



## Two New Species of *Isoetes* (Isoetaceae) from northern Brazil

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### Abstract

*Isoetes serracarajensis* and *I. cangae*, two new species from northern Brazil, are described, illustrated, and compared to similar species. *Isoetes serracarajensis* can be distinguished from other species by a set of characters including triangular to linear microphylls, a reniform-orbicular labium, a rudimentary velum covering up to 1/5 of the sporangial surface, dark brown to red-brown sporangia and verrucate megaspores (verrucae type 1). *Isoetes cangae* presents large microphylls, a reniform-orbicular labium, a rudimentary velum covering only up to 1/4 of the sporangium surface, hyaline sporangia and verrucate (verrucae type 2) to tuberculate megaspores (tubercles type 3). Based on the IUCN criteria, we suggest that *Isoetes serracarajensis* should be classified as vulnerable (VU) and *Isoetes cangae* included as critically endangered (CR).

**Key words:** aquatic plants, lycopsid, Serra do Carajás, spores, taxonomy

### Introduction

The lycopsid genus *Isoetes* Linnaeus (1753: 1100) is a worldwide distributed plant group occurring aquatic in lakes, ponds, streams, estuaries, bogs, or terrestrial on seasonally inundated soil (Taylor & Hickey 1992). The genus comprises ca. 200 species, and South America is considered a center of both morphological and taxonomical diversity for *Isoetes* (Hickey 1990). Particularly in Brazil, the genus seems to be highly diverse with nearly 23 species (Prado *et al.* 2015).

Efforts to understand the taxonomy of *Isoetes* from Brazil recently led authors to publish studies on spores (Lorscheitter *et al.* 2009), on chromosome numbers (Pereira *et al.* 2015), as well as on new species (Pereira *et al.* 2012, Pereira & Labiak 2013, Windisch *et al.* 2014). However, most of these studies were based on southeastern Brazilian taxa, where the genus seems to be more diverse (Prado *et al.* 2015). Meanwhile the genus is almost unknown from northern Brazil.

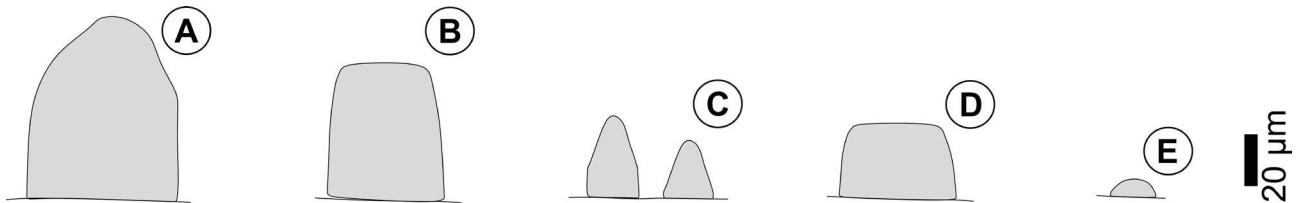
Fieldworks conducted to access the ferns and lycophytes diversity in Brazilian Amazon resulted in the collection of two *Isoetes* species that we recognize as new to science. In this work, we provide descriptions, illustrations, images of the spores, and a taxonomic key to identify these new species.

### Material and Methods

Materials of two new *Isoetes* species were collected in the Serra dos Carajás, Pará, northern Brazil (Fig. 5). Voucher specimens were deposited at B, BHCB and UPCB (acronyms following Thiers 2016). For each locality where these species were collected (see additional specimens examined), we examined spores of at least three specimens. To delimitate these two new species, we also compared them with the original descriptions and type-specimens of *Isoetes*

*amazonica* A. Braun (*Spruce 1081*, K, M), *I. gardneriana* Kunze ex A. Braun (*Gardner 3563*, B, K, E), *I. ovata* N. Pfeiff. (*Goebel s.n.*, NY), and *I. triangular* U. Weber (*Ule 8000*, B, K).

Spore images were generated by transferring the spores to aluminum scanning electron microscope (SEM) stubs coated with a carbon adhesive. The stubs were then coated with gold-palladium-alloy in a sputter-coater for 180 sec, after which the spores were digitally imaged using a Zeiss SIGMA VP. The resulting images were adjusted in Photoshop for contrast and the background was altered to black. To estimate the average measurements of the spores, we used a minimum of 20 spores per sporangium, from at least three sporangia. The terminology used for description of the spores follows that of Punt *et al.* (2007), with some slight modifications. For the macrosculpture of the megaspore surface, we define verrucae as distinct from tubercles by being broader than high (vs. tubercles are higher than broad). Based on analyses of the megaspores of the type materials of the more similar species (data not shown), the tubercles were classified into three types and the verrucae shown two types (Fig. 1).



**FIGURE 1.** Macrosculptural elements on the megaspore surface of *Isoetes*. A. *Isoetes ovata* and *I. triangular*: tubercle type 1. B. *Isoetes serracarajensis* (only distal surface): tubercle type 2. C. *Isoetes gardneriana* and *I. cangae*: tubercle type 3. D. *Isoetes serracarajensis* and *I. amazonica*: verruca type 1. E. *Isoetes cangae*: verruca type 2.

## Taxonomy

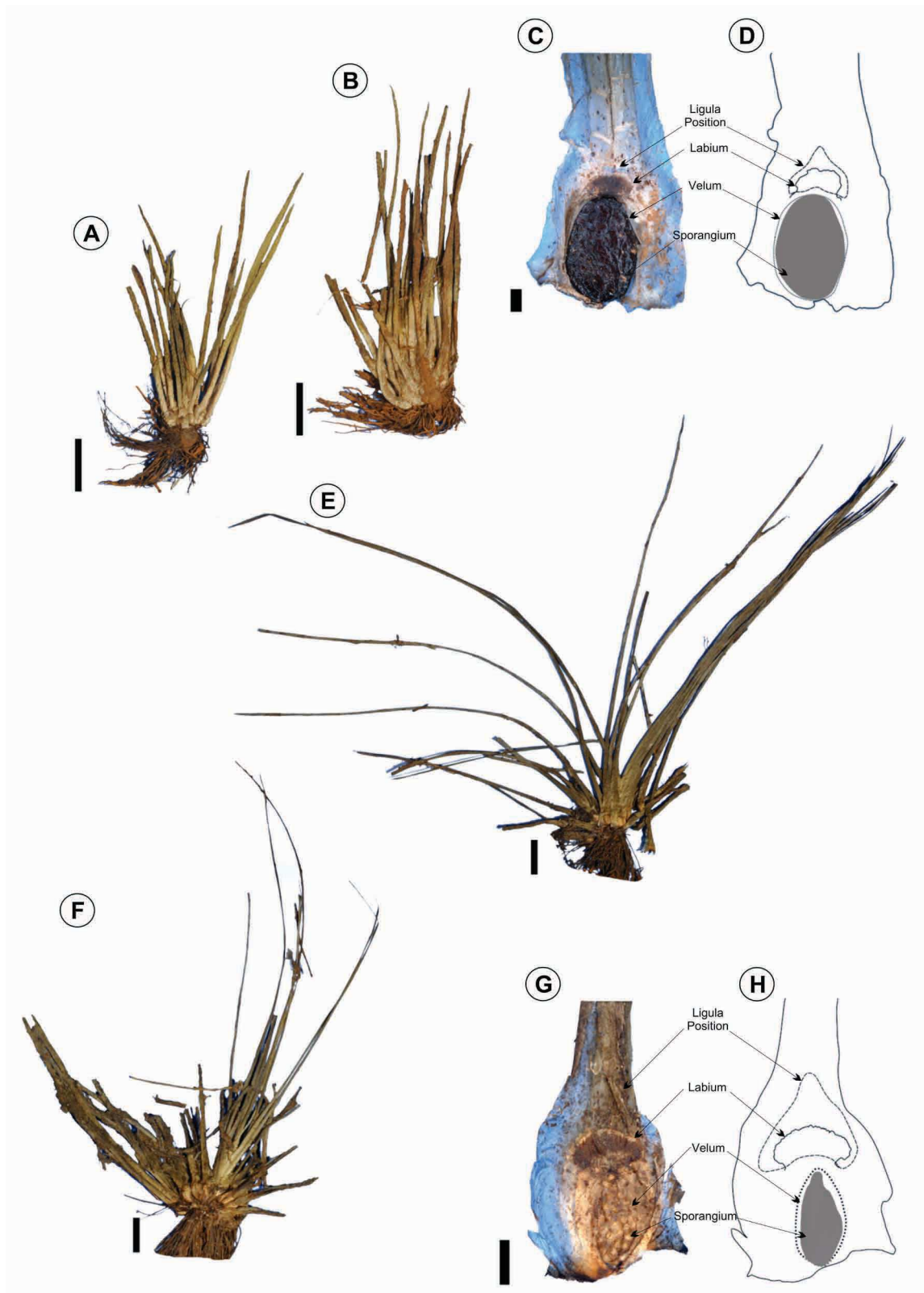
*Isoetes serracarajensis* J.B.S.Pereira, Salino & Stützel, *sp. nov.* (Figs. 2A–D, 3)

*Isoetes serracarajensis* is distinguished from other species of the Amazon Basin by a combination of the following characters: microphylls triangular to linear, labium reniform-orbicular, velum rudimentary to covering up to 1/5 of the sporangial surface, sporangium dark brown to red-brown on adaxial surface, megaspores verrucate (verruca type 1), with the laesures wider than higher or as wide as high and clearly separated from the remaining macrosculptural elements of the proximal surface.

**Type:**—BRAZIL. Pará: Parauapebas; Serra Norte, N6, 06°07'48"S, 50°10'32"W, 694 m, 26 January 2013, *A.J. Arruda 1356 with P.L. Viana, M.O. Pivari, B.P. Leles, F. M. Santos* (holotype B!, isotype BHCB!).

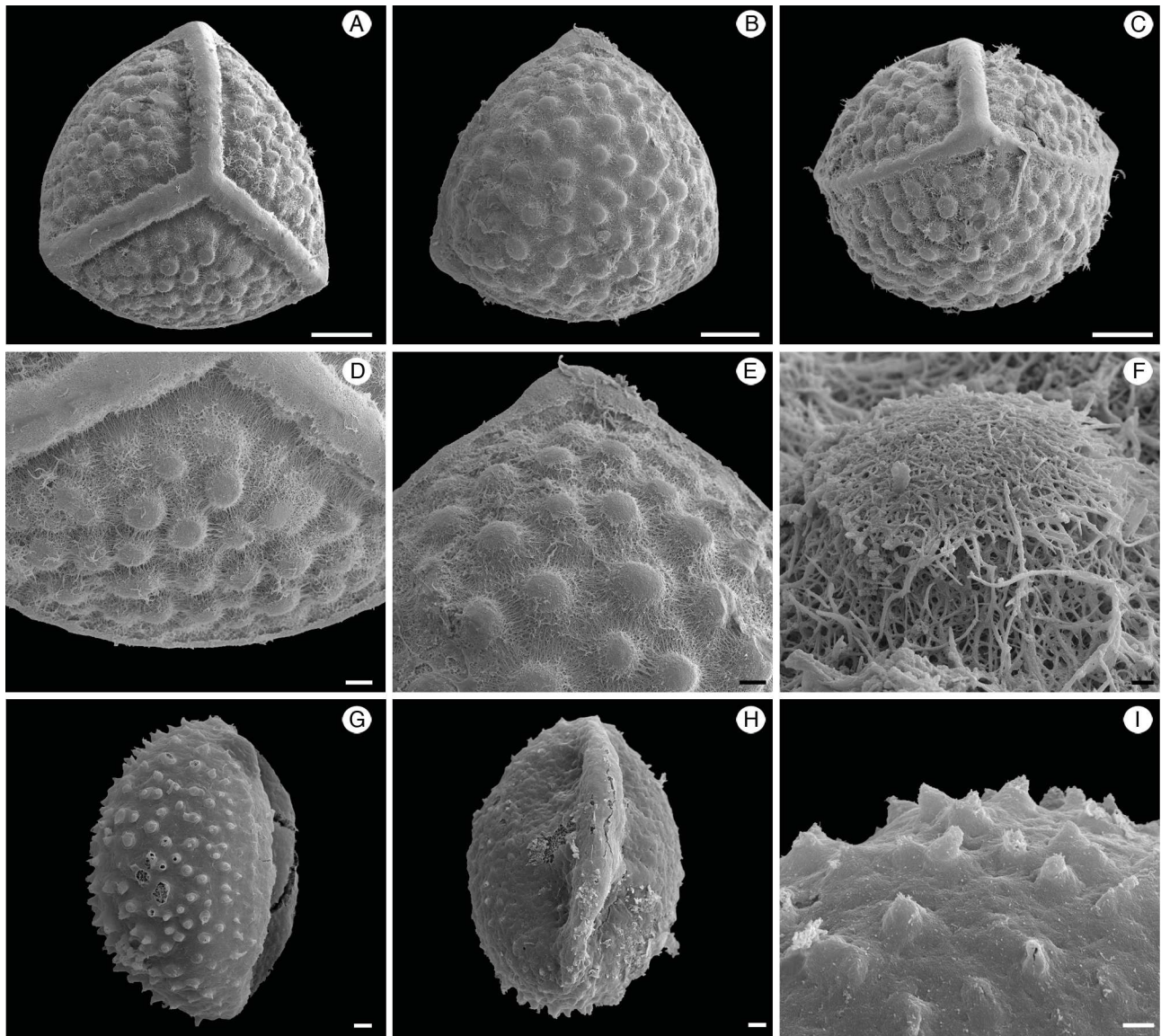
Plants aquatic, submerged, growing in lakes and ponds. Corm globose, 0.4–0.8 cm wide, 3–lobate. Roots conspicuous, dichotomously branched. Leaves 1.0–1.5 mm wide at middle, 4–8(–14) cm long, 14–70 per individual, triangular to linear, straight, erect to ascending, apex acute to attenuate; alae 1.3–1.5 cm long, stretching along ca. 1/4 the length of the leaf, 1.5–2 mm wide at sporangium, hyaline, membranaceous, apex attenuate; subula present, olive green. Labium present, 1.0–2.2 mm wide, 0.8–1.2 mm long, reniform-orbicular. Ligule consisting of a thin membrane, generally not visible in dry material. Velum rudimentary to covering up to 1/5 of the adaxial sporangial surface, 0.2–0.4 mm along the lateral edges, 0.1–0.2 mm along the upper edges of the sporangium. Scales absent. Sporangium 2.2–4.7 mm long, 1.7–3.3 mm wide, elliptic, dark brown to red-brown, at the base of the leaf. Megaspore 470–640  $\mu\text{m}$  diameter ( $\bar{x}$ =490,  $N$ =20), 440–570  $\mu\text{m}$  polar axis, 0.83–0.92 P/E ratio, subspheroidal, trilete, white, no lustrous, heteropolar; laesures 50–59  $\mu\text{m}$  wide, 50–55  $\mu\text{m}$  high, distinct, straight, wider than high or as wide as high, with straight and parallel sides, apex rounded to truncate, no apiculate, the same height along the whole length, slightly separated from the remaining macrosculptural elements; proximal surface macrosculpture verrucate, verrucae type 1 (rarely tubercles type 2), 23–42  $\mu\text{m}$  wide, 10.0–20.0  $\mu\text{m}$  high, microsculpture foveolate with microechinae; equatorial ridges distinct, straight, with straight and parallel sides, apex rounded to truncate; distal surface macrosculpture verrucate, verrucae type 1, 23–42  $\mu\text{m}$  wide, 13.0–23.0  $\mu\text{m}$  high, microsculpture foveolate with microechinae. Microspore 30–36  $\mu\text{m}$  long ( $\bar{x}$ =34,  $N$ =20), light brown to dark brown, monolete, heteropolar; laesure straight, without prominent invagination; proximal surface macrosculpture psilate (rarely with minute echinae), microsculpture comprising microgranula and microbacula; distal surface macrosculpture echinate to tuberculate, microsculpture comprising microgranula and microbacula.

**Etymology:**—The specific epithet refers to the localities where this species was collected, Serra do Carajás, located in southern Pará, Brazil.



**FIGURE 2.** Habitat of the holotypes. A–D. *Isoetes serracarajensis* (Arruda 1356). A–B. Habitat. C. Adaxial view of the base of the microphylls with velum covering less than 1/5 of the sporangial surface. D. Diagram of the adaxial view of the microphylls showing the ligula position, labium, velum and sporangium. E–H. *Isoetes cangae* (Arruda 1329). E–F. Habitat. G. Adaxial view of the base of the microphylls with velum covering less than 1/4 of the sporangial surface. H. Diagram of the adaxial view of the microphylls showing the ligula position, labium, velum and sporangium. Scale bars: A, B, E and F = 1 cm; C–D = 1 mm; G–H = 2 mm.

**Additional specimens examined (paratypes):**—BRAZIL. Pará: Parauapebas, Serra Norte, N7, 06°09'13"S, 50°10'21"W, 692 m, 25 March 2012, *A.J. Arruda et al.* 854 (B, BHCB). Canaã dos Carajás: Serra Sul, 06°21'7"S, 50°23'44"W, 735 m, 23 January 2013, *A.J. Arruda et al.* 1338 (B, BHCB); Floresta Nacional de Carajás, Serra Sul, Corpo B, 06°21'06"S, 50°23'43"W, 733 m, 14 February 2010, *T.E. Almeida* 2157 (BHCB, UPCB); Floresta Nacional de Carajás, Serra Sul, 06°24'32"S, 50°21'04"W, 822 m, 9 December 2014, *V. Giorni & P. Burkowski s.n.* (B, BHCB); Floresta Nacional de Carajás, Serra Sul, 06°24'10"S, 50°18'24"W, 655 m, 16 March 2015, *V. Giorni s.n.* (BHCB, UPCB); Floresta Nacional de Carajás, Serra Sul, 06°22'00"S, 50°23'43"W, 733 m, 17 March 2015, *V. Giorni s.n.* (B, BHCB).

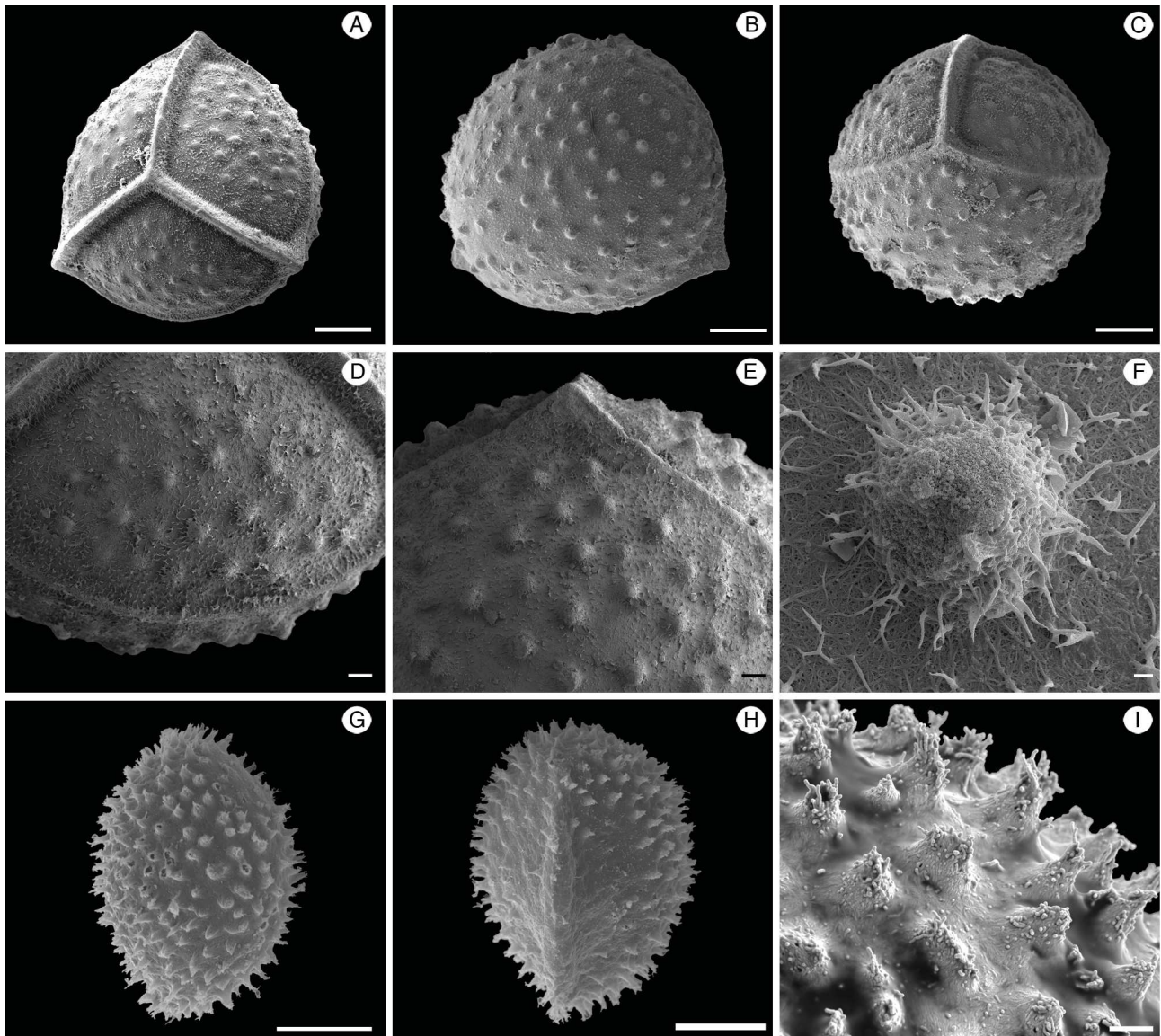


**FIGURE 3.** SEM images of the spores of *Isoetes serracarajensis* (from the holotype). A–F. Verrucate megaspore. A. Proximal view. B. Distal view. C. Equatorial view. D. Detail of the proximal surface. E. Detail of the distal view. F. Detail of the verruca of the distal view. G–I. Microspore. G. Distal view showing the echinate-tuberculate macrosculpture. Lateral view. H. Proximal view showing the psilate macrosculpture. I. Detail of the surface of the microspore. Scale bars: A–C = 100  $\mu$ m; D–E = 20  $\mu$ m; F–H = 2  $\mu$ m; I = 1  $\mu$ m.

**Comments:**—Three species of *Isoetes* with tuberculate or verrucate megaspores occur in the Amazon Basin: *Isoetes amazonica* Braun in Baker (1880: 109), *I. ovata* Pfeiffer (1922: 108) and *I. triangula* Weber (1922: 253). Among these, *I. amazonica* is more similar to *I. serracarajensis* by its microphyll size and verrucate megaspore. However, in *I. amazonica* the microphylls are acicular to filiform, 0.4–0.8 mm wide at middle (vs. triangular to linear microphylls, 1.0–1.5 mm wide at middle), the adaxial surface of the sporangia is hyaline (vs. dark brown to red-brown), the laesures of the megaspore presents convex sides and obtuse apices (vs. parallel sides and rounded to truncate apex), and the macrosculptural elements of the proximal surface are connected to laesures (vs. macrosculptural elements clearly separated from laesures).

**Distribution and Habitat:**—*Isoetes serracarajensis* is only known from four localities (Serra Norte, Serra Sul, Serra do Tarzan, and Serra do Bocaína), that form the mountain range Serra dos Carajás (Fig. 5). It was found narrowly associated to ‘canga’ vegetation and submerged in seasonal lakes, ponds, or terrestrial on wet soil on top of the mountains at 650–822 m.

**Conservation Status:**—This species is recorded from areas in which the soils present high iron contents. The increase of the iron exploration in these localities by mining companies has led to deterioration of the quality of the habitats of *Isoetes serracarajensis* and several subpopulations may have already been extinct. Licensing processes for the construction of new iron mines have been initiated and therefore, a continuing decline of the populations is expected. Based on the IUCN criteria (IUCN 2012, 2010), we suggest that *I. serracarajensis* should be classified as Vulnerable (VU).



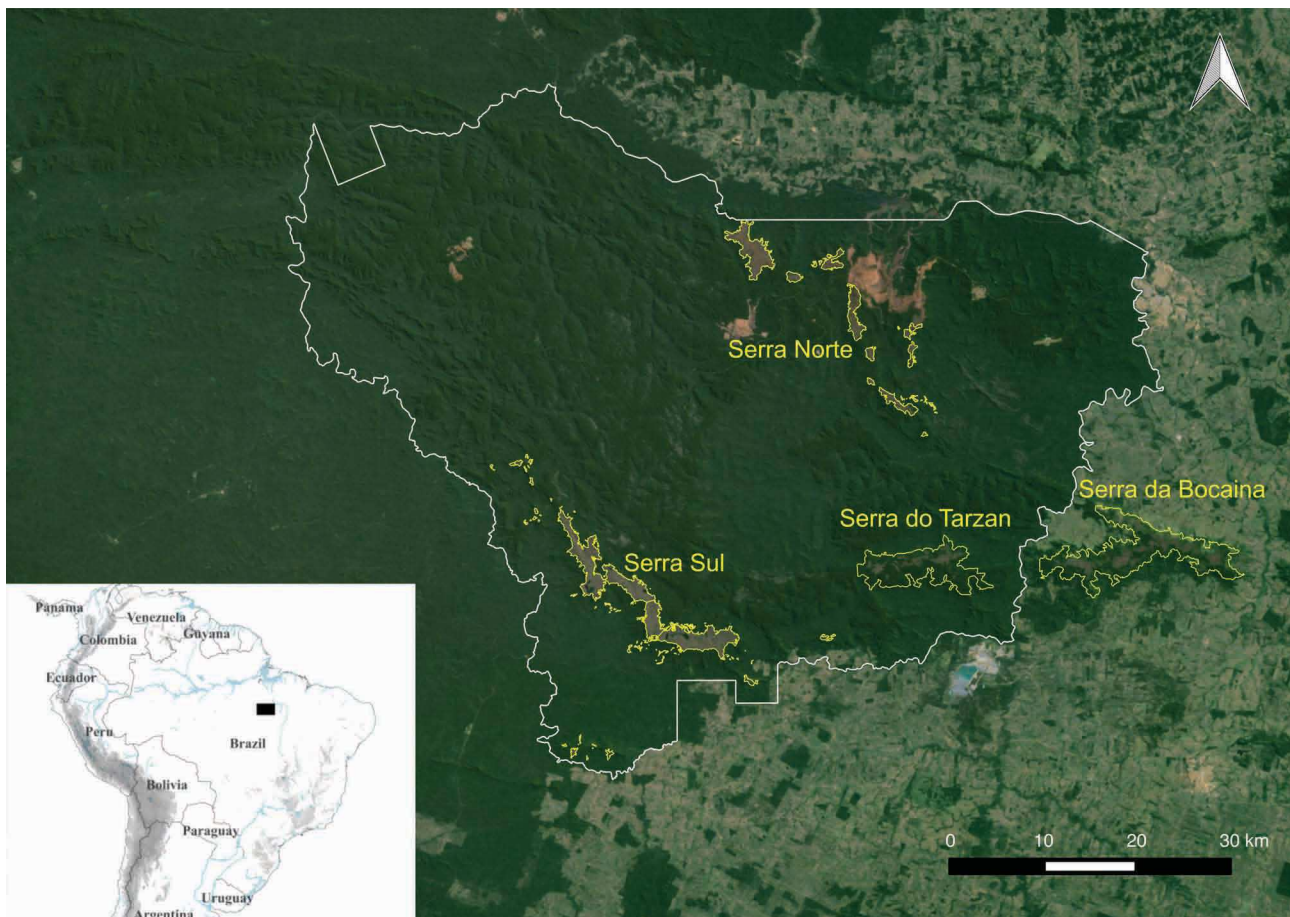
**FIGURE 4.** SEM images of the spores of *Isoetes cangae* (from the holotype). A–F. Verrucate-tuberculate megaspore. A. Proximal view. B. Distal view. C. Equatorial view. D. Detail of the proximal surface. E. Detail of the distal view. F. Detail of the verruca of the distal view. G–I. Microspore. G. Distal view showing the echinate-tuberculate macrosculpture. H. Proximal view showing the inconspicuously echinate macrosculpture. I. Detail of the surface of the microspore. Scale bars: A–C = 100  $\mu\text{m}$ ; D–E = 20  $\mu\text{m}$ ; F = 2  $\mu\text{m}$ ; G and H = 9  $\mu\text{m}$ ; I = 1  $\mu\text{m}$ .

***Isoetes cangae* J.B.S.Pereira, Salino & Stützel, *sp. nov.***

*Isoetes cangae* is distinguished from other species of the Amazon Basin by the combination of the following characters: microphylls large (15–30 cm long), labium reniform-orbicular, velum rudimentary to covering up to 1/4 of the sporangium surface, sporangium hyaline, megaspore white, verrucate (verrucae type 2) to tuberculate (tubercles type 3) (Figs. 2E–H, 4).

**Type:**—BRAZIL. Pará: Canaã dos Carajás, Serra Sul, S11D, 06°23'57''S, 50°22'13''W, 730 m, 22 January 2013, *A.J. Arruda 1329, M.O. Pivari, B.P. Leles, F.M. Santos* (holotype B!; isotype BHCBI!).

Plants aquatic, submerged, growing among rocks in lakes. Corm globose, 0.8–1.3 cm wide, 3-lobate. Roots conspicuous, dichotomously branched. Leaves 0.65–1.2 mm wide at middle, 15–25 cm long, 25–50 per individual, linear, straight, ascending, apex acute; alae 2.5–4.0 cm long, stretching along ca. 1/5 the length of the leaf, 1.5–2.5 mm wide at sporangium, hyaline to light green, membranaceous, apex acuminate; subula present, olive-green. Labium present, 2.6–3.5 mm wide, 1.8–2.4 mm long, reniform-orbicular. Ligule a thin membrane, generally not visible in dry material. Velum rudimentary to covering less than 1/4 of the adaxial sporangial surface, 0.1–0.4 mm along the lateral edges, and 0.1–0.26 mm along the upper edges of the sporangium. Scales absent. Sporangium 2.2–3.0 mm wide, 3.3–4.5 mm long, elliptic, light brown, at the basal of the leaf. Megaspores 490–580 µm diameter ( $x=510$ ,  $N=20$ ), 440–510 µm polar axis, 0.88–0.96 P/E ratio, subspheroidal, trilete, white, slightly lustrous, heteropolar; laesures 31–35 µm wide, 40–52 µm high, distinct, straight, higher than wide, with straight and inclined sides, apex acute to obtuse, not apiculate, the same height along the whole length, slightly separated from the remaining macrosculptural elements; proximal surface macrosculpture verrucate to tuberculate, tubercles type 3 and verrucae type 2, 13–32 µm wide, 10–22 µm high, microsculpture foveolate with microechinae and microgranula; equatorial ridges distinct, straight, with convex sides, apex rounded; distal surface macrosculpture verrucate to tuberculate, tubercles type 3 and verrucae type 2, 15–35 µm wide, 15.0–23.0 µm high, microsculpture foveolate with microgranula and microechinae. Microspores 29–34 µm long ( $x=31.5$ ,  $N=20$ ), light brown, monolete, heteropolar; laesure straight, without prominent invagination; proximal surface macrosculpture inconspicuously echinate, microsculpture comprising microgranula and microbacula; distal surface macrosculpture echinate to tuberculate, microsculpture comprising microgranula and microbacula.



**FIGURE 5.** Serra dos Carajás comprising its four mountain chains (Serra Norte, Serra Sul, Serra do Tarzan and Serra da Bocaina), in the southern part of the Pará State, northern Brazil. White line marks the limits of the Carajás National Park. Type locality of *I. serracarajensis* in Serra Norte. Type locality of *I. cangae* in Serra Sul.

**Etymology:**—The specific epithet refers to the ‘canga’, a superficial iron crust which occurs over ferriferous formations, where *Isoetes cangae* was found.

**Comments:**—*Isoetes gardneriana* Braun (1862: 330) is similar to *I. cangae* in microphyll size, having tuberculate megaspores (tubercles type 3) and a velum covering less than 1/4 of the adaxial sporangial surface. However, in *Isoetes gardneriana* the megaspores are dark brown to grey (vs. white), the laesures are knife-like (vs. sharply triangular), and the distal surface of the microspores is laevigate to papillate (vs. echinate to tuberculate).

**Distribution and Habitat:**—*Isoetes cangae* is only known from its type locality in Serra Sul, Canaã dos Carajás, located in southern Pará, Brazil (Fig. 5). It was found submerged in permanent lakes over ‘canga’ soil, in rupestrian fields at 730 m.

**Conservation Status:**—Although innumerable efforts have been made to find new populations of *I. cangae* in the Serra dos Carajás, this species is currently known from a single locality, and only from a few individuals. *Isoetes cangae* is recorded in an area, which is surrounded by iron mines. The increase of the mining operations in this locality has led to deterioration of the quality of the habitats of *I. cangae* and populations may have already been extinct. Licensing processes for the amplification of iron mines have been initiated and therefore, a drastic decline of this species is expected. Based on the IUCN criteria (IUCN 2012), we suggest that *I. cangae* should be classified as critically endangered (CR).

#### Key to species of *Isoetes* with tuberculate and verrucate megaspores from the Amazon Basin

1.	Leaves longer than 15 cm.....	2
–	Leaves up to 14 cm.....	6
2.	Megaspores 750–900 µm diam.....	<i>I. ovata</i>
–	Megaspores 350–700 µm diam.....	3
3.	Laesures of the megaspores with apex attenuate, acute or obtuse.....	4
–	Laesures of the megaspores with apex truncate to rounded.....	5
4.	Megaspores dark brown, laesures knife-like; microspore papillate; plants from midwestern Brazil (Goiás State)....	<i>I. gardneriana</i>
–	Megaspores white, laesures sharply triangular (straight inclined sides with apex acute); distal surface of the microspore echinate to tuberculate; plants from northern Brazil (Pará State).....	<i>I. cangae</i>
5.	Megaspores oblate (P/E ratio=0.54–0.75), macrosculptural elements with apex rounded (tubercles type 1).....	<i>I. triangula</i>
–	Megaspores subspheroidal (P/E ratio=0.83–0.92), macrosculptural elements with apex truncate (verrucae type 1, or rarely, tubercles type 2).....	<i>I. serracarajensis</i>
6.	Leaves filiform, 0.4–0.8 mm wide; sporangia hyaline; proximal view of the megaspore without distinct separation between the laesures and tubercles, laesures with convex sides and obtuse apex.....	<i>I. amazonica</i>
–	Leaves triangular-linear, 1.0–1.5 mm wide; sporangia dark brown to red brown; proximal view of megaspores with distinct separation between laesures and macrosculptural elements, laesures with parallel sides and rounded to truncate apex.....	<i>I. serracarajensis</i>

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