

## *Isoetes fluitans* sp. nov.: the identity of Spanish plants of '*I. longissimum*'

M. I. ROMERO<sup>1</sup>\*, J. AMIGO<sup>2</sup> and P. RAMIL<sup>1</sup>

<sup>1</sup>University of Santiago, Department of Botany, Escola Politécnica Superior, 15702 Lugo, Spain

<sup>2</sup>University of Santiago, Department of Botany, Facultade de Farmacia, 15865 Santiago, Spain

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Recent revision of North African specimens of *Isoetes velata* A. Braun and the closely related taxon *I. longissimum* Bory, together with Spanish material conventionally designated *I. longissimum*, suggests that the Spanish specimens constitute a new species, *I. fluitans*. This is described and illustrated. The North African taxon *I. longissimum* is probably not specifically distinct from *I. velata*. © 2004 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2004, 146, 231–236.

ADDITIONAL KEYWORDS: aquatic plant – endemism – Iberian Peninsula – Isoetaceae – taxonomy – threatened plant.

### INTRODUCTION

Several species of the genus *Isoetes* L. with a wide distribution in the Mediterranean region were initially described on the basis of specimens from Algeria. Such is the case for *I. velata* A. Braun, a taxon showing marked variability (Pfeiffer, 1922; Reed, 1953; Greuter *et al.*, 1984–89), as appears to be normal for the taxa of this genus (see Kott & Britton, 1983). A closely related taxon initially described from the same Algerian location as *I. velata* (Bory, 1844; Bory & Durieu, 1846–69) is *I. longissimum* Bory [= *I. velata* ssp. *longissima* (Bory) Greuter & Burdet, = *I. velata* f. *longissima* (Bory) Pfeiffer, = *I. velata* var. *longissima* A. Braun, = *I. velata* ssp. *typica* f. *longissima* (A. Br.) Maire]. Since its initial description, however, little has been published about this taxon (but see Pfeiffer, 1922). It is of uncertain rank, difficult to distinguish from *I. velata*, and there is scant herbarium material available. However, it was cited at various European locations in early publications (see Lange, 1860–65; Willkom & Lange, 1870), and has likewise been cited from the north-west Iberian Peninsula more recently (Prada, 1983, 1986; Jermy & Akeroyd, 1993; Romero & Amigo, 1995).

In the present study we aimed to re-assess the taxonomic status of these populations from north-west Iberia, on the basis of comparison of herbarium specimens from North Africa with specimens from north-west Spain. Taxonomy on the basis of morphology alone is difficult in the case of plants such as *Isoetes* with very simple morphology, and as suggested by Hickey (1986) we have therefore additionally taken into account other factors, including autecological and cytological characteristics.

### MATERIAL AND METHODS

#### SPECIES DESCRIPTIONS

*Isoetes velata* is a plant with trilobulate stems bearing 8–24 cm long leaves. The leaves have numerous stomata and broad membraneous basal margins. The ligule is triangular and lanceolate. The sporangium is almost completely covered by a velum. Megaspores are 420–580 µm, with a few large verrucae on the proximal face and mixed large and small verrucae on the distal face. Microspores are 26–33 µm and densely spinulose (Pfeiffer, 1922).

*Isoetes longissimum* was discovered by Durieu in a flooded forest near Lake Oubeira (El-Kala, north-east Algeria), a Ramsar wetland site near the coast. It is characterized by its narrow dark green leaves that

\*Corresponding author. E-mail: byrosma@usc.es

may exceed 65 cm in length. These leaves float like those of an aquatic grass, but do not become desiccated when the water body dries up, and remain orientated in the same direction. The root bulb is proportionally much smaller than in other species of *Isoetes* (Bory, 1844).

#### MATERIAL

For this study we examined herbarium specimens of both *I. longissimum* and *I. velata*, collected at the same time and from the same location as the corresponding type specimens (see Appendix). We also examined *I. velata* specimens from nearby locations, mostly in Algeria. All of these specimens were loaned by the Paris Herbarium (Muséum d'Histoire Naturelle de Paris). The specimens from the north-west Iberian Peninsula were from the SANT Herbarium, together with material newly collected by the authors.

#### MORPHOMETRY

All morphometric determinations were made from herbarium specimens. In all cases we recorded the number of leaves per plant, leaf lengths and corm diameter. From each sheet examined, wherever possible we obtained both megaspore and microspore preparations in Euparal on glass slides. In each preparation we measured maximum diameter (including ornamented spore wall; see Kott & Britton, 1983) of 30 spores selected at random, using a stereomicroscope and following the procedure of Britton & Brunton (1993). Spore shape and orna-

mentation were evaluated by scanning electron microscopy.

## RESULTS AND DISCUSSION

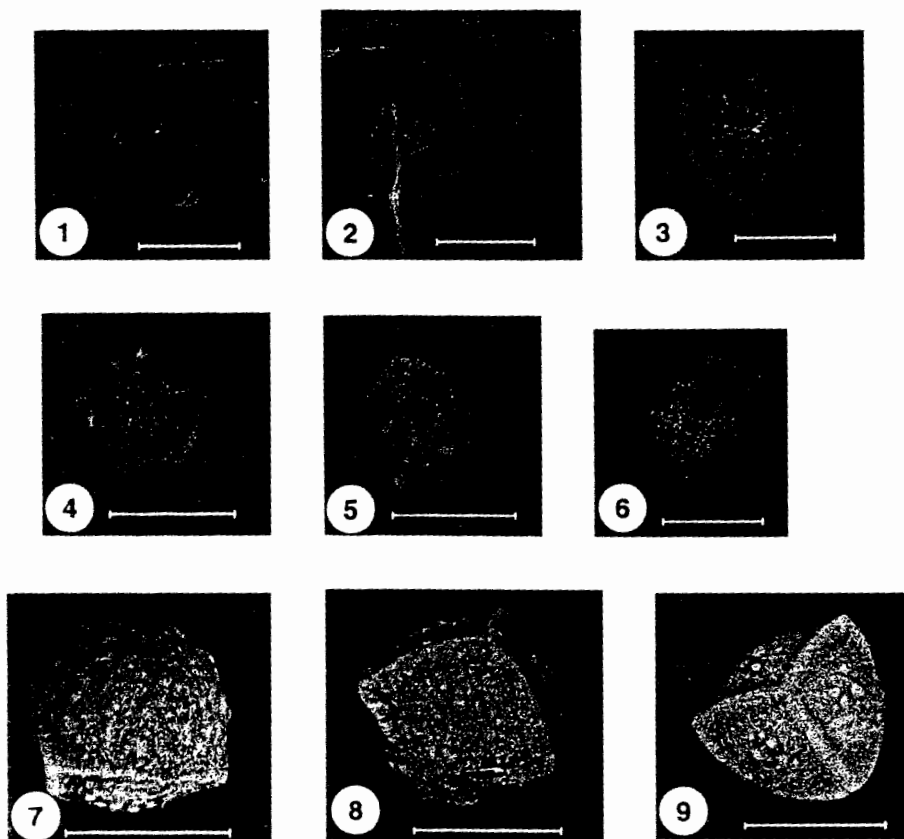
#### MORPHOMETRIC DATA

The spore morphometry data are summarized in Table 1. In the case of *I. velata* (material from Africa, mostly Algeria), the means and ranges of all spore morphometry variables are in line with the data reported by Pfeiffer (1922) and Maire (1952). The corresponding means and ranges for spores from Algerian *I. longissimum* are likewise within the ranges reported for *I. velata*. In contrast, spores from the Spanish specimens are markedly larger than the spores from the North African specimens. Thus, the mean megaspore diameter in Spanish specimens is 580 µm vs. 540 µm in Algerian *I. longissimum*, while the mean microspore length × width (L × l) for the Spanish specimens is 32 × 19 µm vs. 28 × 16 µm in Algerian *I. longissimum*. The megaspore surface ornamentation is similar in the three types of specimen (see Figs 1–9; note that the Spanish spores are more homogeneous), in line with their inclusion in the section *Tuberculatae* (see Pfeiffer, 1922; Fuchs, 1962). The evolutionary relations of this group have been widely discussed (see, e.g. Hickey, 1986; Taylor & Hickey, 1992), but it is increasingly accepted that the section *Tuberculatae* is of polyphyletic origin (Rydin & Wilkström, 2002).

Other characters included in Table 1 (including frond length, number of leaves per plant and corm diameter) are similarly larger in the Spanish specimens than in Algerian *I. longissimum*.

**Table 1.** Comparison of the features of the three *Isoetes* taxa. Microspore measurements are maximum diameters (L and l) as defined by Berthet & Lecocq (1977)

	<i>I. velata</i> (North Africa)	<i>I. longissimum</i> (Algeria)	NW Spanish populations
Megaspores (µm)			
Min.–Max.	346.14–615.36	461.52–615.36	484.6–730.74
Mean ± SE (N)	463.62 ± 53.60 (420)	539.72 ± 40.31 (60)	579.56 ± 54.59 (240)
Microspores (µm)			
Min.–Max. (L × l)	22.5–35 × 12.5–22.5	25–30 × 15–20	27.5–42.5 × 15–25
L Mean ± SE (N)	28.84 ± 2.15 (360)	27.91 ± 1.45 (60)	32.11 ± 2.5 (270)
l Mean ± SE (N)	16.84 ± 1.61 (360)	16.04 ± 1.31 (60)	18.61 ± 1.52 (270)
Leaf length (cm)	18–43	41–43.5	26–84
Mean ± SE (N)	28.79 ± 7.76 (19)	41.9 ± 0.91 (5)	59.54 ± 13.86 (22)
Leaf number	10–34	9–20	18–72
Mean ± SE (N)	19.78 ± 6.50 (19)	15.6 ± 4.22 (5)	36.95 ± 14.74 (22)
Corm diameter (cm)			
Min.–Max.	0.6–1.8	0.8–1.4	0.5–3.5
Mean ± SE (N)	1.16 ± 0.29 (16)	1.12 ± 0.23 (4)	1.79 ± 0.61 (22)



**Figures 1–9.** Distal, lateral and proximal views of *Isoetes* megaspores. Figs 1–3. *I. longissimum*. Scale bars = 200  $\mu$ m. Figs 4–6. *I. velata*. Scale bars = 400  $\mu$ m. Figs 7–9. Spanish plant. Scale bars = 400  $\mu$ m.

#### ECOLOGY AND CYTOLOGY

The available phenological data (see Table 2) suggest that Algerian *I. longissimum* behaves like *I. velata*, which shows leaf and spore development in the spring and leaf dehiscence in summer. This behaviour is frequent in amphibious plants, which take advantage of declining water levels to reproduce sexually. As the water level drops, the aerial parts of the plant dry up and eventually disappear.

In contrast, the Spanish populations are clearly active in summer, and the fronds persist throughout the year. In addition, these plants are strictly aquatic, growing exclusively in permanent watercourses (Romero & Amigo, 1995).

Plants of these Spanish populations are polyploid with  $2n = 44$  (Prada, 1983), vs.  $2n = 22$  for Spanish populations of *I. velata* (Prada, 1979).

#### DESCRIPTION

Our results indicate that North African *I. longissimum* is very similar to North African *I. velata*, with no clearly differential morphological

characters. For this reason, we agree with Pfeiffer (1922) that the so-called *I. longissimum* is a longer-leaved variant of *I. velata* (see Table 1). However, our findings (notably spore size and morphology, corm diameter, leaf persistence and habitat) indicate that the Spanish populations constitute a new species, which we describe as follows.

#### *ISOETES FLUTANS* M. I. ROMERO SP. NOV. (FIG. 10)

*Type:* River Xallas, between Dumbria and Mazaricos (A Coruña Province, Galicia, Spain). 29TMH9856. 25.viii.2002. Leg. M. I. Romero & J. Amigo (SANT 47788, holotype).

*Diagnosis:* Planta perennis cum foliis longis (26–84 cm) et anno toto abundantibus quae in caule robusto et lobulato locatae sunt. Sporangia a velo aperta. Megaspores sphaericae, magnae (484.6–730.74  $\mu$ m) et verrucosae. Microspores spinulosae (27.5–42.5  $\times$  15–25  $\mu$ m), cum crescentis lunae forma. Habitat in alveo fluviorum.

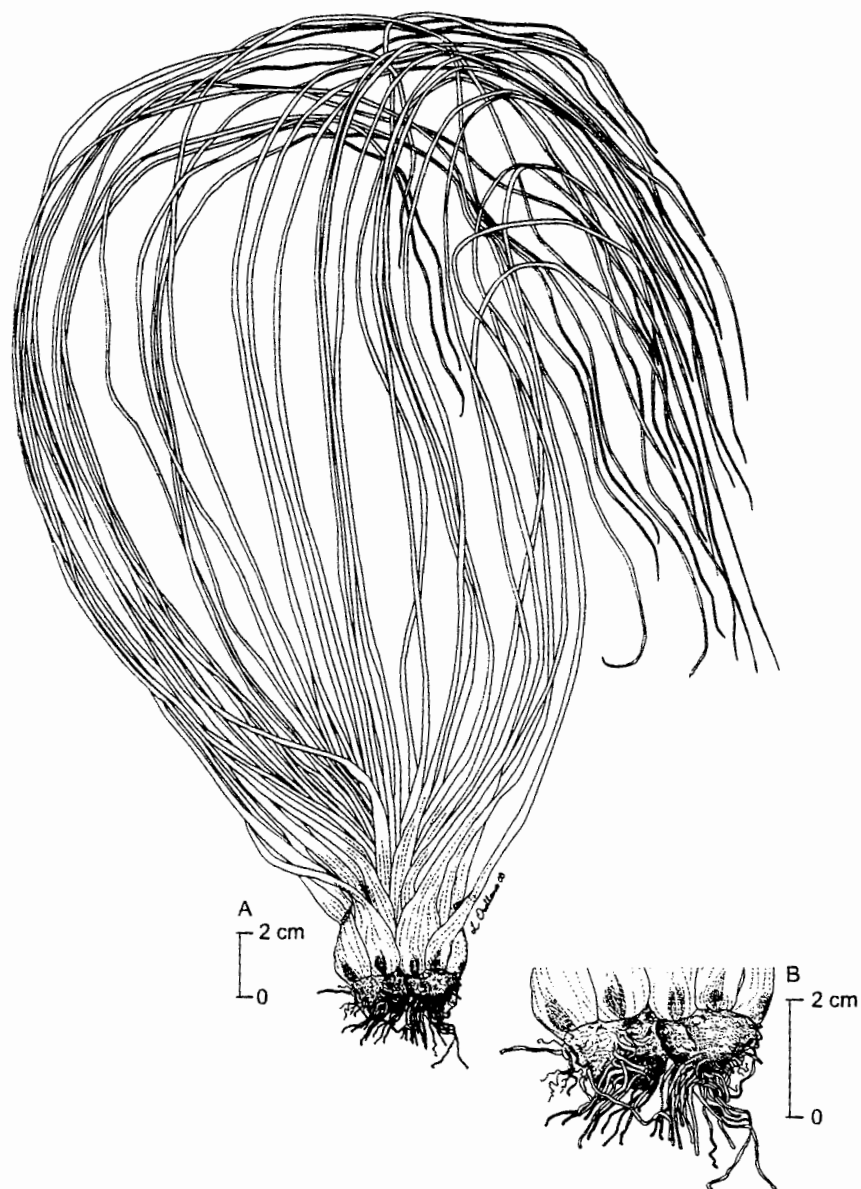


Figure 10. *Isoetes fluitans* sp. nov. A, habit of specimen. B, detail of part of the plant with corm.

Table 2. Differential ecological and karyological characteristics of the three taxa of *Isoetes*

	<i>I. velata</i> (North Africa)	<i>I. longissimum</i> (Algeria)	NW Spanish populations
Phenology	April–May	May	July–August
Fronds	Seasonal	Seasonal?	Persistent
Habit	Amphibious	Amphibious	Aquatic
Habitat	Seasonal pools and temporarily flooded areas	Flooded areas	River-beds
Chromosome number <sup>1</sup>	2n = 22 (Prada, 1979)	????	2n = 44 (Prada, 1983)

<sup>1</sup>Chromosome counts were obtained from Spanish material.

**Description:** Perennial, with abundant long leaves per plant (26–84 cm or more), slender and dark green on a short thick stem, present throughout the year. Sporangia covered with velum. Megaspores spherical, large (484.6–730.74 µm), tuberculate. Microspores (27.5–42.5 × 15–25 µm) spinulose, halfmoon-shaped. Spores develop in summer. Aquatic plant growing in rivers in north-west Iberia (Romero & Amigo, 1995), rooted in the river-bed. Polyploid,  $2n = 44$  (Prada, 1983). Currently categorized as 'Endangered' throughout its natural distribution area (Aizpuru *et al.*, 2000).

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## APPENDIX

Sheets from the Paris Herbarium (Muséum d'Histoire Naturelle de Paris) and SANT Herbarium (University of Santiago, Spain) examined in the present study.

Voucher	Date	Locality	Legit.
P00202849	23.vi.1888	Tunisia (Lake Cejenan)	Cosson & Barratte
P00202850	vii.1888	Tunisia (Mission sci.)	Cosson, Barratte & Duval
P00202844	16.iv.1949	Tunisia (Garra Senhadja)	–
P00202848	23.vi.1888	Tunisia (Lake Cejenan)	Cosson & Barratte
P00202847	23.vi.1888	Tunisia (Lake Cejenan)	Cosson & Barratte
P00202846	01.vii.1888	Algerian border, NW	Cosson, Barratte & Duval
P00202845	09.vi.1949	Tunisia (Garra Sed Jenana)	Pottier
P00202852	v.1841	Algeria (Bois de la Calle)	Bory
P00202851	v.1841	Algeria (La Calle)	Durieu
P00202834	16.vi.1904	Algeria (Foret de la Rhégocia)	Chevallier
P00202833	03.vii.1861	Algeria (Senhadja Bone)	Hicalik
P00202828	01.vi.1912	Morocco (Camp Boulhauht)	D'Alleizette
P00202835	–	Algeria (El Alia)	Cordier
P00202836	16.iv.1953	Algeria (La Rassauta)	Dubuis, Vigit & Callé
P00202840	18.iv.1838	Algeria	Dr Lebel
P00202839	12.iv.1861	Algeria (Boudoum)	Rec. Bourlier
P00202841	24.vi.1844	Algeria (La Calle)	Du Rieu de Maisonneuve & Motelay
P00202842	15.v.1878	Algeria (Bou-Tegart, Oran)	Cosson
P00202843	20.v.1875	Algeria (Bou-Tegart, Oran)	Cosson
P00202829	18.iv.1859	Algeria (Chaïba, c. Coléah)	Rec. Clauson
PO202831	23.iii.1841	Algeria (La Calle)	Durieu
P00202832	31.v.1841	Algeria (La Calle)	Durieu
P00202830	14.v.1844	Algeria (La Calle)	Durieu
SANT28309	16.vii.1991	Hs: A Coruña, Baio	Romero
SANT20132	22.vii.1985	Hs: A Coruña, C. Teixeira	Amigo & Guitian
SANT16456	31.viii.1984	Hs: A Coruña, Brandomil	Horjales & Redondo
SANT22544	9.viii.1992	Hs: A Coruña, Mazaricos	Amigo & Romero
SANT24263	05.ix.1992	Hs: Lugo, Vilalba, Codesido	Soñora
SANT28308	16.vii.1991	Hs: A Coruña, c. Lires	Romero
SANT13485	05.ix.1985	Hs: A Coruña, C. Teixeira	Izco & Amigo
SANT28306	23.vii.1991	Hs: A Coruña, Sta. Comba	Romero
SANT20585	24.vi.1988	Hs: Lugo, Guitiriz	Bárbara
SANT39097	23.vii.1994	Hs: Lugo, Vilalba, Alligal	Amigo <i>et al.</i>
SANT47151	22.viii.2001	Hs: A Coruña, Dumbria	Romero & Amigo
SANT 47789	25.viii.2002	Hs: A Coruña, Mazaricos	Romero & Amigo
SANT 47787	25.viii.2002	Hs: A Coruña, Zas, Brandomil	Romero & Amigo
SANT 47788	25.viii.2002	Hs: A Coruña, Dumbria	Romero & Amigo
SANT 47785	20.viii.2002	Hs: Lugo, Ladra-Parga	Romero, Ramil <i>et al.</i>
SANT 47151	15.viii.2001	Hs: Dumbria-Mazaricos	Romero & Amigo
SANT 47786	20.viii.2002	Hs: Lugo, Outeiro de Rei	Romero, Ramil <i>et al.</i>
SANT 47783	27.viii.2002	Hs: A Coruña, Cee	Romero & Amigo
SANT 47784	27.viii.2002	Hs: A Coruña, Cee-Muxia	Romero & Amigo