



UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu



A morphological and anatomical study of Brazilian *Lygodium* species (Lygodiaceae),
focusing on spore and stomata morphology and spore germination

**(*Lygodium* × *fayae*: abrindo o caminho para uma maior compreensão
sobre híbridos de samambaias brasileiras)**

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Dissertação apresentada ao
Instituto de Biociências, Câmpus de
Botucatu, UNESP, para obtenção do título
de Mestre no Programa de
Pós-Graduação em Ciências Biológicas
(Botânica), Área de concentração
Sistemática Vegetal

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Lygodium ×fayae: abrindo o caminho para uma maior compreensão sobre híbridos de samambaias brasileiras : um estudo morfológico e anatômico sobre as espécies brasileiras de *Lygodium* (Lygodiaceae), focado em morfologia de estômatos e esporos, e germinação de esporos / Rebekah Helen Lindsey. -- Botucatu, 2020

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ABSTRACT

The family Lygodiaceae has always had a contradictory taxonomy, the number of accepted species in Brazil varying from two to twelve through the decades. Lygodiaceae being an old family, this contradictions are believed to be caused by hybrids. Hybrids arise by the crossing of two or more species, and, usually, they are first identified by its intermediate morphological characteristics among the parental species. Normally, early fern hybrids are detected occurring along with its parental species. The spores of hybrid individuals generally are badly formed and/or aborted, thus, not viable, but the few balanced spores could starts the speciation process of a new species. Considering the overlapping of morphological characteristics, it could have occurred in two species of *Lygodium* in Brazil, *L. venustum* and *L. volubile* and the hypothetical hybrid *L. ×fayae*. Due to the difficult in identification of these taxa, our studies herein analysed two different characters (spore and stomata size and morphology) through scanning electronic microscopy, we studies the spore germination and new descriptions and an identification key were done to the accepted species to help clearing the taxonomic delimitation of the two taxa of *Lygodium* and their putative hybrid. According to our results, the species herein classified as *L. ×fayae* on the Brazilian south-east region is not a hybrid, but a morphological variation of *Lygodium venustum*. Additional studies on morphometric analysis with the measures of stomata and spore are being carry out to to give greater support to our results.

Key words: ferns, Lygodiaceae, morphology, Schizaeales, spores, stomata.

1. INTRODUCTION

The order Schizaeales Schimp. has a fossil record dating back to the Jurassic period (Mickel 1962) or even possibly from the Carboniferous (Kenrick & Crane 1997). Order Schizaeales (sensu Smith *et al.* 2008) embraces three families and Anemiaceae Link, with 106 accepted species; Lygodiaceae C. Presi, with 30 species; and Schizaeaceae Kaulf, with 22 accepted species, according to The Plant List (access in 05/13/2020) (Fig. 01). The ferns in order Schizaeales are terrestrial and rupestral ferns, often found in dry habitats (Tryon & Tryon 1982). Some of the synapomorphies (Fig. 1) for the order are the differentiation between fertile and sterile parts of the laminae since the laminae is born and the piriform sporangia, with a transverse, sub-apical and continuous indusium (Tryon & Tryon 1982; Smith *et al.* 2008). There are three families within Schizaeales (sensu Smith *et al.* 2008): Anemiaceae Link, , Lygodiaceae C. Presi, and Schizaeaceae Kaulf (Fig. 01)



Fig. 01. The three families belong to order Schizaeales: A-Anemiaceae, showing fronds, and the different reproductive structures; B-Schizaeaceae, showing fronds with sorophores at the distal part; C-Lygodiaceae, showing reproductive fronds.

The Lygodiaceae is a monogenetic family, widely spread across the Neotropics, represented by the climbing ferns of the genus *Lygodium* Sw. (Fig. 3) (Duck 1978; Tryon & Tryon 1982; Moran 1995; Mickel & Smith 2004; Smith *et al.* 2006; Rabelo & Schwartzburd 2016). It is uncommon for

climbing ferns to reach considerable heights, but individuals of *Lygodium* usually could reach 10 or more meters long (until 30 meters) (Moran 1995).

Morphologically, these ferns could be identified by its terrestrial habit, with subterranean rhizome bearing short trichomes and few to many fibrous roots; short to long-creeping stem, frequently branched; the fronds have undetermined growth (Moran 1995) and tend to be climbing, polymorphic, intertwined, with furcate pinnae, widely alternately pinnate, glabrous to pubescent, the pinnae is short-stalked, pseudodichotomously branched with and arrested bud in the axil, each primary pinna-branch palmately or radiately lobed or branched or pinnate. The pinnulae are 2-pinnate, and the second order of pinnulae can be linear or palmate, they can partially dimorphic, with the fertile bearing marginal lobes; the veins are free or rarely anastomosed without include free veinlets (Fig. 02).



Fig. 02. Fronds of *Lygodium venustum* in different states. A - Sterile frond; B - Sub-apical and continuous indusium; C, D - Details of early fertile fronds. Pictures by A. Simões.

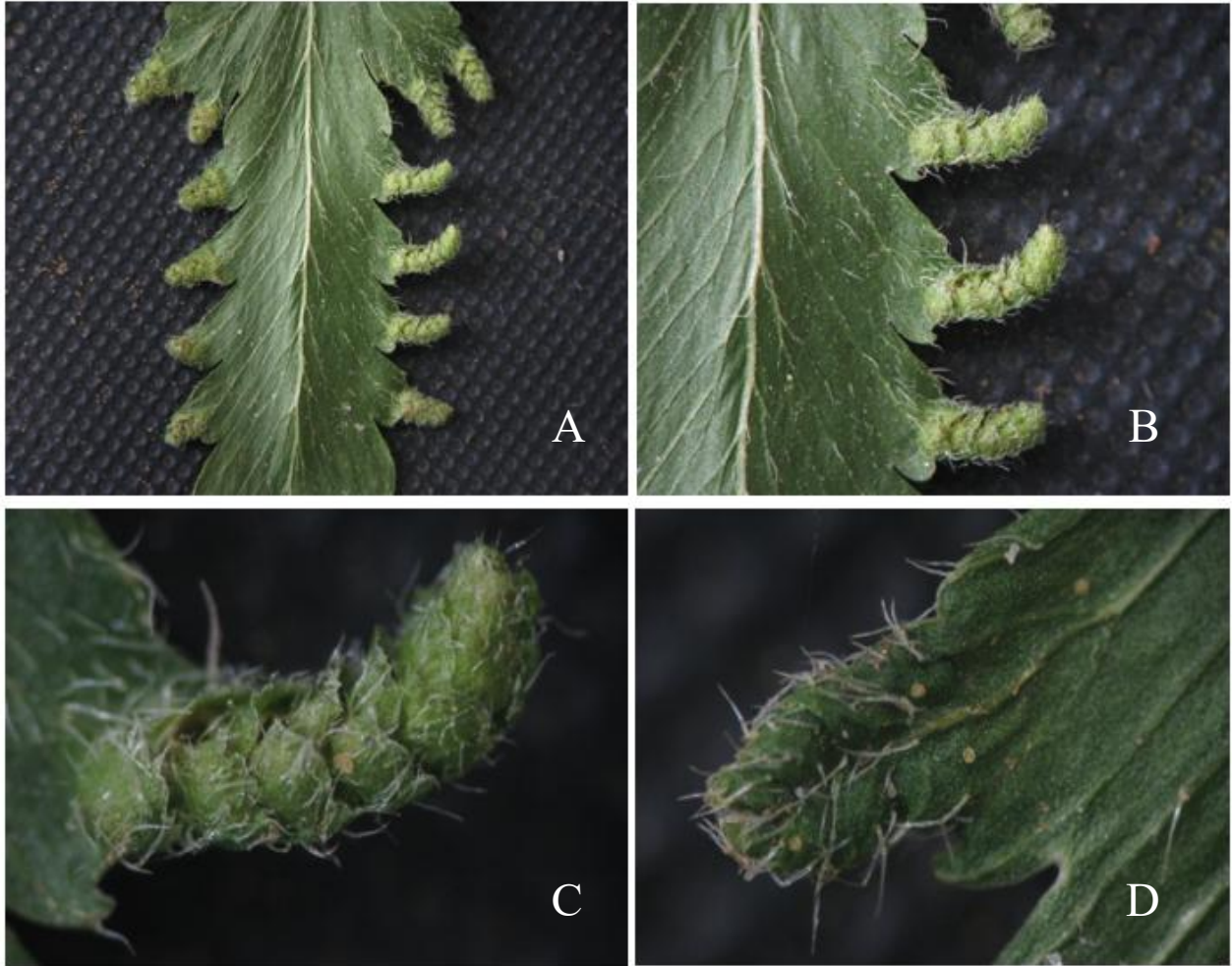


Fig. 03. Different stages of sorophores A, B - closer look on the indusium of *L. venustum*; C - details of trichome; D - spores near the indusium. Pictures by A. Simões.

Lygodium comprises about 30 species with six/eight of them occurring in American tropics (Fig. 04), but just two in Brazil (Tryon & Tryon 1982). The taxonomy of *Lygodium* is still controversial (Tryon & Tryon 1982; Rabelo & Schwartsburd 2011; 2016). The first account of *Lygodium* from Brazil was done in “Flora Brasiliensis” (von Martius *et al.* 1840) presenting 12 species: *L. acuminatum* J.W. Sturm., *L. expansum* Desv., *L. hastatum* (Willd.) Desv., *L. hirtum* Kaulf., *L. lucens* Kaulf., *L. micans* J.W. Sturm., *L. mucronatum* J.W. Sturm., *L. palmatilobum* J.W. Sturm., *L. pohlianum* C. Presl, *L. puberulum* J.W. Sturm., *L. venustum* Sw. and *L. volubile* Sw. However, Sturm never got to see the plants in their natural habitat, characterizing them only by parts that arrived in Europe what may have induced to erroneous circumscriptions.. However, some

names are still used today, and his work still holds great importance. A few years later, Fée (1869), in his “Cryptogames vasculaire du Brésil”, revisited the genus taxonomy and reduced the number of species to five: *L. hastatum*, *L. hirtum*, *L. pohlianum*, *L. venustum*, and *L. volubile*, of which all would occur in south-eastern Brazil, except for *L. hastatum*, which would only be found in the north-east Brazil. Almost one hundred years later, in the year of 1974, Aloysio Sehnem published “Flora Ilustrada Catarinense”, in which he described four species of *Lygodium* distributed in the southern region of Brazil: *L. mucrolatum* J.W. St., *L. polymorphum* (Cav.) HBK, *L. venustum* Sw., and *L. volubile* Sw. After four years, Duek (1978) published “A taxonomical revision of *Lygodium* (Filicinae) on America”, a book with a great impact in the *Lygodium* taxonomy. Duek noticed that, for a genus as plastic and volatile as *Lygodium*, using solely a morphological analysis would not be enough. Applying complicated calculations and considering many different individuals, Duek carried out a morphometric analysis of the genus and based on his results, he concluded that only four species of *Lygodium* could be accepted for Brazil: *L. micans* J.W. Sturm, *L. oligostachyum* (Willd.) Desv, *L. venustum* Sw., and *L. volubile* Sw. Although all the complex methodology, it was a short and descriptive paper. After its publication, most of the *Lygodium* in southern and south-eastern Brazil were considered either *L. venustum* or *L. volubile*. After Duek's work, new revisions were made, always reducing the number of accepted species and, currently, only two species, *Lygodium venustum* and *Lygodium volubile* (Barros *et al.* 2015, Prado *et al.* 2015) are accepted in Brazil.

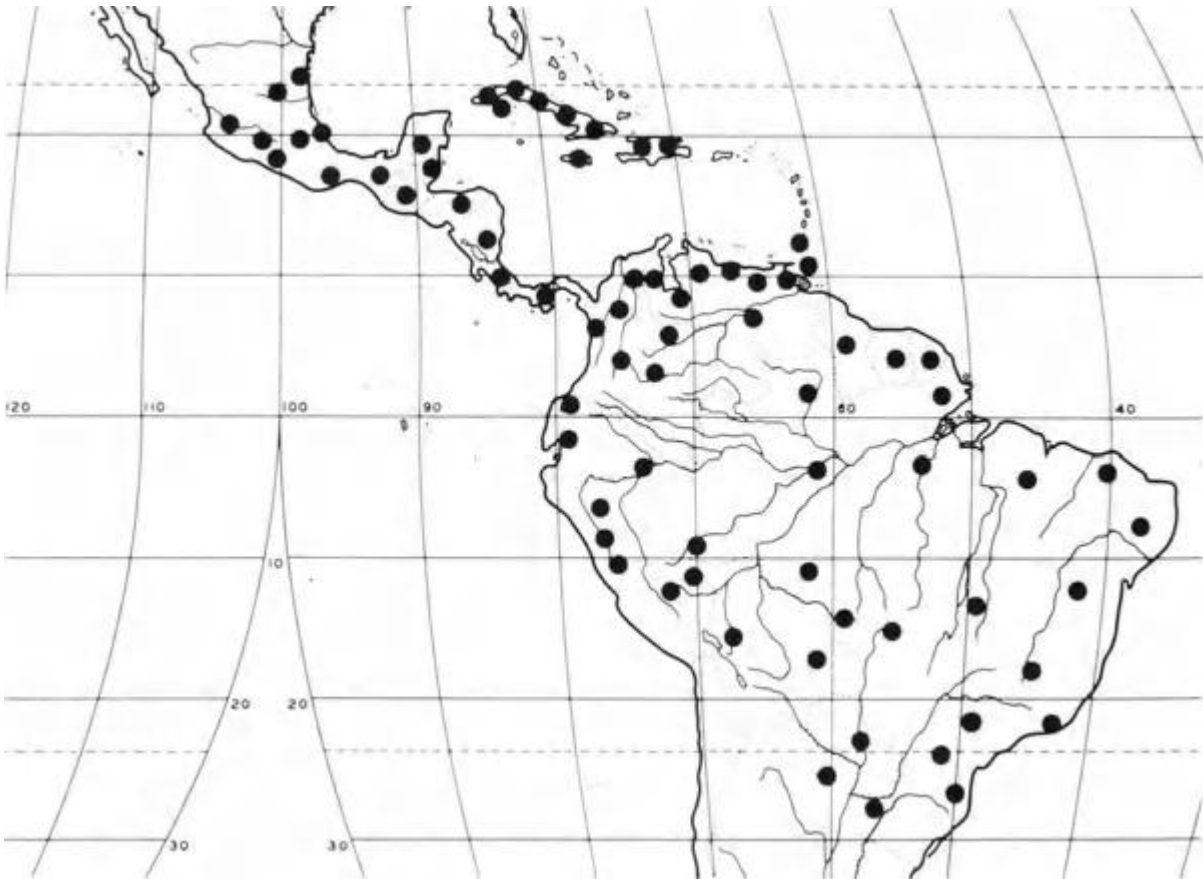


Fig. 04. Map from Tryon & Tryon (1982), showing the distribution of *Lygodium* in America.

Around ten years later, after Duek's paper, in 1984, Alice and Rolla Tryon published a book, which is still very famous and important among the pteridologists: "Ferns and Allied Plants: with special reference to tropical America". In this book, many *Lygodium* species were described, not only based on morphological or morphometric analysis, but also on palynology and chromosome number, which is a challenging technique when working with *Lygodium*. Some articles, like Walker (1964); Roy & Manton (1965); Walker, T. G. (1966); and Tryon & Tryon (1984), mention the chromosome number for *L. volubile* ranging from $n=28$ to $n=90$ and for *L. venustum* $n=58$ (Walker, 1985). Recently, more modern studies and techniques are being used to differentiate and identify different plant species, such as complete or partial DNA sequencing (Madeira *et al.* 2008), but some questions are still open, among them the existence of *Lygodium* hybrids in Brazil.

For over a century, it has been known about the existence of hybrids within ferns (Stebbins, G. L. 1959). Although, it has been poorly studied in the Neotropics, hybridisation is known to be a recent phenomenon, occurring mostly in disturbed habitats (Tryon & Tryon 1982). Hybrids arise by the crossing of two or more species and, usually, have intermediate morphological characteristics among the parental species (Jermy & Walker 1985). Normally, early hybrids only occur in regions where the parental species are present. That happens because the spores of hybrid individuals generally are unbalanced (Jermy & Walker 1985), but once hybrids undergo polyploidisation and restore fertility, it become able to reproduce sexually and could disperse to new regions far from the parental species. Such dispersion could favours hybrid establishment by reducing the competition with the parental species (Reviewed by Rezende *et al. submitted*).

When gametophytes of phylogenetically close species germinate close to each other, the conditions to hybridisation are settle down: when the gametes are mature, a cross fertilization could takes place. Fern hybrids, generally, cannot reproduce sexually, since their spores tend to be unbalanced and usually aborted. It occurs mainly due to differences in DNA repetitive sequences and chromosome numbers, avoiding a proper meiotic pairing (Jermy & Walker 1985). Although most of new fern hybrids cannot reproduce sexually, they still manage to have populations with a reasonable number of individuals through vegetative reproduction, but with low genetic variability. Nevertheless, hybrids may undergo a non-reductional meiosis, which is a common error in which the number of chromosomes is not divided in half during the meiotic process, which leads to a spore with a diploid number of chromosomes (non-reduced gametes/spores) (reviewed by Pelé *et al.* 2018; Rezende *et al. Submitted*). When two non-reduced spores fuse with each other, polyploid individuals emerge and after that, fern hybrids may reproduce sexually. It is possible that ferns with high ploidy (tetraploid, hexaploid, and so on) should arise by hybridization and polyploidy.

Hybrids within the order Schizaeales with large ploidy are very common (Mickel 1962). Ferns with high ploidy tend to have larger spores and stomata, due to the increase of molecular material inside the cell nuclei (Jermy & Walker 1985), and comparing these structures from hybrids

and accepted species has been proved a good method for identifying and studying such hybrids (Schwatsburd *et al.* 2008, Giacosa *et al.* 2013), although molecular studies are also important (Besse, P. 2014).

Although fern hybrids are common, *Lygodium* hybrids were never found in Brazil until recently. Rabelo & Schwatsburd (2016), proposed that a putative hybrid of the two *Lygodium* species currently accepted in Brazil, *L. venustum* and *L. volubile* (e.g. Barros *et al.* 2015; Prado *et al.* 2015). The putative hybrid, *L. ×fayae* Jermy & T.G. Walker, which has been punctually registered in other Neotropical countries (Kramer 1978; Moran 1995), was, according to Rabelo & Schwatsburd (2016), found in Viçosa, Minas Gerais, where both parental species also occurred in sympatry and are common in forest areas. These three taxonomic entities could be identified based on few morphological characters (Rabelo & Schwatsburd 2016; Table 01), however, due to the species plasticity, its identification in the field could be very complicated.

Table 01. Macromorphological characteristics for *L. venustum*, *L. ×fayae* and *L. volubile*. Picture extracted from Rabelo & Schwatsburd (2016).

Characters	<i>L. venustum</i>	<i>L. ×fayae</i>	<i>L. volubile</i>
Size of secondary pinnules	Proximal ones twice or three times longer than the distal ones	Uniform	Uniform
Secondary pinnules articulation	Non articulated	Commonly articulated	Articulated
Petiolules of secondary pinnules	Winged	Commonly winged	Not winged
Shape of secondary pinnules	Palmately pinnate or palmately lobed with 3–5 costate lobes	Slightly lobed to palmately pinnate	Oblong-acuminate
Indument of abaxial surface of costae, veins, and laminar tissue between the veins	Villous	Intermediary condition	Virtually glabrous, but with scattered hairs

Considering the difficulty of delimiting the species of *Lygodium* and their hybrid in Brazil, in this study we asked whether there are two species of *Lygodium*, *L. venustum*, *L. volubile* and the hybrid *L. ×fayae*. To answer it, we have carried out germination and morphological studies, the latter with emphasis in the spores and stomata. By using these tools we aimed to solve the problem of species circumscription of the genus *Lygodium*, contributing to the taxonomic study of ferns.

2. MATERIAL & METHODS

2.1. *Taxonomic and Morphological Studies*

The study was based on observations of specimens in the field, analyses of our own collections, herbarium collections (BOTU, VIC); acronyms are according to Thiers, 2015, and a literature review. The terminology used to describe the morphological features follows Rabelo & Schwartsburd (2016). The descriptions of species to taxonomic studies based on specimens deposited in BOTU and VIC herbariums and from field observations. All analysed specimens occurs in south-eastern of Brazil. The map of the distribution of the studied species in this study was done DIVA-GIS 7.5 (<https://www.diva-gis.org/download>), with GPS data from each collected specimen.

2.2. *Spore and stomata morphology*

Spores were obtained from freshly collected plants in the Municipalities of Viçosa and Presidente Bernardes, Minas Gerais, Brazil. The fieldwork was centred on the remaining patches within the University area (Recanto das Cigarras, Mata da Silvicultura and Mata da Bovinocultura), outside the Federal University of Viçosa (Estação de Pesquisa, Treinamento e Educação Ambiental Mata do Paraíso, Mata do Seu Nico), in exposed rocky sites (Pedreira da Caprinocultura, margins of the river Piranga), and disturbed sites, as viewed previously in the study of Rabelo & Schwartsburd (2016). The voucher specimens were deposited at BOTU and VIC. All the *Lygodium* vouchers deposited at BOTU and VIC were examined (R.H. Lindsey 81 to 112, P.B. Schwartsburd 4591 to 4602) using images obtained from scanning electronic microscopy and the program Scandium, ten stomata were measured from each sample of *Lygodium volubile* (R.H. Lindsey, R.H. Lindsey, 91, VIC 10.184, 15.498), *L. venustum* (R. H. Lindsey 86, 102, 108, VIC 15.974) and the possible hybrids, *L. ×fayae* (R.H. Lindsey 81, 94, 98, 99 BOTU). The stomata were observed on the abaxial side of the pinnules, which were fixed in 2.5% glutaraldehyde in 0.1 mol L⁻¹ phosphate buffer, pH

7.3, for 24 h, dehydrated in a graduated acetone series, critical point-dried using CO₂, mounted on aluminium stubs, coated with gold.

Also using images obtained from scanning electronic microscopy and the program Scandium, the spores from each sample of *Lygodium volubile* (R. H. Lindsey 91, VIC 10.184, 13.011, 15.498, 16.784, 18.621), *L. venustum* (R. H. Lindsey 86, 102, 108, VIC 15.916, 15.974,) and *L. ×fayae* (R. H. Lindsey 90, 94, 96, 98, 99, 107) were collected in identified flasks from fresh plants which were first dried in the laboratory kiln, mounted on aluminium stubs with double sided adhesive tape and coated with gold.

The spores and stomata were studied in order to look for morphological and size difference. Morphological differences between the spores of *L. venustum* and *L. volubile* are visible, but the spores from the putative hybrid have never been studied before. The same is valid for stomata; if there are morphological differences, the taxa could be differentiated with less contradictions. As said before, difference in size can point to polyploidy, which is a important information when studying putative hybrids.

Both, stomata and spores, were examined using a Quanta 200 (FEI Company) scanning electron microscope (SEM) at the Electron Microscopy Centre of the Institute of Bioscience of Botucatu, Brazil. The measurements were done using the program Scandium, also in the Electron Microscopy Centre of the Institute of Bioscience of Botucatu (Table 02-03).

2.3. Germination of spores

Spores germination was studied in order to discover whether the spores from all three taxa were fertile. That showed whether the spores were malformed or aborted, or not. To test this, spores of all three taxa were germinated using individual petri dishes with a bed of sphagnum moss. The moss was sterilized over boiling water for 30 minutes, to eliminate any microorganisms, then placed in a clean petri dish. Spores of three individuals of each taxa were placed in separate dishes, and

each dish was watered, and placed in an airtight container. They were kept in this condition for 25 to 30 days.

The gametophytes were later observed and photographed at Universidade Federal do ABC (UFABC), using Zeiss magnifying lenses, and are shown in Fig. 09.

3. RESULTS & DISCUSSION

3.1.1. *Analysis of spores*

Useful micro-morphological characters include the quantity of spheroids in spores and the texture of the surface: scanning electron microscopy (SEM) analyses of the spores of Brazilian *Lygodium* species revealed a few diagnostic micro-morphological characters that support species circumscriptions of both species *L. venustum* and *L. volubile*, but the spores of the putative hybrids were not very distinct from the other two species.

The three taxa presented trilete, triangular spores (Fig. 07-09) with straight sides, what could be straight to convex in *L. volubile* (Fig. 08-C). In equatorial view, the spore of the three species presented proximal face convex, while the distal face was hemispheric (Fig. 07-09).

Spheroids were seen on the spore surface of all taxa (Fig. 07-09). Very few spheroids were observed in *L. venustum*, and they were abundant especially in the proximal and equatorial view of the spores of *L. volubile*. In what was thought to be *Lygodium* *×fayae* were observed many spheroids, although they were not as organized as in *L. volubile*. This work corroborates the studies of Giacosa *et al.* (2013) that also pointed few spheroids in surface of spores of *L. venustum* and abundant in *L. volubile*. Those spherical depositions are characteristic of the spores of *Lygodium* that occurs in America (Tryon & Tryon, 1982).

Single or fused ridges are seen on the distal face as observed in study of Giacosa *et al.* (2013) and on ridge surfaces, the micro-ornamentation is verrucate-tuberculate, similar to that observed on

the proximal face (Figs. 07-09). An equatorial ridge is present and it delimits facets proximally. In *Lygodium venustum* the equatorial ridge is not so marked as observed in *L. volubile* and *L. ×fayae*.

Through the results of the study with micro-morphology of surface of spore, corroborated by Giacosa *et al.* (2013), it is possible to differentiate the species *Lygodium venustum* and *L. volubile* by the spore surface and quantity of spheroids. The individuals here classified as *L. ×fayae* have intermediate characteristics between the two species, and are more similar to *L. volubile*, although macromorphological characteristics, such as the shape of the laminae, are more similar to *L. venustum*. The means of the equatorial diameter were compared using One Way - ANOVA, and there was no statistical difference (Fig. 5; $p=0.58$; $f=0.55$).

Lygodium venustum

The spore varies from 80 to 102 μm of the equatorial diameter. Its ornamentation is formed principally by the variations of the outer layer, such as tubercles and verrucae, although the surface itself is smooth. The spores of *Lygodium venustum* have abundant perforations of different sizes, and there are many verrucae and tubercles; sometimes fused, forming short ridges. The leasurae have the same kind of ornamentation as the spore surface, such as few spheroids, which are observed particularly on the distal face (Fig. 07; Table 2).

Lygodium volubile

The spore varies from 84 to 109 μm of the equatorial diameter. The spores have abundant perforations of different sizes, and there are many verrucae and tubercles, mostly on the proximal face. On the distal face, there are single or fused ridges. On the ridges surface, it's possible to see micro-ornamentation, which is verrucate-tuberculate, similar to what's observed in the proximal face. An equatorial ridge is delimited facets proximally. The leasurae have the same kind of ornamentation: verrucae and tubercle. There are also numerous perforations of different sizes on the perispore surface. All over the spore, spheroids are seen, single or fused. They are more abundant

on the distal face (Fig. 08). The spore ornamentation is formed principally by the variations of the outer layer, as in the other species, such as tubercles and verrucae. The surface of the spores is rugged, showing prominent ridges (Fig. 08; Table 2).

Lygodium ×fayae

The spore varies from 72 to 102 μm of the equatorial diameter. They have abundant perforations of different sizes, and there are many verrucae and tubercles; sometimes fused, forming short ridges. The leasurae have the same kind of ornamentation as the spore surface, such as few spheroids, which are observed particularly on the distal face. The spore ornamentation is formed principally by the variations of the outter layer, such as tubercles and verrucae. Some spheroids might be seen in the sectioned image, on the spore surface, with varies between a rougher texture and a smoother texture, although it still shows prominent ridges (Fig. 09; Table 2).

Table 02. Morphological characteristics of spores from *Lygodium venustum*, *L. volubile* and *L. ×fayae*

Species	Ornamentation	Equatorial diameter, mean (μm)	Figures
<i>L. venustum</i>	Verrucae and tubercles; sometimes fused, forming short ridges	85.4	Figure 07 A-D
<i>L. volubile</i>	Abundant perforations of different sizes, and there are many verrucae and tubercles, mostly on the proximal face	87.7	Figure 08 A-D
<i>L. ×fayae</i>	Verrucae and tubercles sometimes fused, forming short ridges	86.3	Figure 09 A-D

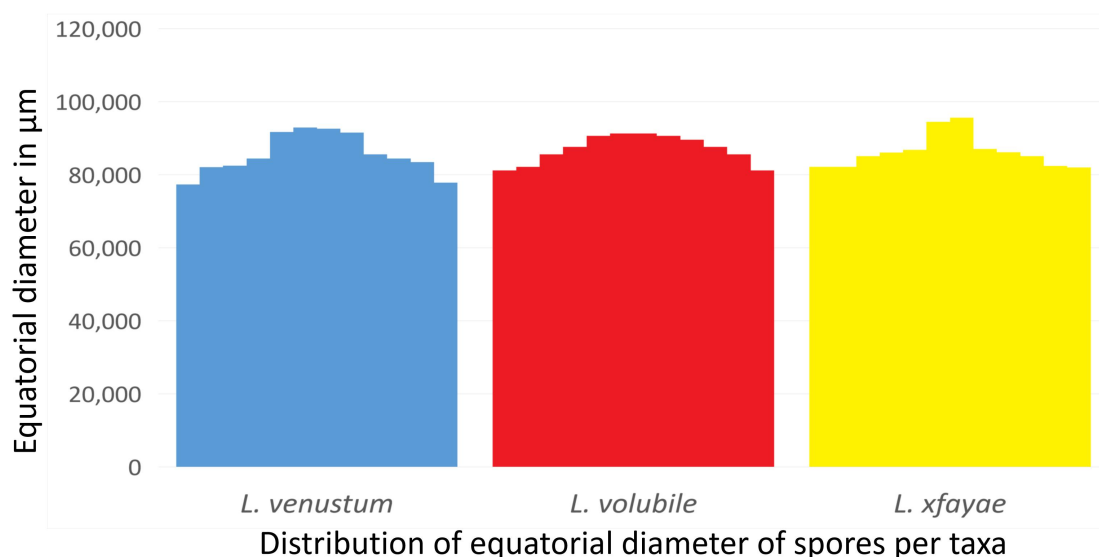


Figure 5. Distribution of equatorial diameter of spores from *Lygodium venustum*, *L. volubile* and *L. xfayae*

3.1.2. Analysis of stomata

The stomata within the three species are simple, occurring only on the abaxial side of the pinnule. The measurements of the ostiole length and stomata area did not differ among species, as compared with One Way ANOVA ($p=0.99$; $f=0.00$). Characteristics as shown on the table (Figure 6; Table 03).

Table 03. Measurements of stomata from *Lygodium venustum*, *L. volubile* and *L. xfayae*.

Species	Guard-cell length, mean (μm)	Figure 11
<i>L. venustum</i>	25.2	A, D
<i>L. volubile</i>	25.0	B, E
<i>L. xfayae</i>	25.5	C, F

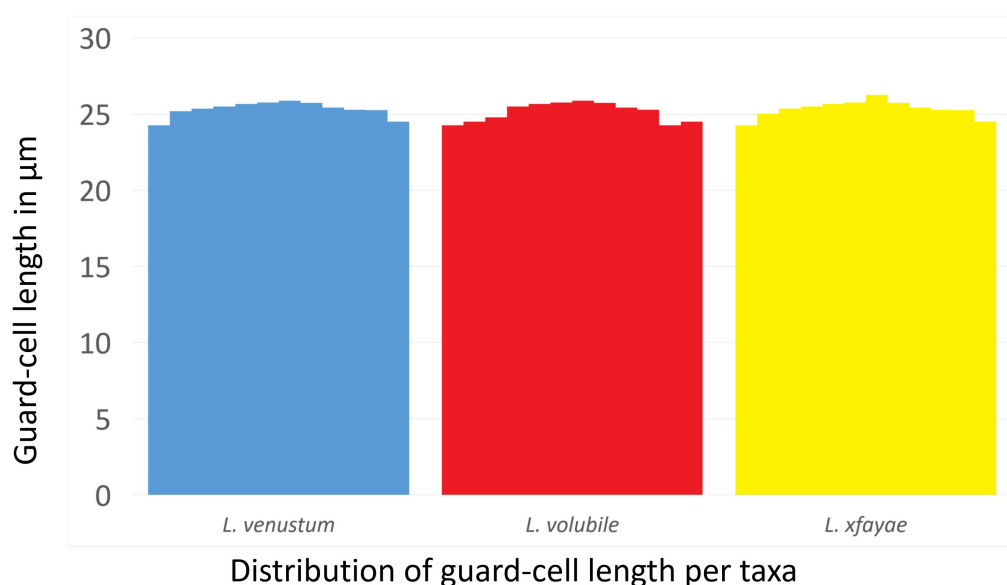


Figure 06. Distribution of guard-cell length per taxa from *Lygodium venustum*, *L. volubile* and *L. xfayae*

3.1.3. Spore germination

Spores from *L. venustum*, *L. volubile* and the putative hybrids germinated, resulting in very similar gametophytes, with no morphological difference. The gametophytes are hearts-shaped, measuring 0.6 mm - 0.8 mm of width, and 0.8 mm - 1 mm of length, as shown in Fig. 09. Although no difference was observed, the germination proved the spores from all taxa were fertile.

3.2. TAXONOMIC TREATMENT

Identification key to species of *Lygodium* in Brazil

1. Pinnules 1- or 2-pinnate; secondary pinnules palmately pinnate or palmately lobe *L. venustum*
1. Pinnules 1-pinnate; the secondary pinnules are oblong-acuminate *L. volubile*

Descriptions of species accepted in our study

3.2.1. *Lygodium venustum* Sw., Journal für die Botanik 1801(2): 303. 1803. Lectotype: Brazil, *Breynius s.n.* (S?); designated by Proctor, *Fl. Lesser Antill.* 2: 51 (1977).

Terrestrial plant, fronds have indeterminate growth. Laminae twining, dividing into several furcate pinnae; rachises terete, 1–2 mm diam., stramineous in colour; pinnae furcate, bearing two pinnules and a dormant bud between them; pinnules 1- or 2-pinnate, 10–25 cm long; secondary pinnules palmately pinnate or palmately lobed, with 3–5 costate lobes, sometimes asymmetrical, proximal pinnules (4–7 x 2–4 cm) being twice or thrice longer than the distal and none of them have articulated petiolule; petiolules winged, villous; costae villous on both sides, adaxial and abaxial; veins free, more villous on the abaxial side than the adaxial side, as is the laminar tissue amongst the veins. Spores are well formed.

Representative specimens examined: BRAZIL. Minas Gerais: Viçosa, Recanto das Cigarras, between the flags and the barbaque space, by the corner of the roundabout, 28th March 2019, R. H. Lindsey 82, 84, 85, 86, 88 (BOTU); Mata do Paraíso, 31st March 2019, R. H. Lindsey 105, 107, 108, 112 (BOTU). Mato Grosso do Sul, Brasilândia, River Verde's wetlands, s.c., s.d., BOTU (23247); idem, s.d., s.c., BOTU (22468); Bonito, by River Formoso, waterfall, near the road MS 178 Bonito-Bodoquena, s.c., s.d., BOTU (19944).

Distribution: in Brazil, the species occurs in North region (Acre, Amazonas, Amapá, Pará, Rondônia, Roraima, Tocantins), north-eastern (Alagoas, Bahia, Ceará, Maranhão, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, Sergipe), Midwest (Distrito Federal, Goiás, Mato Grosso do Sul, Mato Grosso), south-east (Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo), south (Paraná, Rio Grande do Sul, Santa Catarina) [Santiago 2020, under construction].

3.2.2. *Lygodium volubile* Sw., Journal für die Botanik 1801(2): 304. 1803. Holotype: Jamaica, Swartz s.n. (S).

Terrestrial plant, fronds have indeterminate growth. Laminae twining, dividing into several furcate pinnae; rachises terete, 1–2 mm diam., stramineous in colour; pinnae furcate, bearing two pinnules and a dormant bud between them; pinnules 1-pinnate 15–25cm long; secondary pinnules are oblong-acuminate (4–9 x 1.25–2.5 cm) having a uniform size amongst the proximal and distal secondary pinnules, articulated with petiolule; petiolules not winged, villous; virtually glabrous on both sides, but with scattered hairs, as is the laminar tissue amongst the veins, veins free. Spores are well formed.

Representative specimens examined: BRAZIL. Minas Gerais: Viçosa, Recanto das Cigarras, between the flags and the barbaque space, by the corner of the roundabout, 28th March 2019, R. H. Lindsey 83, 87 (BOTU); idem, Road between the Zootechny Farm and Fruit-farming Farm, on the way to the EVA Farm, 29th March 2019, R. H. Lindsey 89, 90, 91, 92, 93 (BOTU); idem, Beside the dam, 31st March 2019, R. H. Lindsey 96, 100, 101, 103, 104 (BOTU); idem, Mata do Paraíso, 31st March 2019, R. H. Lindsey 106, 109, 111 (BOTU). São Paulo: Botucatu, Farm 4 Meninas e Mac, 3475, 3132, 3476, 3137, 3146 (BOTU); idem, Botucatu-Itating road, near River Pardo, 10km from the municipal airport (BOTU 6207); Bertioga, s.d., s.c., (BOTU 2271). Brotas, Caçoroba waterfall, s.d., s.c. (BOTU 19865); Guarujá, s.d., s.c. (BOTU 8832); Ubatuba, s.d., s.c. (BOTU 19101).

Distribution: in Brazil, the species occurs in North region (Acre, Amazonas, Amapá, Pará, Rondônia, Roraima, Tocantins), north-eastern (Alagoas, Bahia, Ceará, Maranhão, Paraíba, Pernambuco, Rio Grande do Norte, Sergipe), Midwest (Distrito Federal, Goiás, Mato Grosso do Sul), south-east (Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo), south (Paraná, Rio Grande do Sul, Santa Catarina) (Santiago 2020, under construction, Flora do Brasil).

4. CONCLUSION

The present study revealed distinctive micro-morphological characteristics for two of the three studied taxa: *Lygodium venustum*, *L. xfayae* and *L. volubile*, which contribute directly to a better understanding of the taxonomy of the group, although the differences in size, both in spore and stomata, do not support the occurrence of a valid hybrid. The putative hybrid has variable characteristics, which, through this study, can be seen to change from lobbed to pinnate pinnules. Further studies should include molecular and genetic approach, to better determine the characteristics of the genus. Also, it is important to study what causes *L. venustum* to present such variable characteristics, since it would give more clarity to understanding the genus as a whole.

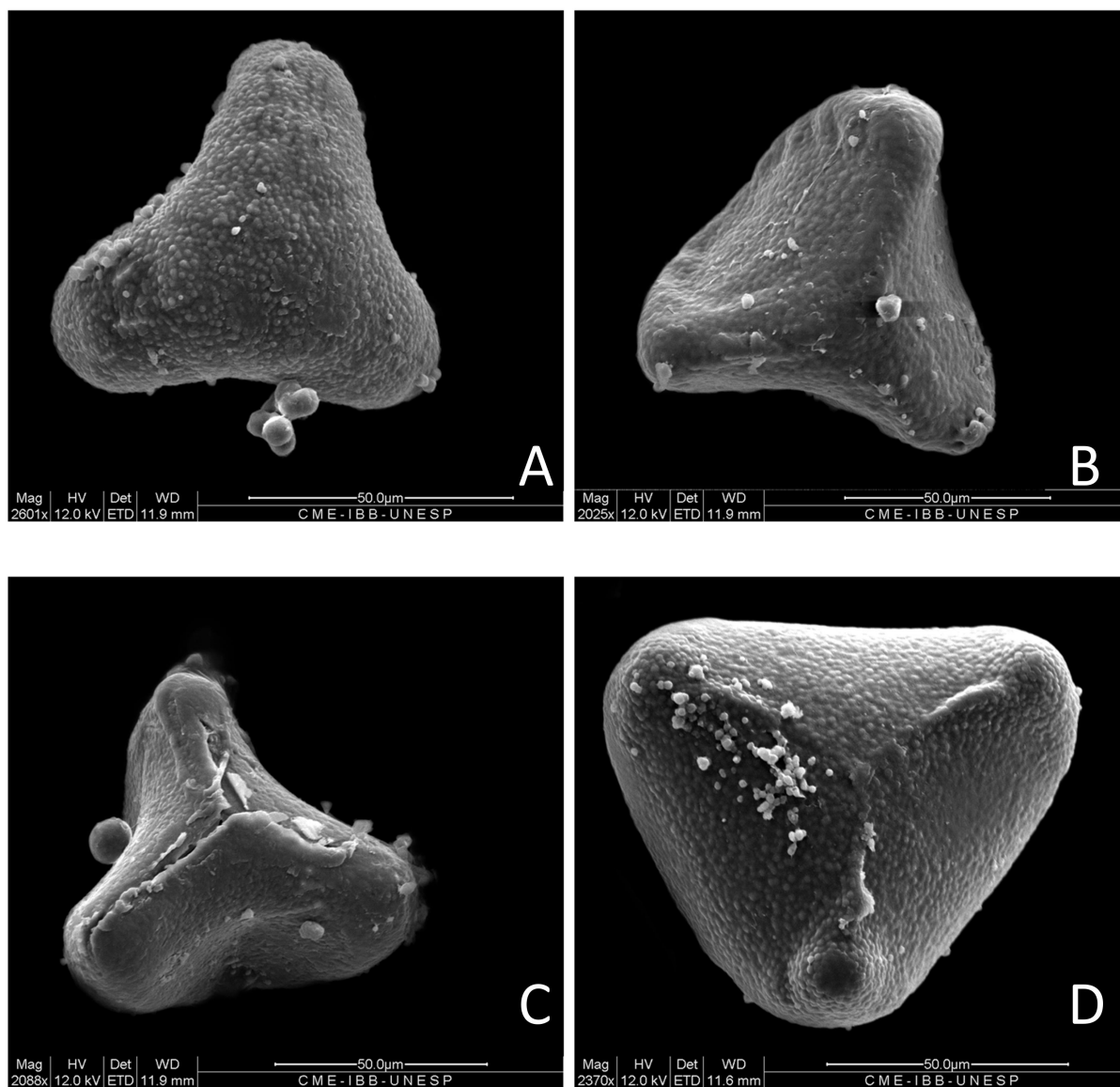


Fig. 07 - Spores of *Lygodium venustum* in SEM. A, C - distal view of a spore. The ornamentation is verrucate-tuberculate with few spheroids observed on the spore surface. B - equatorial view of a spore. D - proximal view of a triangular spore with straight sides.

Images were treated with AdobePhotoshop

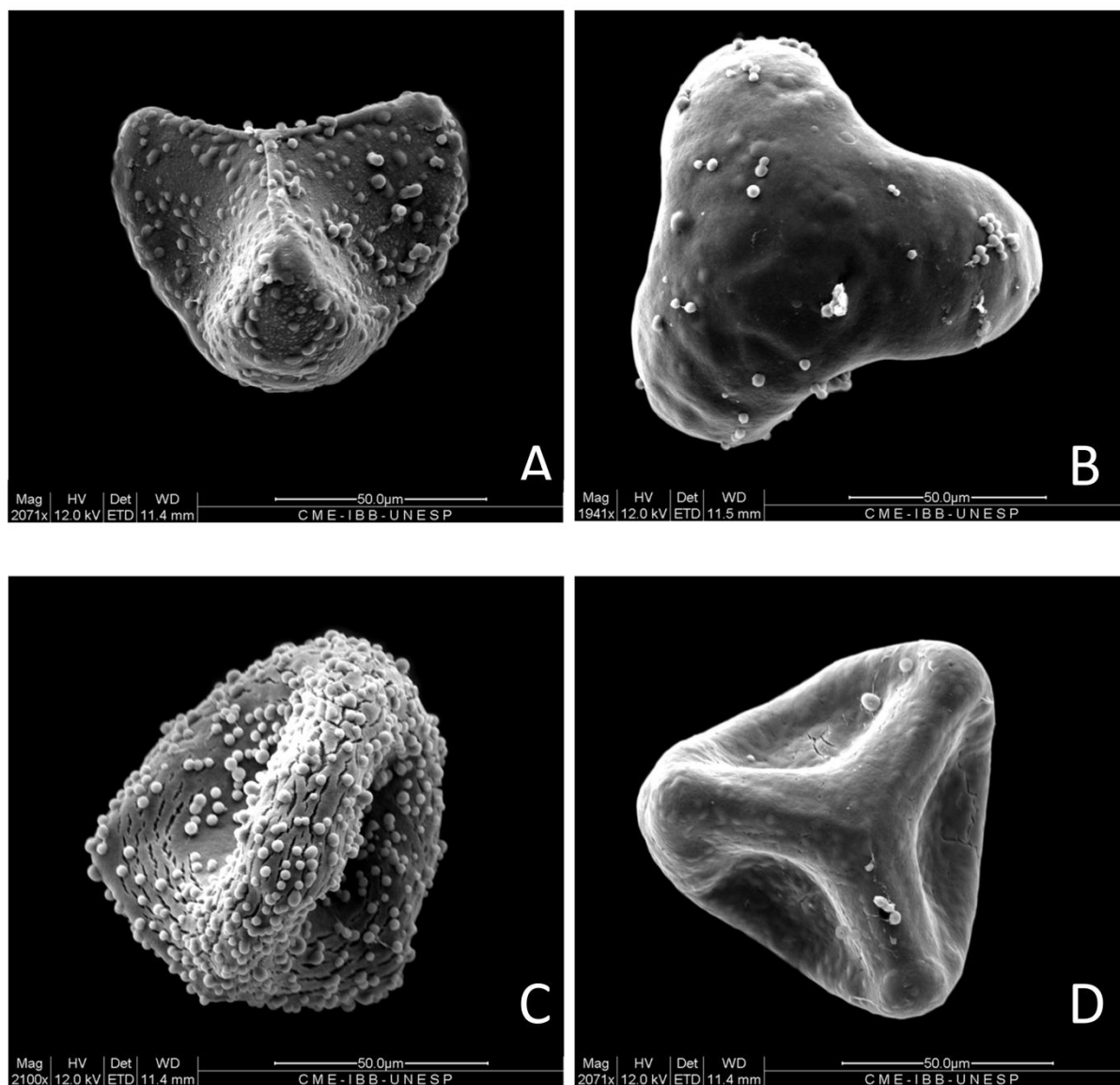


Fig. 08 - Spores of *Lygodium volubile* in SEM. A, D - distal view of a triangular spore with straight sides. Spheroids can be observed on the surface of the spore. The ornamentation is verrucate-tuberculate with many spheroids. B - distal view of a spore. The verrucate surface is very visible on the distal side. C - equatorial view of a spore.

Images were treated with AdobePhotoshop

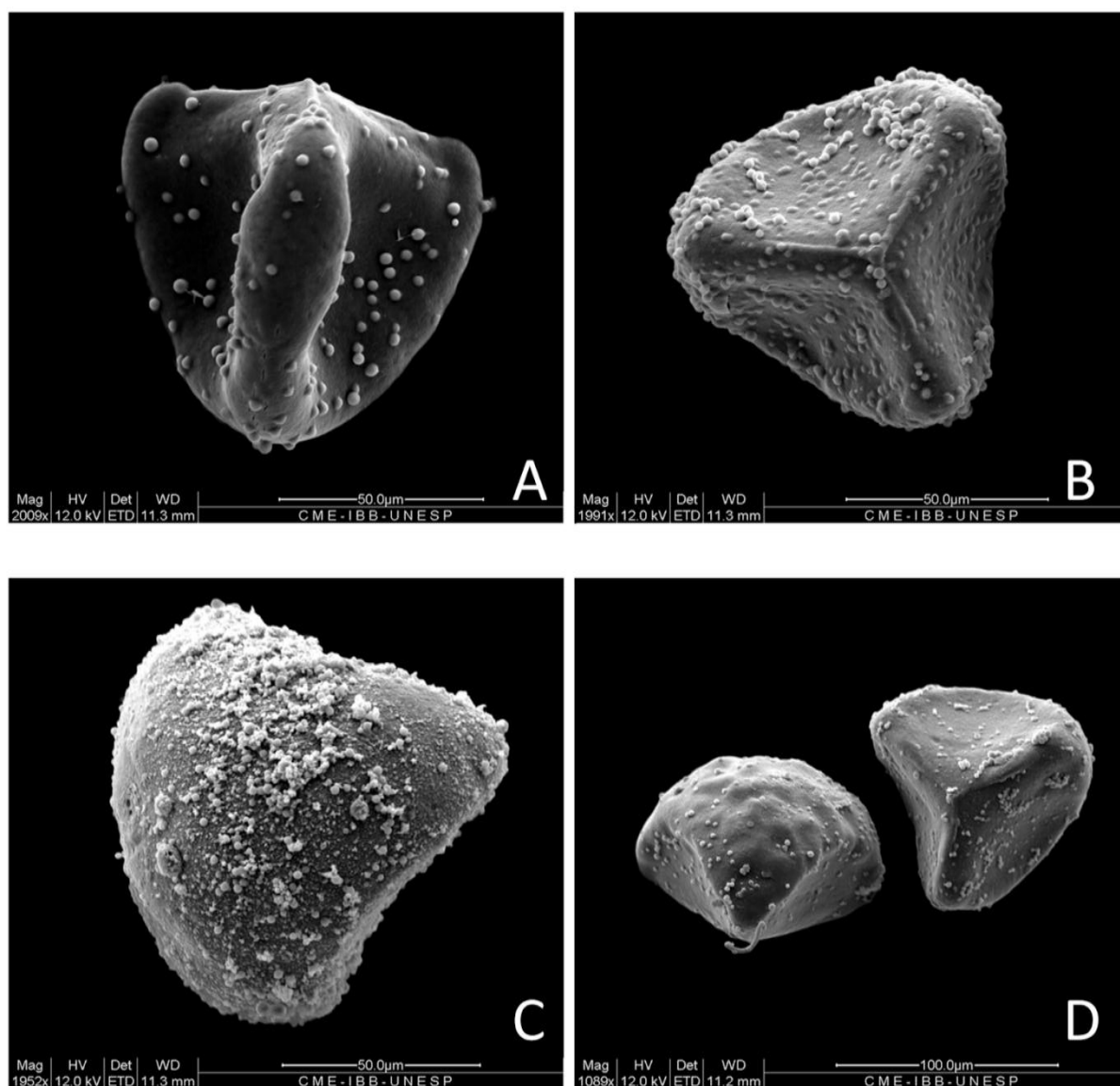


Fig. 09 - Spores of *Lygodium x faya* in SEM. A - equatorial view of a spore with spheroids on the surface of the spore. The ornamentation is verrucate-tuberculate with many spheroids, which can be observed on its surface. B, D - proximal view of a spore with straight sides and spheroids on the surface. C - distal view of a spore.

Images were treated with AdobePhotoshop

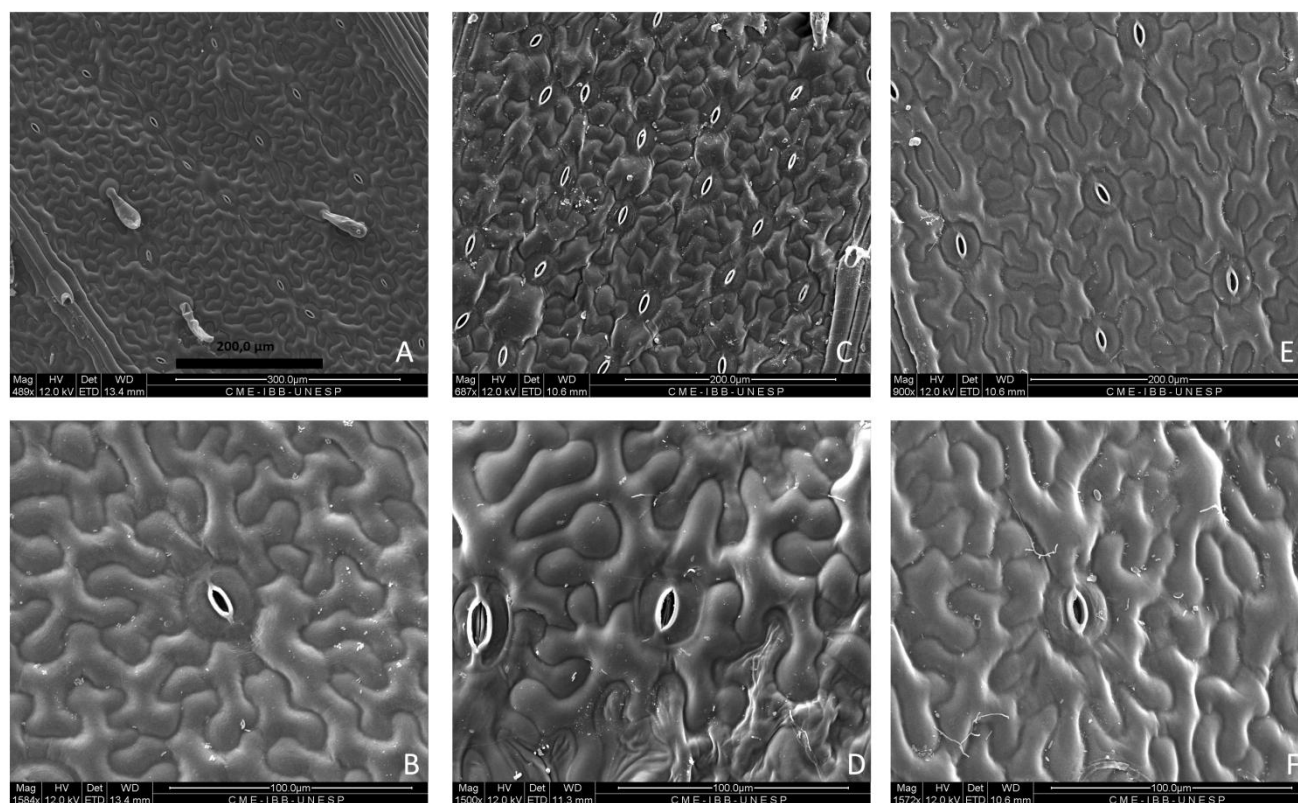


Fig. 10 - Pinnule surfaces of *Lygodium* in SEM, showing stomata. A-B: *L. venustum*; C-D: *L. volubile*; E-F: *L. x fayae*.

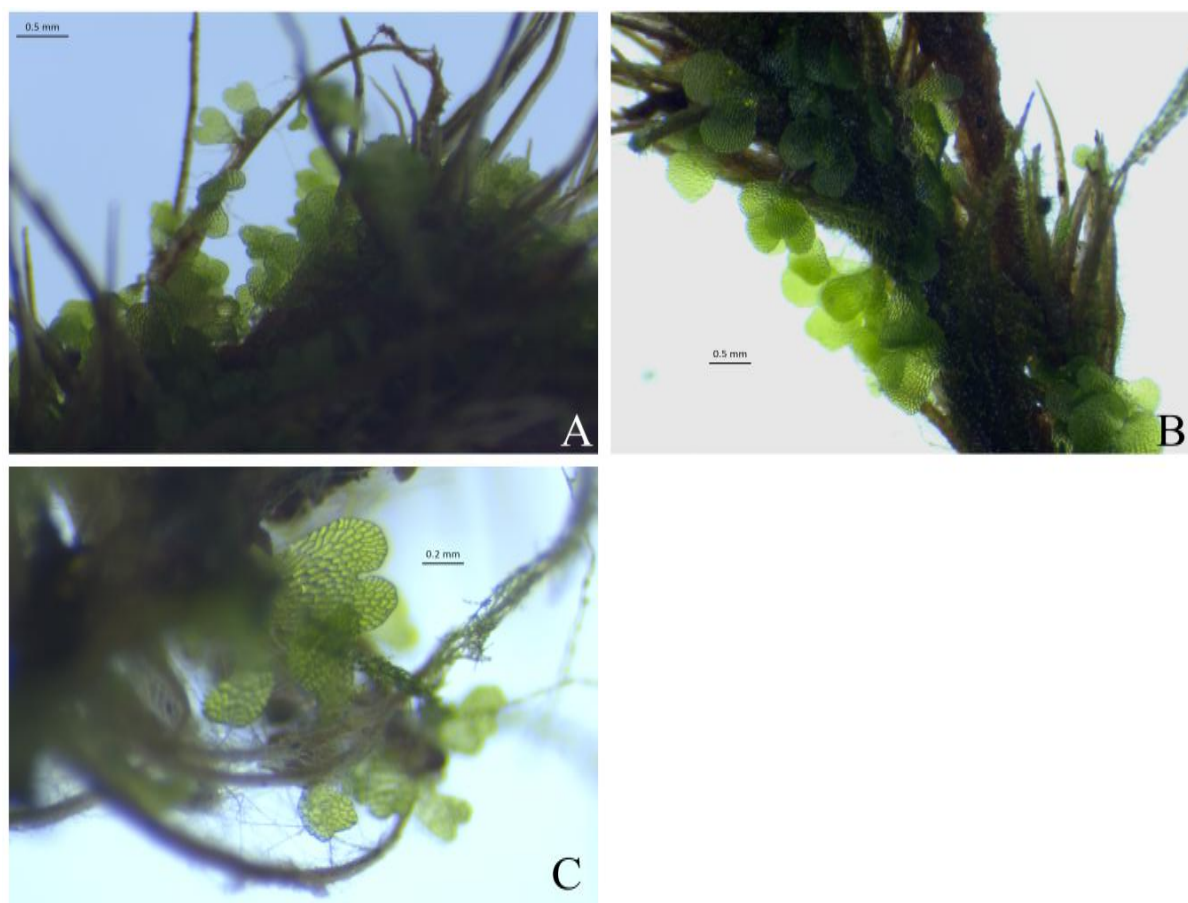


Fig. 11 - Gametophytes through magnifying lenses. A - *Lygodium x fayae*; B - *Lygodium volubile*; C - *Lygodium venustum*

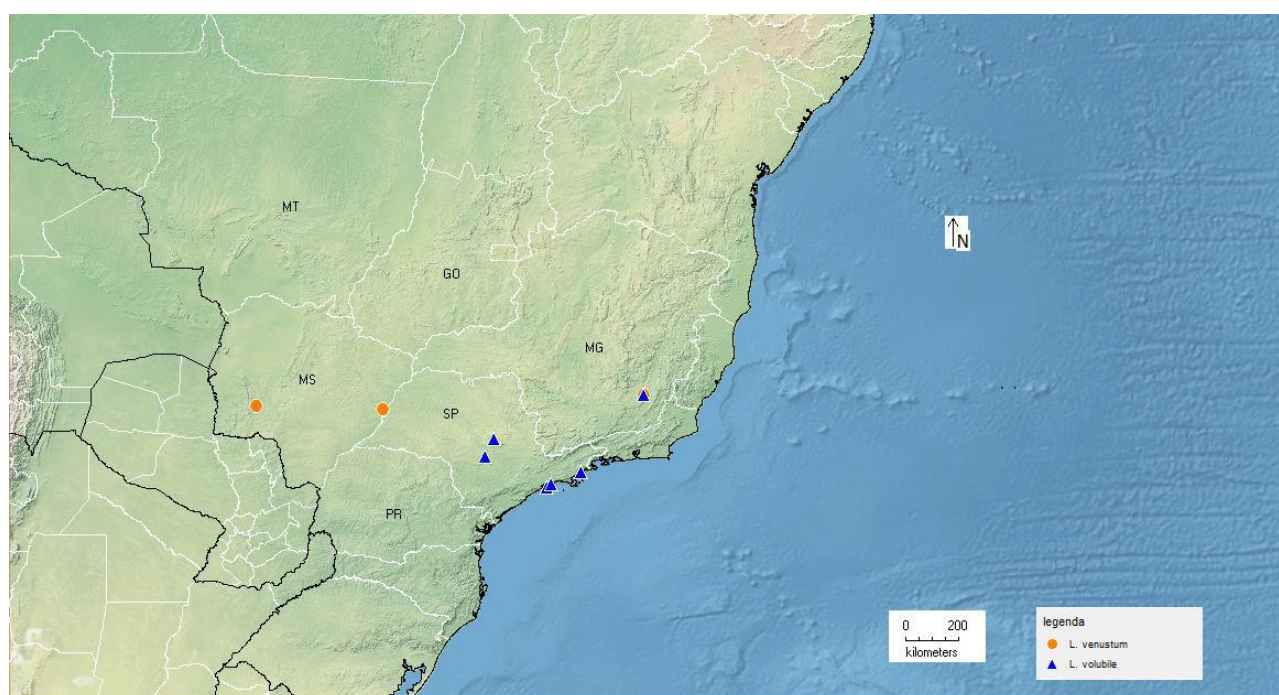


Fig. 12. Map of the representative specimens examined of *L. venustum* and *L. volubile* occurring in the Southeastern of Brazil.

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