The Botanical Review 72(2): 121–134

Microspore Morphology of *Isoetes* Species (Lycophyta) from Southern South America

C. Cecilia Macluf

Palinología and Morfología Vegetal, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n (1900), La Plata, Argentina

Marta A. Morbelli

Palinología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n (1900) La Plata, Argentina

AND

Gabriela E. Giudice

Morfología Vegetal, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n (1900) La Plata, Argentina

Abstract

Microspores of the 24 species of *Isoetes* that grow in southern South America were analyzed under a light microscope and scanning electron microscope. The microspores are monolete, elliptic in polar view, 30–40 μm long, and 20–25 μm wide. A background with various characteristics is seen on each surface. A supra-laesural expansion is present. The perispore is ornamented and has a perforated background. In section, it has a lacunose structure. The exospore is smooth, and it has a compact structure in section. The studied species could be divided into three groups by their perispore ornamentation: equinate, rugulate, and tuberculate. Microspore size was positively correlated with increasing ploidy level, and larger microspores were associated with terrestrial habitats. A convergence in ornamentation was found between spores produced by the studied species and those that grow in regions outside of the area under study.

Copies of this issue [72(2)] may be purchased from the NYBG Press, The New York Botanical Garden, Bronx, NY 10458-5125, U.S.A. Please inquire as to prices.

Issued 23 June 2006

Resumen

Se analizaron con microscopio óptico y electrónico de barrido las microsporas de veinticuatro especies de *Isoetes* que crecen en Sud América Austral. Las microsporas son monoletes, elípticas en vista polar, de 30–40 μm de longitud y 20–25 μm de ancho. En superficie se observa una base con diversas características. Una expansión sobre la lesura está presente. El perisporio está ornamentado y tiene una base perforada. En sección, tiene una estructura lacunosa. El exosporio es liso y en sección tiene estructura compacta. Las especies estudiadas se pueden reunir en tres grupos de acuerdo a la ornamentación del perisporio, estos son: equinado, rugulado y tuberculado. Se ha observado que existe una correlación positiva entre el tamaño de las microsporas y el incremento en el nivel de ploidía y el hábitat. Se observó una convergencia en la ornamentación entre las esporas producidas por las especies estudiadas y las producidas por otras especies que crecen en regiones fuera del área de estudio. Palabras clave: Sud América, *Isoetes*, microsporas, morfología, escultura.

Introduction

The Isoetaceae have a worldwide distribution. They live in temperate to warm regions of all continents, from sea level to 4200 m, most frequently above 2000 m (Tryon & Tryon, 1982). *Isoetes* L. is a cosmopolitan genus of heterosporate Lycophyta that includes about 150 species (Tryon & Tryon, 1982), 24 of which grow in southern South America, distributed in the following countries: Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay. The species growing in this region are *I. alcalophila* Halloy, *I. andicola* (Amstutz) Gomez, *I. boliviensis* Weber, *I. bradei* Herter, *I. brasiliensis* Fuchs, *I. chubutiana* Hickey, Macluf & Taylor, *I. ekmanii* Weber, *I. escondidensis* Halloy, *I. eshbaughii* Hickey, *I. favulata* Hickey, *I. fusco-marginata* Fuchs, *I. gardneriana* A. Braun, *I. herzogii* Weber, *I. hieronymii* Weber, *I. itaboensis* Fuchs, *I. lechleri* Mett., *I. panamensis* Maxon & Morton, *I. pedersenii* Fuchs, *I. ramboi* Herter, *I. savatieri* Franchet, *I. sehnemii* Fuchs, *I. smithii* Fuchs, *I. spannagelii* Fuchs, and *I. weberi* Herter.

Systematic (Pastore, 1936; Capurro, 1969) and floristic (de la Sota et al., 1998) aspects of *Isoetes* in southern South America have received some attention, but little information can be found in those contributions regarding palynological characteristics. The microspores of some species from the study area have been analyzed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) by Macluf et al. (2004) and Musselman (2003). The spores of *I. savatieri*, analyzed with light microscopy (LM) and SEM, have been considered in papers by Morbelli (1980), Hickey (1985, 1986), Hickey et al. (2003), and Macluf et al. (2003).

Spores of other species of *Isoetes* growing in other regions have been examined with TEM and SEM by Lugardon (1973, 1986), Robert et al. (1973), Prada Moral and Saenz de Rivas (1978), Tryon and Lugardon (1991), Taylor (1992, 1993), and Uehara et al. (1991).

Cox and Hickey (1984), Kott and Britton (1983), Hickey (1984), Luebke and Budke (2003), and Troia (2001) analyzed the relationship between micro- and mega-sized spores with ploidy and habitat.

The aim of this work was to study with LM and SEM the general characteristics of microspores of the *Isoetes* species that grow in southern South America to determine whether those characteristics are reliable enough to be used for systematic purposes. In

addition, the microspore assemblage produced by species of the region under study was compared with those of other regions.

Materials and Methods

Spores were obtained from fresh and herbarium specimens. The consulted herbaria are indicated by the initials used by Holmgren et al. (1990) as follows: Museo Argentino de Ciencias Naturales Bernardino Rivadavia (BA), Instituto de Botánica del Nordeste (CTES), Instituto-Fundación "Miguel Lillo" (LIL), Museo de La Plata (LP), Conservatoire et Jardin Botaniques de la Ville de Geneve (G), Harvard University (GH), Milwaukee Public Museum (MIL), Swedish Museum of Natural History (S), Instituto de Botánica Darwinion (SI), Museo Nacional de Historia Natural (SGO), Instituto de Botánica (SP), and Smithsonian Institution (US).

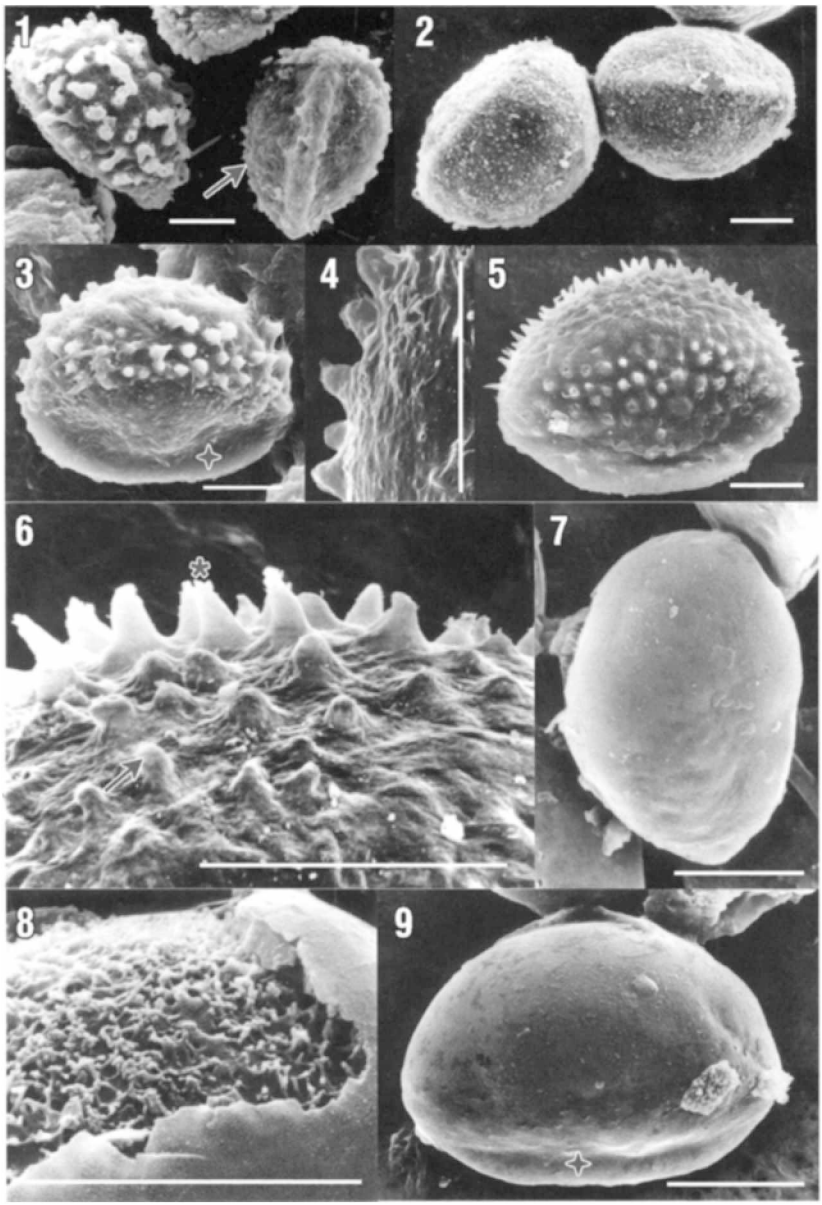
The analysis was performed by LM and SEM. For LM, the material was mounted in glycerine jelly without any chemical treatment. Dimensions were estimated for 25 spores per specimen. The minimum and maximum values in micrometers are given in the text and in Table I.

For studies with SEM, the spores were handled with moist brushes without any chemical treatment and placed on double-stick tape on bronze stubs. The samples were coated with gold-palladium and examined under a Jeol JSM-35 CF microscope at the

Table I

Microspore characteristics, ploidy, and habitat of *Isoetes* species from southern South America

Species	Microspore length (μm)	Microspore width (μm)	Ornamentation	Ploidy	Habitat
I. alcalophila	30–34	17–20	Tuberculate	2n = 22	Aquatic
I. andicola	34 (35) 36	17 (30) 30	Echinulate	2n = 44	Terrestrial
I. boliviensis	26 (35) 40	20 (25) 30	Tuberculate	2n = 22	Aquatic
I. bradei	42–43.6	28.4–29	Echinate		Aquatic
I. brasiliensis	26.4	16	Rugulate		Aquatic
I. chubutiana	33 (41) 41.3	26.2 (29) 33.8	Echinate	2n = 66	Aquatic
I. ekmanii	28–32	19–20	Rugulate		Aquatic
I. escondidensis	36	24	Echinate		Aquatic
I. eshbaughii	35–40	24–25	Echinate	2n = 44	Aquatic
I. favulata	38.9–41	27–28.4	Rugulate		Aquatic
I. fusco-marginata	32	22	Rugulate		Palustral
I. gardneriana	30–32	20–25	Rugulate		Amphibious
I. herzogii	34 (37) 38	22 (23) 25	Tuberculate	2n = 44	Aquatic
I. hieronymii	32–34	19–24	Echinate		Aquatic
I. itaboensis	26–29	19–20	Rugulate		Aquatic/terrestrial
I. lechleri	29 (35) 36	24 (25) 26	Tuberculate	2n = 44	Aquatic
I. panamensis	27 (30) 40	20 (26) 33	Rugulate	2n = 44	Aquatic
I. pedersenii	30–35	20–24	Echinate		Terrestrial
I. ramboi	30–33	21.2–25.2	Rugulate		Aquatic
I. savatieri	35 (40) 40	24 (28) 29	Echinulate	2n = 66	Aquatic
I. sehnemii	25	18.4	Rugulate		Aquatic
I. smithii	27.3–30	18	Rugulate		Aquatic
I. spannagelli	30	18.4	Rugulate		Aquatic
I. weberi	20–27	11–16	Echinate		Amphibious



Figs. 1–9. Microspores as seen by scanning electron microscope (SEM). **1.** *Isoetes alcalophila*. Distal (left) and proximal (right) views. The distal face is tuberculate. Some tubercles are fused and form ridges. The proximal face is rugulate. There is a projection at the equator (arrow). Scale bar: 10 μm. **2.** *Isoetes andicola*. Equatorial view. The whole surface shows echinulae and low cones. An equatorial projection is evident (asterisk). Scale bar: 10 μm. **3.** *Isoetes boliviensis*. Equatorial view showing the supralaesural over the laesura (star). The distal ornamentation consists of tubercles. The proximal and equatorial ornamentation is composed of low rugulae. Scale bar: 10 μm. **4.** *Isoetes boliviensis*. Detail of the distal and equatorial sculptures showing tubercles and rugulae. Scale bar: 10 μm. **5.** *Isoetes bradei*.

SEM laboratory of the Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Argentina.

MATERIAL STUDIED

Isoetes alcalophila. ARGENTINA. Tucumán, Tafí, Laguna Nostra, Huaca Huasi, Cumbres Calchaquíes, Halloy 171 (LIL).

I. andicola. PERU. Lima, Huarochiri, Saunders 1154 (GH).

I. boliviensis. PERU. Huamachuco, Hutchison, Wright & Straw 6143 (US).

I. bradei. BRAZIL. Brade 8119 (S).

I. brasiliensis. BRAZIL. Sao Leopold, Río Grande do Sul, Reitz 128 (LIL).

I. chubutiana. ARGENTINA. Río Negro, Parque Nacional Nahuel Huapi, Lago Mascardi, Taylor 6172 (MIL); Idem, Lago Guillelmo, Taylor 6169 (MIL).

I. ekmanii. ARGENTINA. Delta, Zanja de Correa, Burkart 4003 (BA 8231).

I. escondidensis. ARGENTINA. Tucumán, Laguna Escondida Media, Cumbres Calchaquíes, Halloy A 311 (LIL).

I. eshbaughii. BOLIVIA. Cochabamba, Hickey & Eshbaugh 824 (GH).

I. favulata. Chile, Nahuelbuta National Park, Nishida et al. 75003 (SGO).

I. fusco-marginata. BRAZIL. Brasilia, Rambo 44875 (LIL).

I. gardneriana. BRAZIL, Goyaz, Gardner 3563 (G).

I. herzogii. BOLIVIA, Cochabamba, Hickey & Eshbaugh 822 (GH).

I. hieronymii. ARGENTINA. Córdoba, Sierra Achala, Laguna de la Cumbre, Potrerillos, Hieronymus s/n (BA 473).

I. itaboensis. PARAGUAY. Alto Paraguay, Río Itabó, Shade s/n (CTES).

I. lechleri. BOLIVIA. Cochabamba, Hickey & Eshbaugh 821 (GH).

I. panamensis. PARAGUAY. Guarupé, Balansa 3294 (G).

I. pedersenii. ARGENTINA. Corrientes, Departamento de Mburucuyá, Estancia Santa María, Pedersen 8105 (LP).

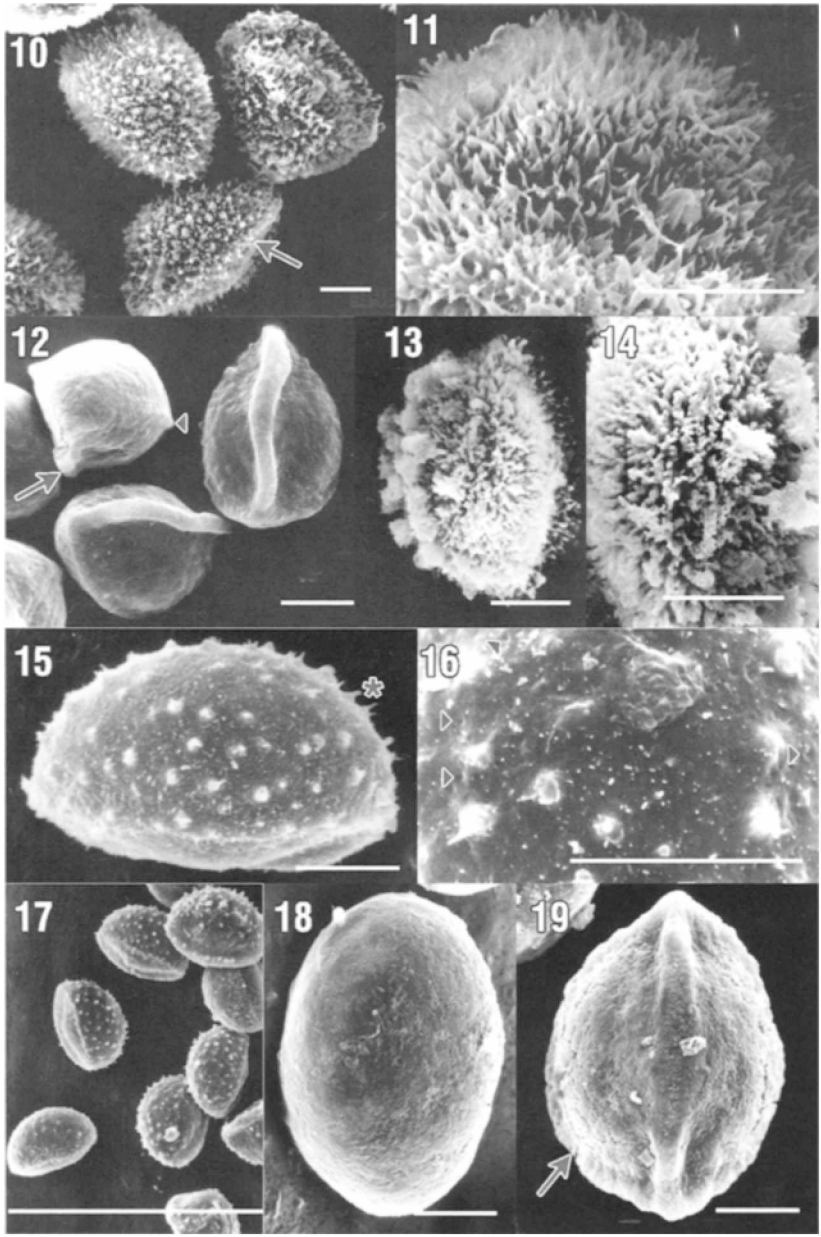
I. ramboi. BRASIL. Estado de Santa Catarina, Spannagel 475 (SP).

I. savatieri. ARGENTINA. Neuquén, Lago Huechulafquen, Fontana s/n (LP). Tierra del Fuego, Laguna Maravilla, Borge 110 (S).

I. smithii. BRAZIL. Río Grande do Sul, Pachinal Preto, Bom Jesus, Reitz 3305 (LIL).

I. weberi. BRAZIL. Porto Alegre, Rambo s/n (LIL 173624).

Figs. 1–9, *continued*
The ornamentation is echinate over the whole surface. The distal echinae are the tallest. The echinae become lower toward the equator. Scale bar: 10 μm. **6.** *Isoetes bradei*. Detail of the echinate distal sculpture. Some echinae have a furcate tip (asterisk) and verrucae toward the proximal face (arrow). Scale bar: 10 μm. **7.** *Isoetes brasiliensis*. Distal view. The surface is rugulate. Scale bar: 10 μm. **8.** *Isoetes brasiliensis*. A fracture that exposes the elements of the middle layer of the perispore below a thin, scabrate outer perispore layer. Scale bar: 10 μm. **9.** *Isoetes brasiliensis*. Equatorial view. A supralaesural expansion (star) is evident at the bottom. Scale bar: 10μm.



Figs. 10–19. Microspores as seen with SEM. **10.** *Isoetes chubutiana*. Spores in distal (top, left corner) and equatorial (top, right corner, and bottom) views. An equatorial projection is evident (arrow). The ornamentation consists of echinae of variable height, densely distributed over the whole surface. Scale bar: 10 μm. **11.** *Isoetes chubutiana*. Detail of the distal echinate sculpture. Scale bar: 10 μm. **12.** *Isoetes ekmanii*. Several microspores in different views. Equatorial view, at the left and bottom, showing a well-developed supra-laesural expansion (arrow) and an equatorial projection (arrowhead). The orna-

In cases such as *I. spannagelii* and *I. sehnemii*, for which herbarium specimens were not available, the descriptions by Fuchs-Eckert (1986) were used.

The ploidy levels of the studied species were taken from Cox and Hickey (1984), Troia (2001), and Hickey et al. (2003).

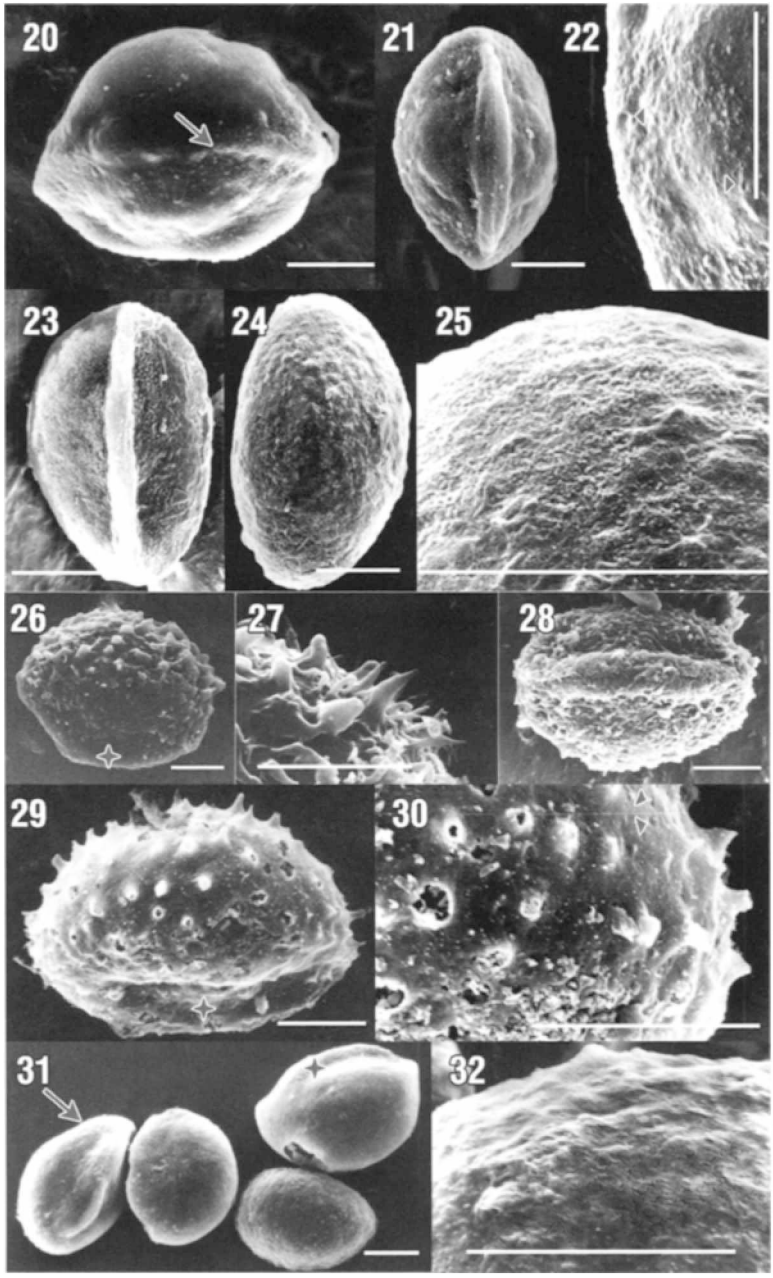
Results

The microspores produced by the studied species are 30–40 μm long and 20–30 μm wide, monolete, elliptic in polar view, and biconvex in equatorial view (Table I). A supra-laesural expansion is always present proximally. A swelling (cf. Musselman, 2003) perpendicular to the laesura was seen in some species (Figs. 21, 34). In section, the wall, from outside to inside, is composed of perispore, para-exospore, exospore, and endospore. The perispore of *Isoetes savatieri* (Macluf et al., 2003) is 0.5–2.8 μm thick in equatorial and distal areas and thins proximally; it has a lacunose structure. The para-exospore is formed of tangentially arranged bars and varies in thickness from 0.3 to 1 μm, depending on the area of the spore measured. The exospore is 0.2–0.5 μm thick. In section, it has a smooth margin and a compact structure.

With respect to the characteristics of the microspore surface, the equatorial area always shows particular features. Thus, some species show a projection, which is evident as an edge (= crest) or a blunt (= rounded) surface, and others show an ornamentation transition. Another typical characteristic of the microspores of this genus is the presence of a supra-laesural expansion, forming a kind of vestibule of variable height. In the studied spores, the supra-laesural expansion ranges from less than 1 μm to more than 5 μm in height. Thus, in spores of *I. favulata*, there is a poorly developed proximal expansion; in *I. brasiliensis* (Fig. 9), it is 2.5 μm high; in *I. eshbaughii* and *I. herzogii*, it is 3 μm high; in *I. itaboensis* (Fig. 31), it is 3 to 4 μm high; in *I. ekmanii* (Fig. 12), *I. gardneriana*, and *I. lechleri*, it is 4 μm high. In *I. pedersenii*, it is 4.5 μm high, in *I. andicola* and *I. panamensis* (Fig. 37), it is 5 μm high, and in *I. hieronymii*, it is 5.5 μm high (Fig. 29).

An equatorial projection is present in *I. alcalophila* (Fig. 1), *I. andicola* (Fig. 2), *I. chubutiana* (Fig. 10), *I. ekmanii* (Fig. 12), *I. eshbaughii* (Fig. 15, 17), *I. favulata* (Fig. 19), *I. fusco-marginata* (Figs. 20, 21), *I. gardneriana* (Fig. 23), *I. hieronymii* (Fig. 29), *I. itaboensis* (Fig. 31), *I. lechleri* (Fig. 34), *I. savatieri* (Fig. 44), *I. smithii* (Figs. 46, 47), and *I. weberi* (Fig. 48). In microspores of *I. favulata*, there is an equatorial projection 2.6 μm thick and 2 μm wide.

Figs. 10–19, *continued*
mentation consists of rugulae over the whole surface. Scale bar: 10 μm. **13.** *Isoetes escondidensis*. Equatorial view. The difference in ornamentation of the two surfaces is evident. Scale bar: 10 μm. **14.** *Isoetes escondidensis*. Detail of the surface at the equator. There is a transition of the ornamentation in this area; there are echinae on the proximal face. Some echinae are fused at the equator. Scale bar: 10 μm. **15.** *Isoetes eshbaughii*. Equatorial view. The whole surface is echinate. The distal echinae (asterisk) are taller and more widely spaced. Scale bar: 10 μm. **16.** *Isoetes eshbaughii*. Detail of the distal sculpture showing large echinae and echinulae on a perforated (arrowheads) background. Scale bar: 10 μm. **17.** *Isoetes eshbaughii*. Several microspores in proximal, equatorial, and distal views. The difference in ornamentation between the two surfaces is evident. The proximal sculptural elements are low. Both the supra-laesural expansion and the equatorial projection are evident. Scale bar: 100 μm. **18.** *Isoetes favulata*. Distal face. The perispore sculpture is rugulate. Scale bar: 10 μm. **19.** *Isoetes favulata*. Proximal view. The rugulae are distributed over the whole surface, and they are also present on the supra-laesural expansion. An equatorial projection is evident (arrow). Scale bar: 10 μm.



Figs. 20–32. Microspores as seen with SEM. **20.** *Isoetes fusco-marginata.* Equatorial view showing the well-developed supra-laesural expansion and the equatorial projection (arrow). Scale: 10 μm. **21.** *Isoetes fusco-marginata.* Proximal view. Swelling perpendicular to the laesura. Scale bar: 10 μm. **22.** *Isoetes fusco-marginata.* Detail of the distal sculpture showing rugulae and granules on a perforated

A transition in ornamentation in the equatorial area is present in *I. escondidensis* (Fig. 13) and *I. herzogii* (Fig. 26).

When the spore surface is analyzed at high magnification, the background can be distinguished. It is generally composed of perforations and low sculptural elements. The shape of the latter may be the same as or different from the larger ones, as in *I. chubutiana* (Fig. 10), *I. escondidensis* (Figs. 13, 14), *I. boliviensis* (Fig. 3), *I. lechleri* (Fig. 35), and *I. weberi* (Fig. 48). In spores with a levigate surface, the background is composed only of perforations as in *I. fusco-marginata* (Fig. 22), *I. hieronymii* (Fig. 30), and *I. eshbaugii* (Fig. 16).

The perispore sculptural elements are varied: cones, echinae, echinulae, tubercles, rugulae, and granules. These elements vary in type, size, and distribution. In *I. bradei* (Figs. 5, 6), *I. eshbaughii* (Figs. 15, 16, 17), *I. hieronymii* (Figs. 29, 30), *I. pedersenii* (Figs. 40, 41), and *I. weberi* (Fig. 48), the echinae are sparsely distributed over the whole surface. There is variability in the size and density of the echinae on the different spore faces. They are generally higher and more densely distributed distally.

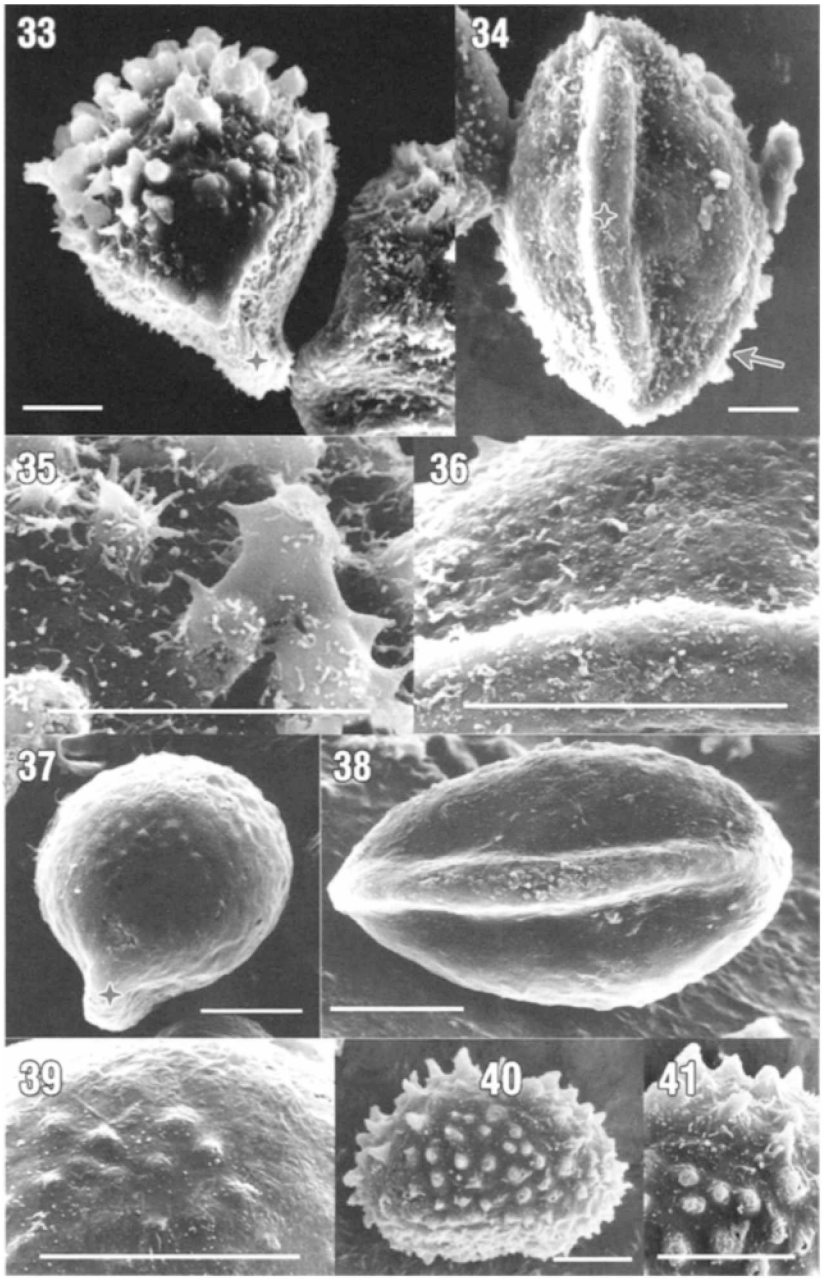
In *I. bradei,* there are echinae on the equatorial and distal faces and verrucae on the proximal face; some of these are branched at the apex (Fig. 6).

In *I. eshbaughii,* the echinae are 1 to 3 μm high. In *I. hieronymii,* the proximal echinae are 1 to 3 μm high. In *I. pedersenii,* the echinae are 1 to 3 μm high and 0.3 to 0.5 μm in diameter at the base.

The perispore has high echinae in spores produced by *I. escondidensis,* which are uniformly and densely distributed over the whole surface. Some of these processes have a blunt apex (Fig. 14), and others are fused at their bases and form high processes on the distal face (Fig. 13). The surface of the supra-laesural expansion has echinulae.

The spores of *I. chubutiana* show a perispore with echinae of different sizes, densely distributed over the whole surface (Figs. 10, 11), while verrucae are also present in equatorial and distal areas. The spores of *I. andicola* (Fig. 2) and *I. savatieri* (Figs. 44, 45) show a perispore with echinulae and small cones over the whole surface.

Figs. 20–32, *continued*
background (arrowheads). Scale bar. 10 μm. **23.** *Isoetes gardneriana.* Proximal view with the equatorial projection and a well-developed supra-laesural expansion. The perispore surface is rugulate. Scale bar: 10 μm. **24.** *Isoetes gardneriana.* Distal view of a spore with a rugulate perispore. Granules are present over the whole surface. Scale bar: 10 μm. **25.** *Isoetes gardneriana.* Detail of the distal sculpture showing rugulae and granules. Scale bar: 10 μm. **26.** *Isoetes herzogii.* Equatorial view showing the proximal supra-laesural expansion (star). The difference in ornamentation between the two surfaces is evident. Scale bar: 10 μm. **27.** *Isoetes herzogii.* Detail of the equatorial sculpture showing densely distributed echinae. Scale bar: 10 μm. **28.** *Isoetes herzogii.* Proximal view. The perispore sculpture is composed of echinulae, which are also present on the laesura. Scale bar: 10 μm. **29.** *Isoetes hieronymii.* Equatorial view showing a prominent supra-laesural expansion (star). The ornamentation consists of echinae uniformly distributed over the whole surface. Scale bar: 10 μm. **30.** *Isoetes hieronymii.* Detail of the distal surface. The background is composed of perforations (arrowheads). Some echinae have their tips broken, and the slender elements of the middle perispore layer are exposed. Scale bar: 10 μm. **31.** *Isoetes itaboensis.* Several microspores in proximal, distal, and equatorial views. Top right, equatorial view. The prominent supra-laesural expansion is marked by a star. At the left, the proximal view shows a blunt equatorial projection (arrow). The ornamentation consists of rugulae distributed over the whole surface. Scale bar: 10 μm. **32.** *Isoetes itaboensis.* Detail of the distal surface showing rugulae, granules, and small verrucae. Scale bar: 10 μm.



Figs. 33–41. Microspores as seen with SEM. **33.** *Isoetes lechleri*. Equatorial view showing the supra-laesural expansion (star). The difference in ornamentation between the two polar surfaces is evident. The distal ornamentation consists of tubercles, and the proximal of echinulae. Scale bar: 10 μm. **34.** *Isoetes lechleri*. Proximal view. The equatorial projection (arrow) and the supra-laesural expansion (star) are

In the spores of *I. alcalophila* (Fig. 1), *I. boliviensis* (Fig. 3), *I. herzogii* (Fig. 28), and *I. lechleri* (Figs. 33, 34), the proximal and equatorial areas are rugulate, whereas tubercles are seen distally (Figs. 4, 26, 33). In *I. boliviensis,* the tubercles are 1 to 3 μm high proximally and 2 to 3 μm high distally, with a sharp or truncate apex. In *I. lechleri,* there are echinulae and rugulae proximally. These elements are also present on the supra-laesural expansion. The distal tubercles are 3 to 4 μm high.

The perispore shows rugulae and granules distributed over the whole surface in spores produced by *I. brasiliensis* (Fig. 7), *I. ekmanii* (Fig. 12), *I. favulata* (Figs. 18, 19), *I. fusco-marginata* (Fig. 22), *I. gardneriana* (Figs. 24, 25), *I. itaboensis* (Figs. 31, 32), *I. panamensis* (Figs. 37, 39), *I. ramboi* (Figs. 42, 43), and *I. smithii* (Figs. 46, 47). In *I. panamensis,* there are verrucae sparsely distributed on the distal surface (Fig. 37). The background is composed of perforations and granules (Fig. 39).

According to the observations of Fuchs-Eckert (1986), the microspores of *I. sehnemii* and *I. spannagelii* show rugulae on the surfaces of both polar faces.

Discussion and Conclusions

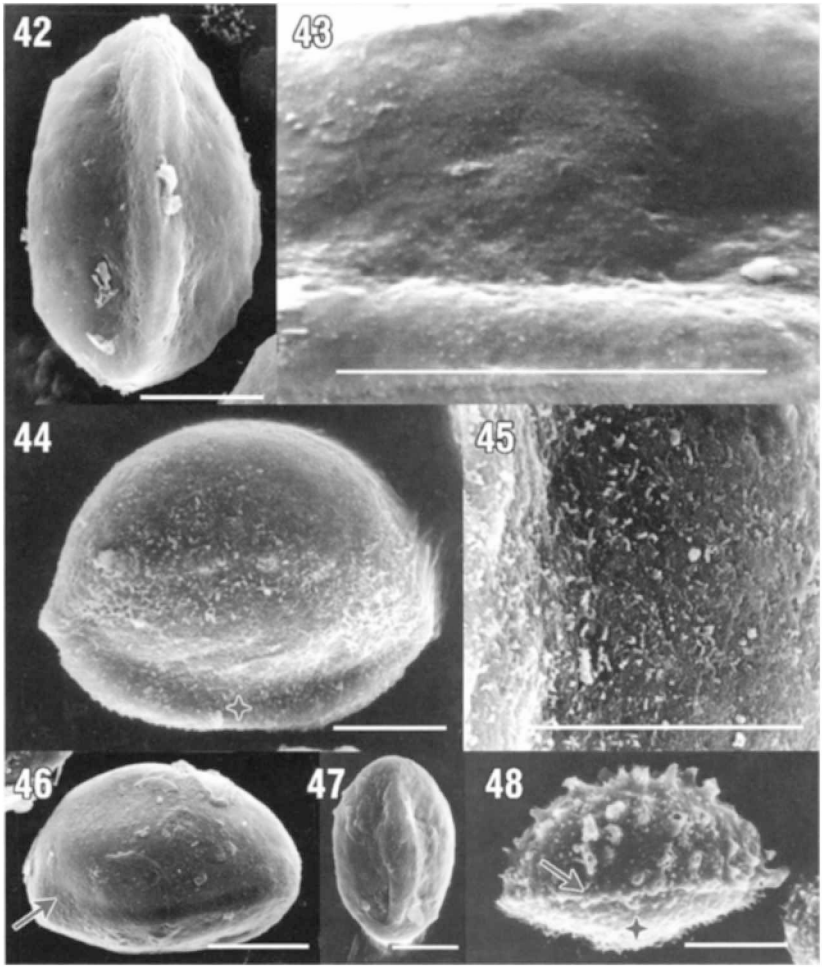
In a previous study (Macluf et al., 2003) and in this study, it was found that characteristics such as ornamentation type, morphology and location of the sculpture elements, and the characteristics of the equatorial area that can be useful for systematic purposes at the infrageneric level.

Among the characteristics analyzed, ornamentation seems to be the one that allows the species to be gathered into groups, while the rest of the characteristics studied should be considered secondarily in order to further differentiate among them. These second-level characteristics are size, the height of the supra-laesural chamber, the presence or absence of a transversal swelling, and the nature of the equatorial differentiation.

The 24 species studied could be grouped according to the dominant element of their perispore sculpture into three main patterns: echinate, rugulate, and tuberculate. The **echinate pattern** is characteristic of the spores produced by the following species: *I. bradei, I. eshbaughii, I. hieronymii, I. pedersenii; I. weberi, I. escondidensis, I. chubutiana, I. savatieri,* and *I. andicola.* The **rugulate pattern** is characteristic of the spores produced by the species *I. brasiliensis, I. ekmanii, I. favulata, I. fusco-marginata, I. gardneriana, I. itaboensis, I. panamensis, I. ramboi, I. sehnemii, I. smithii,* and *I. spannagelii.* The **tuberculate pattern** is characteristic of the spores produced by *I. alcalophila, I. boliviensis, I. herzogii,* and *I. lechleri.* The tuberculate is the least frequent pattern among the analyzed species.

In most species, there is variation in the ornamentation between the two polar faces.

Figs. 33–41, *continued*
evident. **35.** *Isoetes lechleri.* Detail of the distal sculpture showing tubercles on a background composed of perforations and echinulae. Scale bar: 10 μm. **36.** *Isoetes lechleri.* Detail of the proximal perispore sculpture. It is composed of perforations, echinulae, and rugulae. Scale bar: 10 μm. **37.** *Isoetes panamensis.* Equatorial view showing the supra-laesural expansion (star). There are rugulae on both surfaces. Scale bar: 10 μm. **38.** *Isoetes panamensis.* Proximal view. The supra-laesural expansion is evident. Scale bar: 10 μm. **39.** *Isoetes panamensis.* Detail of the surface at the equator showing large rugulae and granules. Scale bar: 10 μm. **40.** *Isoetes pedersenii.* Equatorial view showing echinate sculpture. The proximal echinae are lower than the distal ones. Scale bar: 10 μm. **41.** *Isoetes pedersenii.* Detail of the surface at the equator. The ornamentation consists of large echinae and sparsely distributed cones. Scale bar: 10 μm.



Figs. 42–48. Microspores as seen with SEM. **42.** *Isoetes ramboi*. The proximal sculpture is rugulate. A blunt supra-laesural expansion is evident. Scale bar: 10 μm. **43.** *Isoetes ramboi*. Detail of the proximal surface, which has rugulae and granules. They are also present on the supra-laesural expansion. Scale bar: 10 μm. **44.** *Isoetes savatieri*. Equatorial view. The ornamentation consists of echinulae and small cones on a perforated background. The supra-laesural expansion (star) has the same sculpture as the rest of the spore. Scale bar: 10 μm. **45.** *Isoetes savatieri*. Detail of the proximal sculpture showing echinulae and perforations over the whole surface. Scale bar: 10 μm. **46.** *Isoetes smithii*. Equatorial view. The equatorial projection is evident (arrow). The ornamentation consists of rugulae and granules. Scale bar: 10 μm. **47.** *Isoetes smithii*. Proximal view showing rugulae and granules. Scale bar: 10 μm. **48.** *Isoetes weberi*. Equatorial view. The equatorial projection (arrow) and the supra-laesural expansion (star) are evident. The ornamentation consists of large echinae on a echinulate perforated background. Scale bar: 10 μm.

Thus, the ornamentation elements are generally lower and more spaced proximally, and they may be similar to or different from those of the distal face.

Variation in the size of the sculpture elements was also recognized within the established groups. Those variations occur with respect to height, base width, and apex shape of the elements. Thus, for instance, within the echinate pattern, *I. savatieri* and *I. andicola* have spores with echinulae and small cones over the whole surface, and *I. chubutiana* has echinae of different sizes densely distributed over the whole surface.

It was noticed that the microspores of the studied species have a similar ornamentation to that of the microspores of some other species that grow in other regions, outside of the area of study. Thus, *I. savatieri* and *I. hieronymii* have echinate ornamentation similar to that described by Musselman (2003) as aculeate in *I. boomii*, *I. saccharata* (USA), *I. maritima* (Canada), and *I. abyssinica* (Kenya). The microspores of *I. ekmanii* are rugulate and similar to those of *I. engelmanii* and *I. hopei*, which grow in the United States and New Guinea, respectively. They were described by Musselman (2003) as psilate.

Isoetes chubutiana produces echinate microspores similar to those of *I. andina* Hook, which grows in Equador, and *I. jamaicensis* Hickey (Tryon & Lugardon, 1991), which grows in Jamaica. This similarity in ornamentation between the microspores studied and those produced by nonrelated species suggests a probable convergence in the ornamentation patterns. Thus, it is necessary to collect data on, for example, the chromosome number and the DNA in order to corroborate whether the species compared represent different evolutionary lineages.

From the available chromosome data, it was possible to positively correlate an increase in the ploidy level with an increase in the microspore size (Table I), as is shown by a comparison of the ploidy levels with the sizes of microspores produced by *I. chubutiana* and *I. alcalophila*. Luebke and Budke (2003) came to the same conclusion, comparing *I. tennesseensis* and *I. lacustris*. Kott and Britton (1983) also found that the mean size of microspores and megaspores of *Isoetes* generally are in agreement with the ploidy level.

In this study, it was found that within a given ploidy level, terrestrial species produce larger microspores than aquatic species.

In a second contribution, the ultrastructure of the sporoderm is analyzed for the different ornamentation patterns recognized in this study (Macluf et al., 2006).

Acknowledgments

The authors thank Rafael Urrejola of the Servicio de Microscopía Electrónica de Barrido del Museo de Ciencias Naturales de La Plata and the institutions that sent the herbarium material. This work was supported by grants from the National Council of Scientific and Technological Research (CONICET), Buenos Aires (PIP 5044), the National Agency of Scientific and Technological Promotion (ANPCyT) (PICT 12758), and UNLP (project 363). Part of the results of this study were presented at the 11th International Palynological Congress, held in Granada, Spain, which was made possible by a grant (to C.C. Macluf) from the congress organizing committee.

Literature Cited

Capurro, R.H. 1969. División Pteridophyta. Pp. 123–146 *in* A.L. Cabrera, (ed.), Flora de la provincia de Buenos Aires. Colección Científica Instituto Nacional de Tecnología Agropecuaria 4 (1), Buenos Aires, Argentina.

Cox, P.A. & R.J. Hickey. 1984. Convergent megaspore evolution and *Isoetes*. Amer. Naturalist 124: 437–441.

de la Sota, E.R., M. Ponce, M.A. Morbelli & L. Cassá. 1998. Pteridophyta. Pp. 282–369 *in* Flora Patagónica. Colección Científica Instituto Nacional de Tecnología Agropecuaria 8, Buenos Aires, Argentina.

Fuchs-Eckert, H.P. 1986. Isoetaceas. Pp. 1–42 *in* R. Reitx (Ed.), Flora Ilustrada Catarinense, Santa Catarina, Brazil.

Hickey, R.J. 1984. Chromosome numbers of neotropical *Isoetes*. Amer. Fern J. 74: 8–13.

———. 1985. Revisionary studies of neotropical *Isoetes*. Ph. D. diss., Univ. of Connecticut, Storrs, CT.

———. 1986. *Isoetes* megaspore surface morphology: nomenclature variation and systematic importance. Amer. Fern J. 76: 1–16.

———, **C. Macluf & W.C. Taylor.** 2003. A re-evaluation of *Isoetes savatieri* Franchet in Argentina and Chile. Amer. Fern J. 93 (3): 126–136.

Holmgren P.K., N.H. Holmgren & L.C. Barnett. 1990. Index Herbariorum. Part I: The Herbaria of the World. New York Botanical Garden, New York.

Kott, L. & D.M. Britton. 1983. Spore morphology and taxonomy of *Isoetes* in northeastern North America. Canad. J. Bot. 61: 3140–3163.

Luebke, N.T. & J.M. Budke. 2003. *Isoetes tennesseensis* (Isoetaceae), an octoploid Quillwort from Tennessee. Amer. Fern J. 93 (4): 184–190.

Lugardon, B. 1973. Palynologie. Nomenclature et structure fine des parois acéto-resistantes des microspores d'*Isoetes*. Compt. Rend. Acad. Sci. Paris, Sér. D 276: 3017–3020.

———. 1986. Données ultrastructurales sur la fonction de l'exospore chez les Pteridophytes (ultrastructural data of the exospore function in pteridophyte spores). Pp. 251–264 *in* S. Blackmore & I.K. Ferguson (Eds.), Pollen and spores: form and function. Linnean Society Symposium Series No. 12, Academic Press, London.

Macluf, C.C., M.A. Morbelli & G.E. Giudice. 2003. Morphology and ultrastructure of megaspores and microspores of *Isoetes savatieri* Franchet (Lycophyta). Rev. Palaeobot. Palynol. 126: 197–209.

———, ——— & ———. 2004. Morphology and ultrastructure of microspores of *Isoetes* species (Lycophyta) from southern South America. International Congress of Palynology Abstracts, Polen 14: 323.

———, ——— & ———. 2006. Microspore morphology of *Isoetes* species (Lycophyta) from southern South America. Part II. TEM analysis of some selected types. Bot. Rev. 72(2): 135–152.

Morbelli, M.A. 1980. Morfología de las esporas de Pteridophyta presentes en la región fuego-patagónica de la República Argentina. Opera Lilloana 28: 1–138.

Musselman, L.J. 2003. Ornamentation of *Isoetes* (Isoetaceae, Lycophyta) microspores. Bot. Rev. 68: 474–487.

Pastore, A.L. 1936. Las Isoetaceas argentinas. Revista Mus. La Plata, Secc. Bot. 1: 3–30.

Prada Moral, C. & C. Saenz de Rivas. 1978. Estructura de la esporodermis en las especies españolas de los géneros *Isoetes* L. (Isoetales) y *Cheilanthes* Schwarz (Filicales). Anales Inst. Bot. Cavanilles 35: 245–259.

Robert, M.D., F. Roland-Heydacker, J. Denizot, J. Laroche, P. Fougeroux & L. Davignon. 1973. La paroi mégasporale de L'*Isoetes setaceae* Bosc, Ex Delile. Étude en microscopies photonique et électroniques. Localisation et nature de la silice entrant dans sa constitution. Adansonia 2: 313–332.

Taylor, W.A. 1992. Megaspore wall ultrastructure development in *Isoetes melanopoda*: morphogenetic post-initiation changes accompanying spore enlargement. Rev. Palaeobot. Palynol. 72: 61–72.

———. 1993. Megaspore wall ultrastructure in *Isoetes*. Amer. J. Bot. 80: 165–171.

Troia, A. 2001. The genus *Isoetes* L. (Lycophyta, Isoetaceae): synthesis of karyological data. Webbia 56: 201–218.

Tryon, A.F. & B. Lugardon. 1991. Spores of the Pteridophyta. Surface, wall structure and diversity based on electron microscope studies. Springer, New York.

Tryon, R.M. & A.F. Tryon. 1982. Ferns and allied plants. Springer, New York.

Uehara, K., S. Kurita, N. Sahashi & T. Ohmoto. 1991. Ultrastructural study on microspore wall morphogenesis in *Isoetes japonica* (Isoetaceae). Amer. J. Bot. 78: 1182–1190.