



## ECOSYSTEMS

# *Isoetes candelariensis*, a new species of Isoetaceae (Lycopodiidae) from Argentina

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**Abstract:** *Isoetes candelariensis* is a new species of Isoetaceae from Misiones, Argentina. This species is ephemeral, and grows on basaltic bedrock outcrop pools, in Urutau Reserve from Candelaria Department. This taxon differs from other aquatic *Isoetes* in this region by the unique combination of characters of its leaves, ligule, labium and megaspore ornamentation.

**Key words:** Isoetes, new species, lycophyte, Argentina.

## INTRODUCTION

*Isoetes* L., the only genus of Isoetaceae, constitutes a cosmopolitan genus of approximately 200 species (Troia et al. 2016). *Isoetes* species occur on all continents but Antarctica, found in a wide variety of permanent and ephemeral wetlands habitats from sea level to 4200 m (Pfeiffer 1922, Tryon & Tryon 1982, Jermy 1990, Brunton & Troia 2018). Many species are terrestrial, while others are submerged in shallow water along lake margins, or grow as true aquatics to depths of 4-5 m in lakes or streams. Under optimum conditions, the plants may form dense lawns. While typically reproducing sexually, some *Isoetes* taxa reproduce vegetatively by gemmae (Hickey 1986a). This last condition is rare, if not unknown, in South America.

*Isoetes* is characterised by leaves with four air-chambers, and single micro- or mega sporangia inserted into inner leaf bases, which typically are covered by the velum (Hickey et al. 2009, Pereira et al. 2012, 2019). The megaspores and microspores ornamentation is very important in taxonomic identification within the genus; scanning electron microscopy

(SEM) images have proven very helpful in the interpretation of subtle but informative spore variations (Kott & Britton 1983, Brunton & Troia 2018). Cytology and more recently DNA sequencing, have provided invaluable insights into *Isoetes* diversity, evolution and taxonomic affinities (Hoot et al. 2006, Wood et al. 2020).

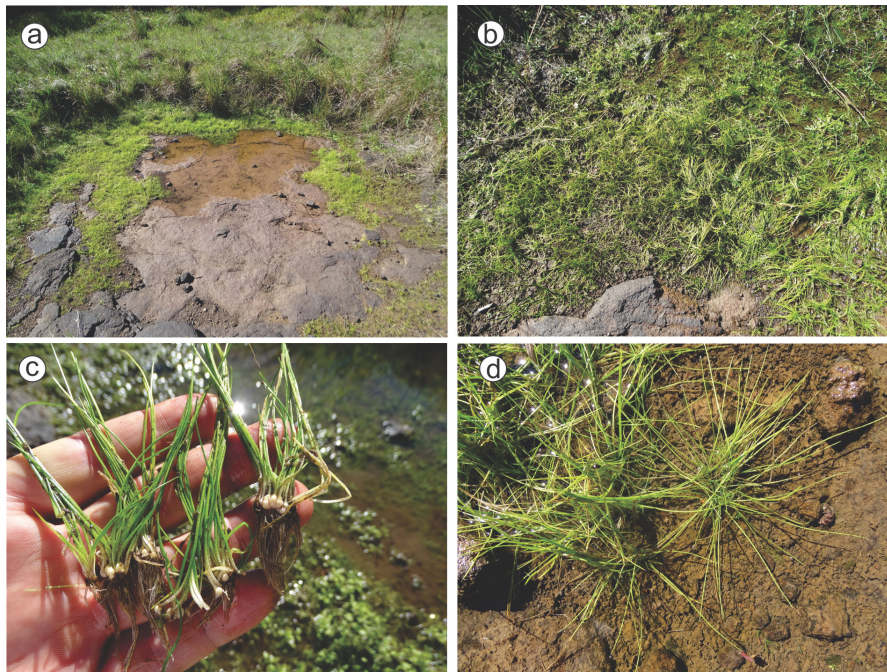
Eight *Isoetes* species are reported in Argentina from the provinces of Buenos Aires, Misiones, Corrientes, Córdoba, Tucumán, Catamarca and the Patagonian region (Weber 1922, Macluf 2016).

During a vascular flora survey in the Urutau Reserve and surrounding areas, near the city of Candelaria in Misiones Province, Argentina, some populations of *Isoetes* were found in small pools on a basaltic bedrock outcrop (Figures 1, 2). Investigation about that material revealed a combination of morphological attributes inconsistent with species described to date.

On the basis of a comparative study of closely associated species from the region, the present investigation provides evidence that this population constitutes a previously undescribed species.



**Figure 1.** Distribution of *Isoetes candelariensis* (●), *Isoetes ekmanii* (■) and *Isoetes pedersenii* (◆).



**Figure 2.** Pictures of the living individuals and landscape where the species grows. (a) Typical basalt bedrock outcropping where *I. candelariensis* grows, (b) population of *I. candelariensis*, (c) and (d) some live individuals of *I. candelariensis*.

## MATERIALS AND METHODS

Dried herbarium material was studied with a Wild M5 stereoscopic microscope (SM) and a scanning electron microscope (SEM). For studies with SEM, the spores were handled without

any chemical treatment and placed with a moist brush on doublestick tape attached to bronze stubs. Samples were coated with gold and examined under a Jeol JSM-35 CF at the SEM laboratory of the Facultad de Ciencias Naturales y Museo, Universidad Nacional de

La Plata, La Plata, Argentina. To estimate the average measurements of the spores, we used a minimum of 20 spores per sporangium, from at least three sporangia. Terminology for the discussion regarding spore morphology follows Hickey (1986b).

## RESULTS

### Taxonomic treatment

*Isoetes candelariensis* C.C. Macluf & G.J. Marquez, *sp. nov.* TYPE Argentina, Misiones province: Department Candelaria, Reserva Urutaú, 27° 29' 20.33" S, 55° 43' 47.15" W, Charcos de agua en basalto aflorante [pools on a basaltic bedrock outcrop], 5 July 2019, H. A. Keller & G. Ruiz Díaz 13900 (Holotype LP!, Isotype CTES!). (Figures 1, 2, 3).

Plants herbaceous (less than 15 cm tall). Roots numerous, dichotomously branched. Corm bilobed, globose, 4-5 mm wide, 5 mm long. Leaves 45-50, spirally arranged, erect

and recurved distally, 9-14 cm long, 0.5-2.5 mm wide at the base, 0.2-1 mm wide at mid length; alae hyaline and chartaceous proximally, dark green and membranaceous distally, 1 mm wide at the height of the sporangium, 12-14 cm long (extending to 80% of the leaf length); subula acute, dark green, their apex long acuminate; scale leaves present. Sporangia elliptic, hyaline, concolorous, 4-5 mm long, 3-4 mm wide. Velum incomplete, extending 60 % across sporangium from the top. Ligule 1-1.5 mm high, 0.5 mm wide; black, delicate, ephemeral; deltate to widely ovate. Megaspores white, 342 ([387.75]  $\mu\text{m}$  (N=20, SD=29.47, 3 plants) 425  $\mu\text{m}$  in diameter, laevigate to obscurely rugulate (in distal surface) with velutinous perispore (spore surface) and no equatorial band (girdle); equatorial and proximal ridges straight, as high as broad (Figures 4a, 4b, 4c). Microspores dark brown *en masse*, 28 ([30]  $\mu\text{m}$  (N=20, SD=1.45, 3 plants)) 32  $\mu\text{m}$  long, densely echinate with broad-based spines (Figures 4d, 4e, 4f).

### Etymology

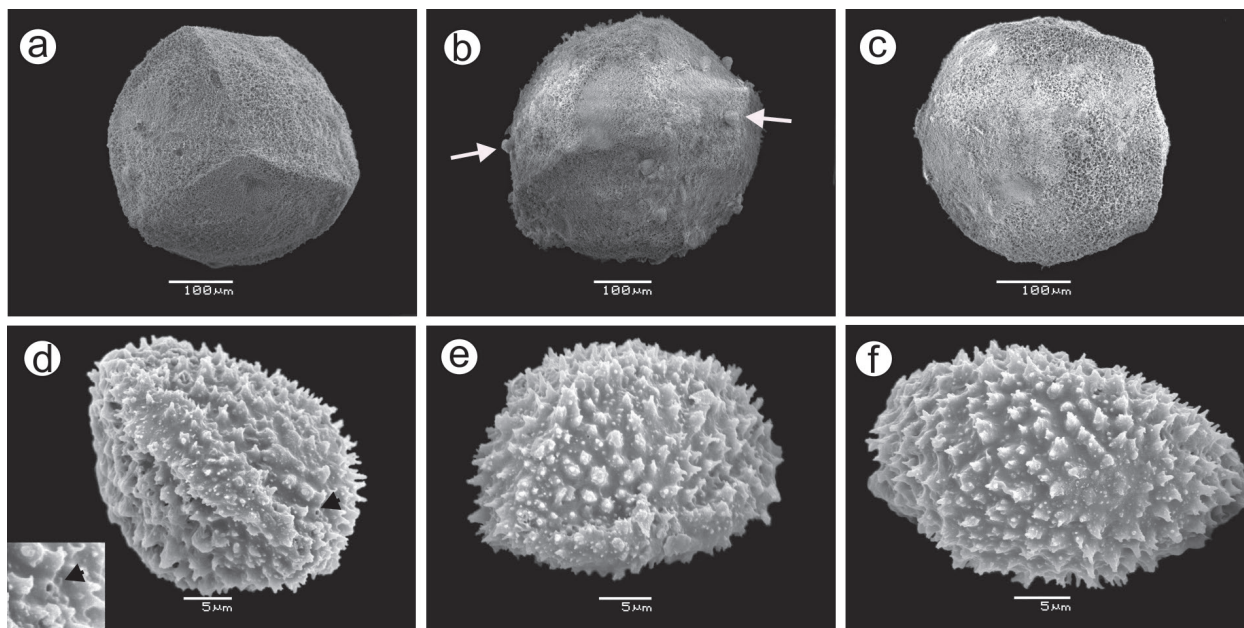
The proposed epithet refers to the Department Candelaria locality where the type material was collected.

### Additional specimens examined

Argentina, Misiones province: Department Candelaria, Reserva Urutaú, 27° 30' 25.4" S, 55° 47' 20.6" W, 9 June 2022, H. A. Keller & G. Ruiz Díaz 15116 (LP, CTES); *idem*, 27° 30' 20.34" S, 55° 47' 14.12" W, 5 August 2022, H. A. Keller & G. Ruiz Díaz 15231 (LP, CTES); *idem*, 27° 30' 28.33" S, 55° 47' 16.84" W, 5 August 2022, H. A. Keller & G. Ruiz Díaz 15232 (LP, CTES); *idem*, 27° 30' 26.24" S, 55° 47' 21.01" W, 5 August 2022, H. A. Keller & G. Ruiz Díaz 15233 (LP, CTES); *idem*, 27° 29' 20" S, 55° 48' 47" W, 5 August 2022, H. A. Keller et al. 15234 (LP, CTES); *idem*, 27° 29' 24" S, 55° 43' 44" W, 5 August 2022, H. A. Keller & G. Ruiz Díaz 15235 (LP, CTES).



**Figure 3.** Holotype specimen of *Isoetes candelariensis* (H. A. Keller & G. Ruiz Díaz 13900, LH).



**Figure 4.** Spores of *Isoetes candelariensis*. Megaspores in proximal (a), equatorial (b) and distal (c) views. Microspores in proximal (d), equatorial (e) and distal (f) views. Arrows indicate microspores on the megaspore surface (b). Arrowheads shows the hollow into the spines in microspores (d)

### Distribution and habitat

This species occurs in Reserva Urutaú (Misiones, Argentina) (Figure 1), and grows in shallow, ephemeral pools and seepage areas on basalt bedrock outcropping (Figures 2a, 2b). The individuals are small grasses that grow on the waterlogged basalt, with the leaves grow outside water, and can be seen together with other swamp species (Figures 2c, 2d). Exist three populations, and each has less than 50 mature individuals, which appears during a short annual period (June to August).

### DISCUSSION

Eight species of *Isoetes* grow in Argentina: *I. alcalophila* S. Halloy (Halloy, 1979), *I. chubutiana* R.J. Hickey, C.C. Macluf & W.C. Taylor (Hickey et al. 2003), *I. ekmanii* U. Weber (Weber, 1922), *I. escondidensis* S. Halloy (Halloy, 1979), *I. gardneriana* Kunze ex Mett. (Mettenius, 1859). *I. hieronymii* Weber (Weber, 1922, Macluf et al. 2009), *I. pedersenii* H.P. Fuchs ex E. I. Meza T. &

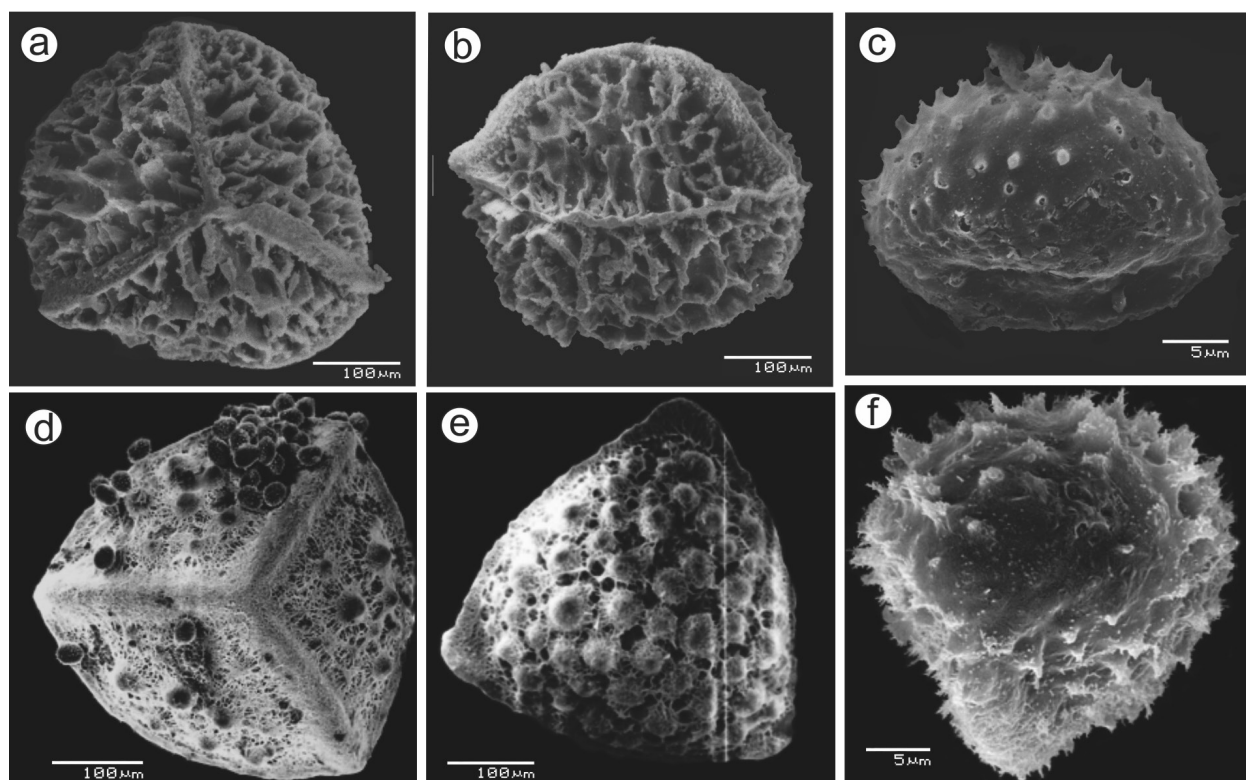
C.C. Macluf (Macluf et al. 2010) and *I. savatieri* Franchet (Franchet, 1884). *I. candelariensis* is closest in geographic distribution and habitat with *I. ekmanii* and *I. pedersenii* (Figure 1).

*Isoetes ekmanii*, which occurs in lowland from Argentina and Brazil (Giudice et al. 2011), grows both as an emergent in silty river banks and as a permanently submerged aquatic (Macluf 2012). *I. candelariensis* has similar leaf features with *I. ekmanii* and both of them grow in the Candelaria department (Misiones). However, *I. ekmanii* have megaspores typically reticulate (Table I, Figures 5a, b). Microspores are similar in *I. candelariensis*, *I. ekmanii* and *I. pedersenii* (Figures 4d, e, f, 5c, f).

*I. pedersenii* grows in flooded land of Dep. Mburucuyá, Corrientes province, and probably it is found in the adjacent regions of Argentina, Paraguay and Brazil (Macluf et al. 2010). The major differences with *I. candelariensis* is the megaspores with verrucae ornamentation (Table I, Figures 5d, e).

**Table I. Morphological comparison of *Isoetes candelariensis*, *I. ekmanii* and *I. pedersenii*.**

	<i>I. candelariensis</i>	<i>I. ekmanii</i>	<i>I. pedersenii</i>
Site/ habitat	Ephemeral bedrock outcrop pool at low elevation (<150 m s. n. m.)	Emergent or aquatic by permanent water body at low elevation (<100 m s. n. m.)	Low generally flooded land (<100 m s. n. m.)
Labium	Inconspicuous to absent	Yellowish-white, membranous, 1.6 mm wide and 0.92 mm high.	Conspicuous, 3.0 mm high and 1-1.5mm wide.
Ligule	Deltate to widely ovate, delicate, ephemeral, 1-1.5 mm high, 0.5 mm wide.	Triangular, slightly elongated, delicate, 2 mm high, 1 mm wide.	Deltoid, auriculate at base, 1.3 mm high, 1.85-2 mm wide.
Microspores size	28 [30] $\mu\text{m}$ long	28 [32] $\mu\text{m}$ long	30 [35] $\mu\text{m}$ long
Microspores ornamentation	echinate with broad-based, occasionally hollow spines	obscurely echinate	echinate
Megaspores size	342 [387.75] $\mu\text{m}$	400 [500] $\mu\text{m}$	395 [410] $\mu\text{m}$
Megaspore ornamentation	Laevigate to low rugulate (sometimes in distal face). Microsculpture: velutinous (low-matted)	Low-reticulate	Verrucate
Equatorial band (girdle)	Absent	Echinate	Absent



**Figure 5. Spores of *Isoetes ekmanii* and *Isoetes pedersenii*. *I. ekmanii*, megaspores in proximal (a), equatorial (b) and microspore in equatorial (c) views. *I. pedersenii*, megaspores in proximal (d), distal (e) and microspore in equatorial (f) views.**

The megaspore and microspore ornamentation of *Isoetes candelariensis* is somewhat similar to that in *Isoetes maxima* Hickey, Macluf & Link-Perez, from southeastern Brazil. However, megaspore ornamentation of *I. maxima* is rugulate to tuberculate in distal surface (Hickey et al. 2009, Pereira et al. 2017), and the equatorial diameter size is higher (525–650  $\mu\text{m}$ ) than *I. candelariensis* (342–425  $\mu\text{m}$ ). In addition, other characters, like leaves, alae, velum and sporangium (Table I), differentiate the species. Another ecological characteristic to differentiate both species is that *I. maxima* grows submerged in streams at high elevations (900 to 1200 m. a.s.l.) while *I. candelariensis* live in seepage areas on basalt bedrock outcropping to no more than 150 m. a.s.l. (Hickey et al. 2009).

As it has been reported with *Isoetes pedersenii* (Macluf et al. 2010), some microspore spines on *I. candelariensis* are hollow (arrowheads and detail in Fig. 4d). This characteristic has also been reported from India (Srivastava 1995) as well as in *Isoetes* plants in Canada, the United States, Australia and several countries in eastern Mediterranean (D. F. Brunton pers. comm.). The taxonomic and/or physiological significance of this is unknown.

*Isoetes weberi* Herter, *I. maxima* and *I. quiririensis* J. B. S. Pereira & P.H. Labiak (Pereira & Labiak 2013) are the only species from southern Brazil with non-reticulate megaspores like *I. candelariensis*. Accordingly, *I. candelariensis* may be considered to have at least some morphological affinity with these Brazilian species, which live in the same biogeographical region, the Atlantic Forest.

### Conservation status

*Isoetes candelariensis* is currently known only from type locality. According to IUCN Red List Criteria (IUCN 2012, 2022) this species may qualify Data Deficient (DD), because the

geographic distribution and possible threats are so imperfectly known. Regardless, the limited availability of potential habitat suggests this will be a taxon of significant conservation concern at some level. For example, in 2011 the elevation of the Yacyreta dam to 83m changed the dynamics of the Paraná River, flooding many sectors of the Department of Candelaria. Future environmental changes associated with the reservoir of this dam may be a potential hazard.

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