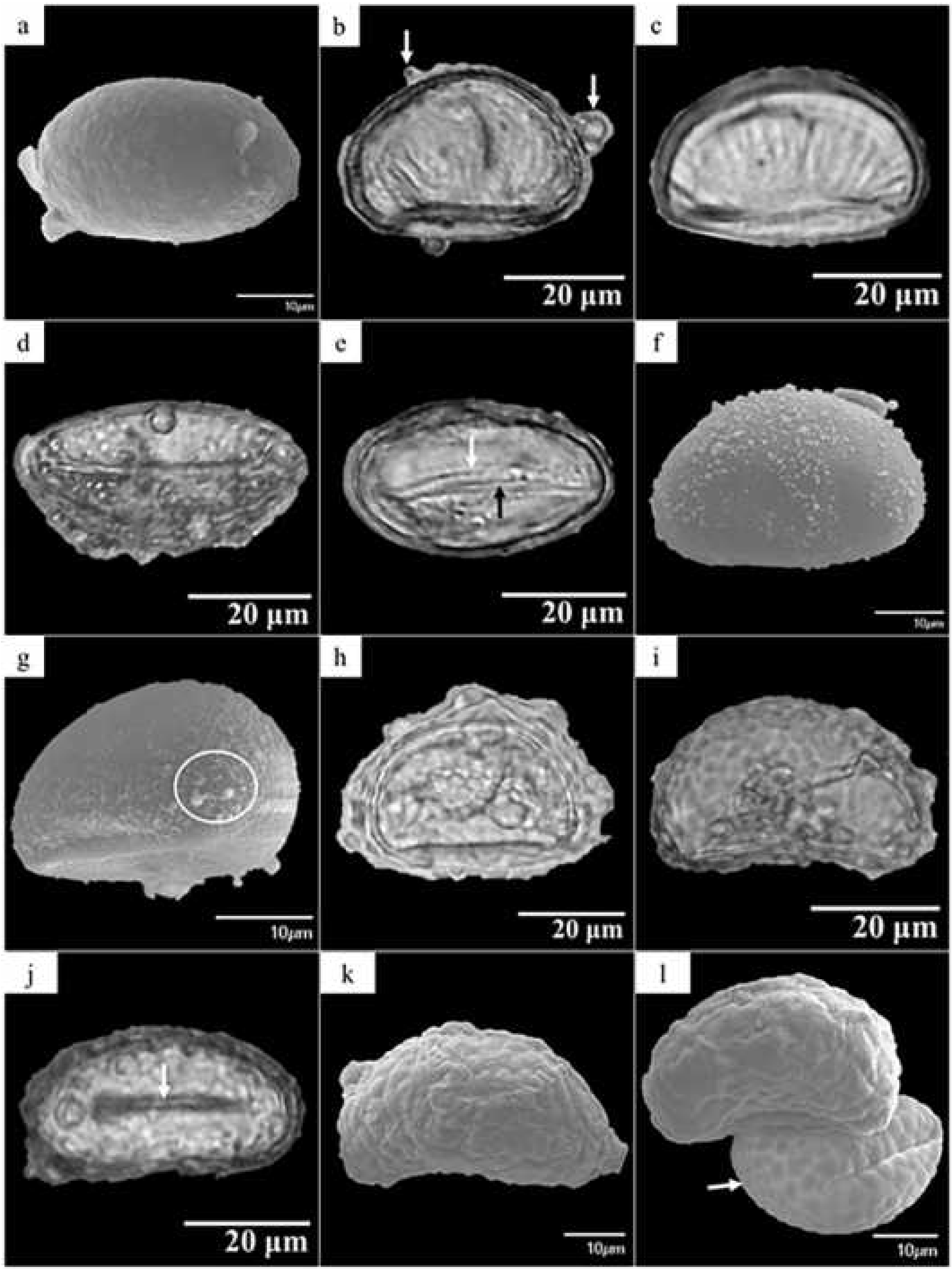

Figure 8 – a-l. Photomicrographs (scale equivalent to 20 µm) and electromicrographs (scale equivalent to 10 µm) of spores. a. *Rumohra adiantiformis*: Distal polar view (MEV). b-g. *Schizaea elegans*: b. Spheres of different sizes (arrow) (MO); c. Equatorial view (MO); d. Papillate surface (MO); e. Proximal polar view (white arrow indicates labium, black arrow indicates commissure) (MO); f. Papillate surface (MEV); g. Spheres of different sizes (circle) (MEV). h-l. *Serpocaulon latipes*: h. Equatorial view, outer perisporium (MO); i. Distal polar view, inner perisporium (MO); j. Proximal polar view (arrow indicates *laesura*) (MO); k. Equatorial view, hamulate perisporium (MEV); l. Spore with and without perisporium (arrow indicates verrucate exosporium) (MEV).

Examined material: BRAZIL. SANTA CATARINA: São Francisco do Sul, Acarai State Park. 20.XII.2017, N. P. Vieira Junior and G. R. Schroeder 12 (JOI). Spores trilete; large in size, meridional outline triangular convex; amb subcircular; shape spheroidal; convex-hemispheric in equatorial view; *laesuras* slightly ondulate, straight and long, forming a triangle when extremely open, ornamented with spicles; sides convex; apex slightly acute; exosporium echinate. Commissure (µm) = 21.07 ± 1.32.

Identification Key

1.Trilete spores
2.Exosporium echinate …..……. ***Trichomanes cristatum***
2'.Exosporium psilate; perisporium covered by three-dimensional network of free or fused rodlets, when fused forming small spicules of various sizes
3.*Laesura* straight, narrow, medium in size..........................***Cyathea phalerata***
3'.*Laesura* straight to slightly ondulate, medium in size, forked on the extremities, labium thick and high................***Lindsaea lancea***
1'.Monolete spores
4.Alate spores
5.Perisporium with irregular folds forming irregular reticles with cones and/or spicules on crest.........................***Asplenium brasiliense***
5'.Perisporium with thick folds and irregular projections…………………………......***Rumohra adiantiformis***
4'.Spores not alate
6.Exosporium microgranulate, gemmate or verrucate-tuberculate
7.Perisporium perforate and reticulate, with *baculae* on crest...***Macrothelypteris torresiana***
7'.Perisporium papillate with small spheres of different sizes. …………………………………………......***Schizaea elegans***
6'.Exosporium verrucate
8.Ornamentation with globules present, isolated or in masses
9.Outer perisporium perforate and reticulate (susceptible to loss after acetolysis)...........................***Niphidium crassifolium***
9'.Perisporium psilate
10.Spores medium in size…...***Campyloneurum acrocarpon***
10'.Spores large in size, with or without warts..***Pleopeltis lepidopteris***
8'.Ornamentation without globules
11.Perisporium microgranulate......***Microgramma vacciniifolia***
11'.Perisporium with or without granules
12.Outer perisporium with folds and irregular projections; inner perisporium hamulate................***Serpocaulon latipes***
12'.Perisporium tenuous, with or without proeminences
13.Spores medium in size............***Pecluma chnoophora***
13'.Spores large in size…….***Pecluma pectinatiformis***

DISCUSSION

Thirteen of the fourteen studied species are native, with the exception of *M. torresiana*, which is an exotic sub-spontaneous species (Gasper and Salino 2015). Among the four orders, Polypodiales was represented by the largest number of species (11), which belong to three suborders: Lindsaeineae, Aspleniineae and Polypodiineae. All of the studied species of Polypodiales have monolete spores except *Lindsaea. lancea,* which has trilete spores. Thus, there is no definite pattern of trilete or monolete spores among the species of the studied Polypodiales. These results confirm the uncertainty that regards the classification of the family Lindsaeaceae within the order Polypodiales (Smith *et al.* 2006; PPG I 2016). The perisporium of spores of *Lindsaea.lancea* (Lindsaeaceae) are ornamented with rodlets, which is very similar to that of *Cyathea phalerata* (Cyatheaceae). Both species also have trilete spores, indicating a possible relationship between Lindsaeaceae and Cyatheaceae. Tryon and Lugardon (1991) also described the perisporium of *Lindsaea lancea* as ornamented.

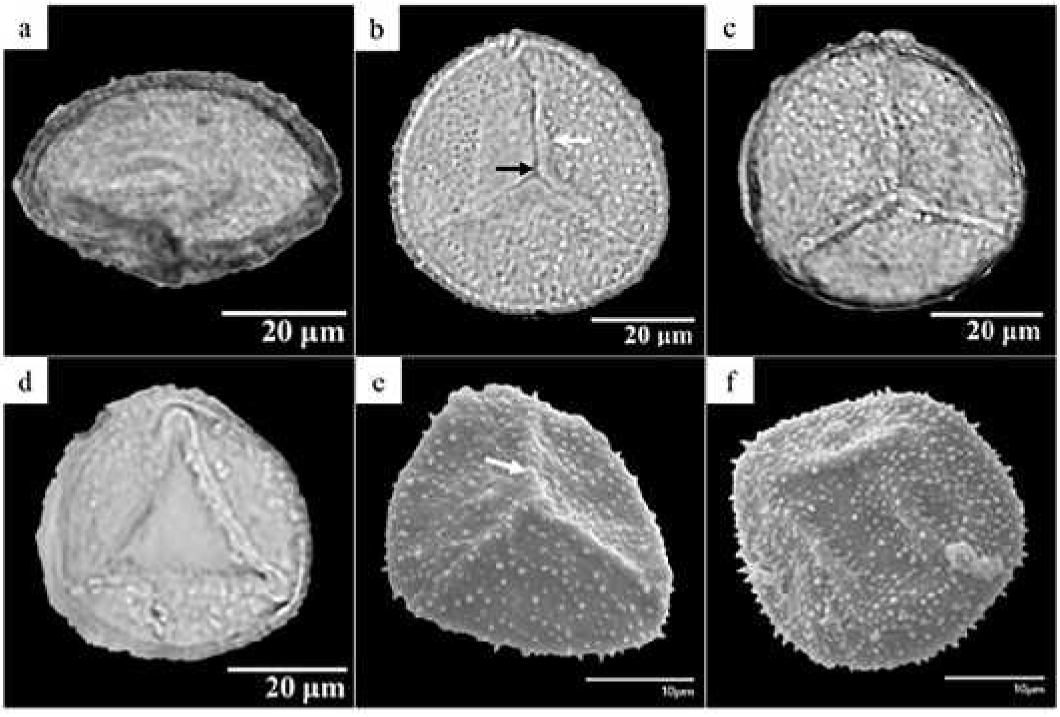

Figure 9. a-f. Photomicrographs (scale equivalent to 20 µm) and electromicrographs (scale equivalent to 10 µm) of spores of *Trichomanes cristatum*. a. Equatorial view (MO); b. Echinate surface (white arrow indicates labium, black arrow indicates commissure) (MO); c. Proximal polar view (MO); d. Open laesura in the shape of a triangle (MO); e. Proximal polar view (arrow indicates detail of the laesura) (MEV); p. Distal polar view, echinate exosporium (MEV)

On the other hand, the present study found the exosporium of this species to be psilate, while these authors described it as gemmate. Esteves and Coelho (2007) also described the exosporium of this species, however, they reported the perisporium to have rugulate ornamentation, but in the present work it was found to be covered by a three-dimensional network of rodlets. The ornamentation of the perisporium and exosporium, and the other morphological characteristics reported in the present work for *Cyathea phalerata*, are consistent with the description provided by Márquez *et al.* (2010) and the description for the genus proposed by Tryon and Lugardon (1991). The results found here for *Rumohra adiantiformis* confirm the classification of the family Dryopteridaceae within the order Polypodiales.

The ornamentation of the perisporium and exosporium of *Rumohra adiantiformis* described here is in agreement with that reported by Tryon and Lugardon (1991), as are the other morphological data. The psilate exosporium, and the other morphological characteristics described here, are also concordant with those described by RCPOL (2018). Both *Asplenium brasiliense* and *Macrothelypteris torresiana* belong to the suborder Aspleniineae (PPG I 2016), and although they differ in the ornamentation of the perisporium and exosporium, they both present granules in one of the layers. *Asplenium brasiliense* is more similar to *Rumohra adiantiformis*, both having alate spores and with the inner perisporium possessing granules, which suggests they have a close phylogenetic relationship and supports the monophyly of Aspleniineae; findings that corroborate Smith *et al.* (2006) and PPG I (2016). The morphology and ornamentation of the external perisporium described here for *Asplenium brasiliense* a similar to that reported by Gamen *et al.* (2013). Additionally, the exosporium described herein is consistent with the description for the genus by Tryon and Lugardon (1991), while other data described here agree with the findings of Ybert *et al.* (2016) for other species of the genus *Asplenium.* Tryon and Lugardon (1991) described the perisporium of *Macrothelypteris torresiana* as having irregular folds and perforations. The present study described it as perforate and reticulate. The same authors also report the presence of reticles for the genus and describe the exosporium as gemmate. The present study add the possibility of this part also be microgranulate. The other morphological characteristics described here corroborate other reports in the literature (Vijayakanth and Sathish 2016). The other studied species of Polypodiales are placed in the family Polypodiaceae and presented great homogeneity, including a verrucate exosporium, which supports the monophyly of the family. The found data bring close *Campyloneurum acrocarpon*, *Niphidium crassifolium* and *Pleopeltis lepidopteris*, species that show a psilate perisporium with small globules that are isolated or in masses. The morphological data obtained for *Campyloneurum acrocarpon* in the present study are in accordance with those proposed by Tryon and Lugardon (1991) for the genus and resemble some of the findings of Coelho and Esteves (2011) for the species *Campylonerum angustifolium* Fée. The morphology found for *Niphidium crassifolium* is also in agreement with that described by Tryon and Lugardon (1991). However, the outer perforate and reticulate perisporium described herein has not been reported previously, which may be explained by the fact that it is easily lost after acetolysis. The morphological characteristics found for *Pleopeltis lepidopteris*, including ornamentation of the perisporium and exosporium, are in agreement with those proposed by Tryon and Lugardon (1991) and Coelho and Esteves (2011) for the genus. The morphological data described herein for *Trichomanes cristatum* are also in accordance with the characteristics described by Tryon and Lugardon (1991) for the genus. The ornamentation the exosporium of *Trichomanes cristatum* differentiates it from the other studied species, with which it only shares a trilete pattern. *Schizaea elegans* did not exhibit great similarity with the other studied species, from which it differed mainly in ornamentation. The data obtained for the exosporium/ sporoderm differ from those proposed by RCPOL (2018), where it is described as gemmate.

However, the morphology of the perisporium is in agreement with the proposal of Tryon and Lugardon (1991). The morphological data obtained for the spores of *Microgramma vacciniifolia* are in agreement with the data obtained by Tryon and Lugardon (1991), Scherer and Lorscheitter (2008), Macedo *et al*. (2009) and Cancelli *et al*. (2012). The spores of *Pecluma pectinatiformis* and *Pecluma chnoophora* are very similar morphologically, differing only in metric data. The morphological data for the two species are in agreement with that proposed by Tryon and Lugardon (1991) for the genus. The data for *Pecluma pectinatiformis* also matches that found by Scherer and Lorscheitter (2008) and Cancelli *et al.* (2012). The morphological characteristics described for the spores of *Serpocaulon latipes* in the present study are consistent with the data presented by Tryon and Lugardon (1991) and Coelho and Esteves (2011). The metric data obtained here were not used in these comparisons since, for the same species, different measurements may be obtained in the same specimen and among specimens (Caldeira *et al.*, 2006; Coelho 2011). This variation in metrics is not related to the phytophysiognomies where the plants grow; in other words, it does not represent biotic variants (Coelho and Esteves 2011). Variation in size can be due to other factors, such as hybridization between individuals and polyploidy (Tryon and Tryon 1982; Tryon and Lugardon 1991). Thus, spore size and other metrics are of limited significance (Salgado-Labouriau 1973; Barth and Melhem 1988), and should not be used as primary taxonomic characters. The descriptions of the analyzed spores added new data to sporopalynological morphology, contributing to systematic studies of ferns, and further studies in palynological sites.

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