

A comparative study of two endemic *Isoëtes* species from South Italy.

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Abstract

Two *Isoëtes* taxa (*Isoëtaceae*, *Pteridophyta*) have recently been described in Puglia and Sicily (South Italy). Though morphologically similar (Ernandes, 2011), they differ in diagnostic characters and habitat preferences.

In this paper we highlight the differences and similarities between *Isoëtes iapygia* Ernandes, Beccarisi *et* Zuccarello (Ernandes *et al.* 2010) and *Isoëtes todaroana* Troia *et* Raimondo (Troia and Raimondo, 2009). Individuals are described in terms of selected diagnostic characters. Morphometric differences and other distinguishing characteristics suggest that *I. iapygia* and *I. todaroana* should be considered as separate species.

Keywords *Isoëtes*, endemic species, Mediterranean temporary ponds, SEM analysis, spore microornamentation

Introduction

The *Isoëtaceae* are a small, cosmopolitan family of aquatic, heterosporous pteridophytes which are related to the extant *Selaginellaceae* and *Lycopodiaceae* families. The family is monotypic and comprises 350 species (Hickey 1997; Hickey et al. 2003) which are classified mainly on the basis of megaspore surface morphology (Fuchs-Eckert 1981; Hickey 1986; Pfeiffer 1922). In recent years, a number of investigations have sought to determine the phylogenetic relationships among *Isoëtes* species based on morphological, ecological (Prada 1979, 1980, 1983; Rolleri and Prada 2004; Prada and Rolleri 2005; Romero and Real 2005; Liu et al. 2006), karyological (Hickey 1984; Troia 2001) and biomolecular (Hickey 1997; Hoot and Taylor 2001; Hoot et al. 2004; Prada and Rolleri 2005; Hoot et al. 2006) characteristics.

Macrospore surface morphology provides a ready means for species sorting and is of great importance in species identification (Taylor et al. 1975; Croft 1980; Hickey 1981; Boom 1982; Kott and Britton 1983; Stolze and Hickey 1983). Surface features differ markedly among species and therefore have long been used to distinguish taxa. Also known as “sculpturing”, spore ornamentation is the most frequently used taxonomic criterion in flora (Brown 1960). Together with other features and habitat preferences, macrospore characteristics are also useful for determining natural species alliances (Hickey 1986). Microspores, on the other hand, have been largely neglected in taxonomic schemes until recently (Musselman 2003).

In the last few years, interest in the “quillwort” genus has led to investigation of other morphological features including leaf anatomy, ligules, corm cross-sections and lobes, velum cover and scales (Musselman, 2003). Plant morphology is considered to be relevant to plant systematic, ecology, genetics and physiology (Kaplan, 2001).

Italy is home to 7 species of *Isoëtes*, 3 of which are protected at national level and listed as endangered due to habitat loss, extension of agricultural land and invasion by exotic species (Conti et al. 2005; Frattini et al. 2010; Barni et al. 2010).

Two new taxa have been recently described in southern Italy. They appear morphologically similar (Ernandes, 2011) but differ in terms of diagnostic characters and habitat preferences.

In this paper we highlight the differences and similarities between these two taxa: *Isoëtes iapygia* Ernandes, Beccarisi *et* Zuccarello (Ernandes *et al.* 2010) and *Isoëtes todaroana* Troia *et* Raimondo (Troia and Raimondo, 2009).

Materials and methods

The study area is located in two regions in the south of Italy (Puglia and Sicily), considered the most important hotspots in the Mediterranean Basin (Medàil and Quézel, 1999). Especially in terms of climate and geological characteristics, the area where *I. iapygia* is located is classified as “Hills of Murge and Salento” (Costantini *et al.* 2004; L’Abate *et al.* 2011). The climate and pedoclimate are Mediterranean-subcontinental to continental. The mean annual air temperature is 14-20°C and mean annual precipitation is 420-700 mm. The rainiest months are October and November while the driest months are from June to August. The soil moisture and temperature regimes are xeric, and to a lesser extent dry xeric or thermic. The geological substrate is characterized by Mesozoic limestone, marl and residual deposits. The mean altitude is 191 metres a.s.l. and the mean slope is 3% (L’Abate *et al.* 2011). The main soil types are: shallow and eroded soils, soils with carbonates, clays and sandy soils (L’Abate *et al.* 2011). The area where *I. todaroana* is located is classified as “Hills of Sicily on Tertiary clayey flysch, limestone, sandstone and gypsum, and coastal plains” (Costantini *et al.* 2004; L’Abate *et al.* 2011). The climate and pedoclimate are Mediterranean-subtropical. The mean annual air temperature is 16-20°C. The mean annual precipitation is 450-670 mm, and the rainiest months are November and January while the driest months are from May to September. The soil moisture and temperature regimes are xeric and dry xeric or thermic. The geological substrate is characterized by Tertiary clayey flysch, sandstone and gypsum. The mean altitude is 247 meters a.s.l. while the mean slope is 12% (L’Abate *et al.* 2011). The main soils are

characterised by accumulation of carbonates and more soluble salts, clay and alluvial deposits (L'Abate et al. 2011).

Individuals were described on the basis of the following 20 diagnostic characters: number of corm lobes, corm width, shape of corm section, root shape, number of leaves, leaf length and shape, presence and shape of phyllopodia or scales, air chambers, stomata, shape and length of ligule, velum, spore diameter, spore morphology, spore ornamentation, perine microornamentation, laesura arms and equatorial ring. In addition, information was collected on habitus, substrate characteristics (pH of soil, type of soil and geological substrate), type of habitat, community species and geographical range (sites of presence, chorology). Both fresh and dried individuals of *I. iapygia* and *I. todaroana* were analysed, and all data are shown in Table 1.

Macrosporangia and microsporangia were selected for each individual, randomly selecting spores in accordance with Ernandes et al. (2010). All morphological and anatomical characteristics were observed by SEM, after being treated in accordance with protocols (Ernandes et al. 2010). Sporal characteristics were defined in accordance with Ferrarini et al. (1986), Musselmann (2003), Prada (1979) and Hickey (1986).

Results and discussion

The most obvious visible differences between *I. iapygia* and *I. todaroana* are in spore morphology and ornamentation. The SEM analysis showed that *I. iapygia* has larger tuberculate macrospores than *I. todaroana*. These are also round in polar view, with numerous tubercles attached to each other and a rudimentary, undulate equatorial ridge (Fig. 1A, B). The laesura arms are flattened and do not form a prominent girdle. In contrast, *I. todaroana* has tuberculate macrospores with aculeate tubercles, triangular in polar view, with a well developed equatorial ridge, raised laesura arms and a prominent girdle, giving the plant its characteristic shape (Fig. 1C, D).

Of great interest is the comparison at higher magnification (reported here for the first time), which shows the microornamentation of the macrospores. The perine of *I. iapygia* has densely fimbriate structures (Fig. 2A, B) that differ from those described in the literature (Prada 1979; Musselman, 2003; Prada and Rolleri 2005; Choi et al. 2008; Bagella et al. 2011); the perine of *I. todaroana* has elongated filaments, welded together, similar to what has been reported for *Isoëtes histrix* Bory (Fig. 2C, D) (Prada 1983; Bagella et al. 2011; Troia et al. 2011a).

The two taxa also differ in the size and ornamentation of microspores, ligule shape and length, leaf shape and number and corm structure (Tab.1). The corm of *I. iapygia* is larger than that of *I. todaroana* and the shape of its cross-section is significantly different (Fig. 3A): in the *I. iapygia* cross-section the secondary cortex is hexagonal, the lateral meristem becoming trilobed at the base. In *I. todaroana* the shape of the secondary cortex is that of an irregular polygon and the lateral meristem seems to be more rounded than lobed (Fig. 3B).

Another obvious difference is in the scales and phyllopodia. These two structures, which are sometimes confused in the literature, represent two independently derived mechanisms for resistance to desiccation (Taylor and Hickey, 1992). Phyllopodia are the sclerified remnants of the bases of fully developed leaves. The presence of phyllopodia was in part the basis for assigning some *Isoëtes* species to the *Terrestres* section (Hickey 1983; Bolin et al. 2008). Scales are complete leaf primordia which become arrested early in their development and are in general unsclerified (Bolin et al. 2008). By this definition, *I. iapygia* has a small number of minute, brown, translucent scales (Ernandes et al. 2010) at the base of the naked corm (Fig. 4A) while *I. todaroana* has black, hardened scales that are similar to phyllopodia, with two rounded lateral lobes and one short central spine-like lobe (Troia and Raimondo 2009) (Fig. 4B).

The geographical range of *I. iapygia* is well defined, limited to 10 sites in the southern part of Puglia (Ernandes 2011) that do not overlap with the range of other congeners. The information on *I. todaroana* is insufficient to define its true geographical range because it has so far only been found in the *locus classicus*, in an area of about 200x100 m (Troia and Raimondo 2009).

The habitat of both taxa is Mediterranean temporary ponds (3170*), considered an international priority (European Commission DG Environment 2007). The type locality of *I. todaroana* is described as temporary wetland with a mosaic of communities characterized by aquatic macrophytes such as *Bolboschoenus maritimus* (L.) Palla, *Eleocharis palustris* (L.) Roem. & Schult., *Scirpus cernuus* Vahl, *Mentha pulegium* L., *Damasonium alisma subsp. bourgaei* (Coss.), *Oenanthe* sp., *Lythrum* sp., *Tamarix* sp. and *Romulea* sp. (Troia and Raimondo 2009; Troia et al. 2011b). *I. iapygia* is found in very small areas, inside rock pools or on a thin layer of mosses (such as *Pleurochaete squarrosa* (Brid.) Lindb. and *Cheilotela chloropus* (Brid.) Broth.), in which the community is dominated by microphytes characteristic of the *Isoëto-Nanojuncetea* phytosociological class, including *Ranunculus sardous* Crantz, *Polypogon maritimus* Willd., *Romulea bulbocodium* (L.) Sebast. et Mauri, *Romulea columnae* Sebast. et Mauri.

The different specificity of the habitat of the two varieties is also seen in the type of soil and geological substrate: *I. todaroana* grows on a distinctive geological substrate of calcareous sandstone with a thin layer of clay on top and an alkaline pH (Tab.1) (Troia and Raimondo 2010). In contrast, *I. iapygia* grows on a thin layer of soil or mosses on a limestone substrate with a neutral or sub-basic pH (Tab.1).

The two taxa share a number of traits including dichotomous roots, complete velum, presence of stomata and two air chambers. However it has been widely documented that at least for *I. iapygia*, the reduction in leaf area and the presence of two air chambers represent an adaptation to xeric environments (Ernandes et al. 2010). Indeed, *I. iapygia* is a terrestrial species, never submerged by water. In all sites where it is recorded the characteristic layer of mosses is episodically soaked, retaining sufficient moisture for the development of the plant, though for several months of the year the habitat is characterised by aridity and high temperatures (Ernandes et al., 2010).

In contrast, the type locality of *I. todaroana* is a temporary wetland that dries out only in summer. It is a remnant of a wider wetland, most of which has been reclaimed and converted to farmland that

now surrounds the “type locality”. Thus it has an amphibious habitus, with both emergent and submerged growth in temporary ponds (Troia and Raimondo, 2009).

Conclusions

The morphometric differences and other distinguishing characteristics suggest that *I. iapygia* and *I. todaroana* should be considered as separate species. The presence of scales and phyllopodia, megaspore ornamentation and habitus suggest a connection between these two species and the Mediterranean “terrestrial” section of the genus, including *I. histrix*, *I. subinermis*, *I. olympica*, *I. setacea*, *I. duriei* and the recently identified *I. libanotica* (Bagella et al. 2010; Bolin et al. 2008, 2011; Jermy and Akeroyd 1993; Bolòs 1996; Coste 1937; Derrick et al. 1997; Greuter et al. 1984). On the other hand, the fibrillose surface of the megaspores suggests a relationship with the amphibious *I. velata* group, although the terrestrial *I. histrix* also has this characteristic (Prada 1983, Bagella et al, 2011).

The formulation of hypotheses is impeded by the documented morphological convergence and phenotypic plasticity of the genus (Hickey et al. 1989). *I. iapygia* has probably evolved gradually, as the result of spatial isolation and low genetic exchange with congeners, due to the low dispersal ability of macro- and microspores. This is reflected in its characteristic morphological traits, including densely fimbriate macrospores, arched and filiform leaves, translucent scales, two air chambers and the extreme specificity of its habitat.

It would be interesting to compare the relationships between these two species and other Mediterranean taxa using the cladistic approach, which may help to identify a common ancestor or clarify their evolution, and to evaluate other original hypotheses.

Targeted studies in this direction are underway and it is likely that new species will be found in the future.

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Table I Characters used to compare and describe individuals.

Morphometric characteristics	<i>Isoëtes iapygia</i>	<i>Isoëtes todaroana</i>
Corm lobes	trilobed	rounded
Corm width (cm)	0.8	0.5
Shape of corm section	hexagonal	irregular
Root shape	dichotomous	dichotomous
Leaf shape	filiform, arched	lanceolate, erect
Leaf number	20	25
Velum	complete	complete
Stomata	present	present
Number of air chambers	2	2
Scales	present	absent
Phyllopodia	absent	present
Macrosore macroornamentation	tuberculate	tuberculate
Microsore macroornamentation	coniculate-echinate	aculeate
Macrosore microornamentation	densely fimbriate	filamentous
Macrosore diameter (μm)	450	440
Macrosore profile	rounded	triangular
Microsore length (μm)	30	25
Ligule shape	ovate	lanceolate
Ligule length (mm)	1.2	1.0
Laesura's arms	flattened	raised
Equatorial ring	little pronounced	pronounced
Ecological characters		
Habitus	terrestrial	amphibious
pH of soil	7.3	8.7

type of soil	clay	clay
geological substrate	limestone	sandstone
Habitat		
Type (Natura 2000 Code)	3170*	3170*
	Pleurochaete squarrosa (Brid.)	
	Lindb., Cheilotela chloropus (Brid.)	Bolboschoenus maritimus (L.) Palla,
	Broth., Ranunculus sardous Crantz,	Eleocharis palustris (L.) Roem. & Schult.,
Characteristic species of community	Polypogon maritimus	Scirpus cernuus Vahl, Mentha
	Willd., Romulea bulbocodium (L.)	pulegium L., Oenanthe sp., Lythrum sp.,
	Sebast. et Mauri, Romulea columnae	Tamarix sp., Romulea sp.,
	Sebast. et Mauri.	
Geographical range		
Sites of presence	10	1
Chorology	southern Puglia	insufficient data

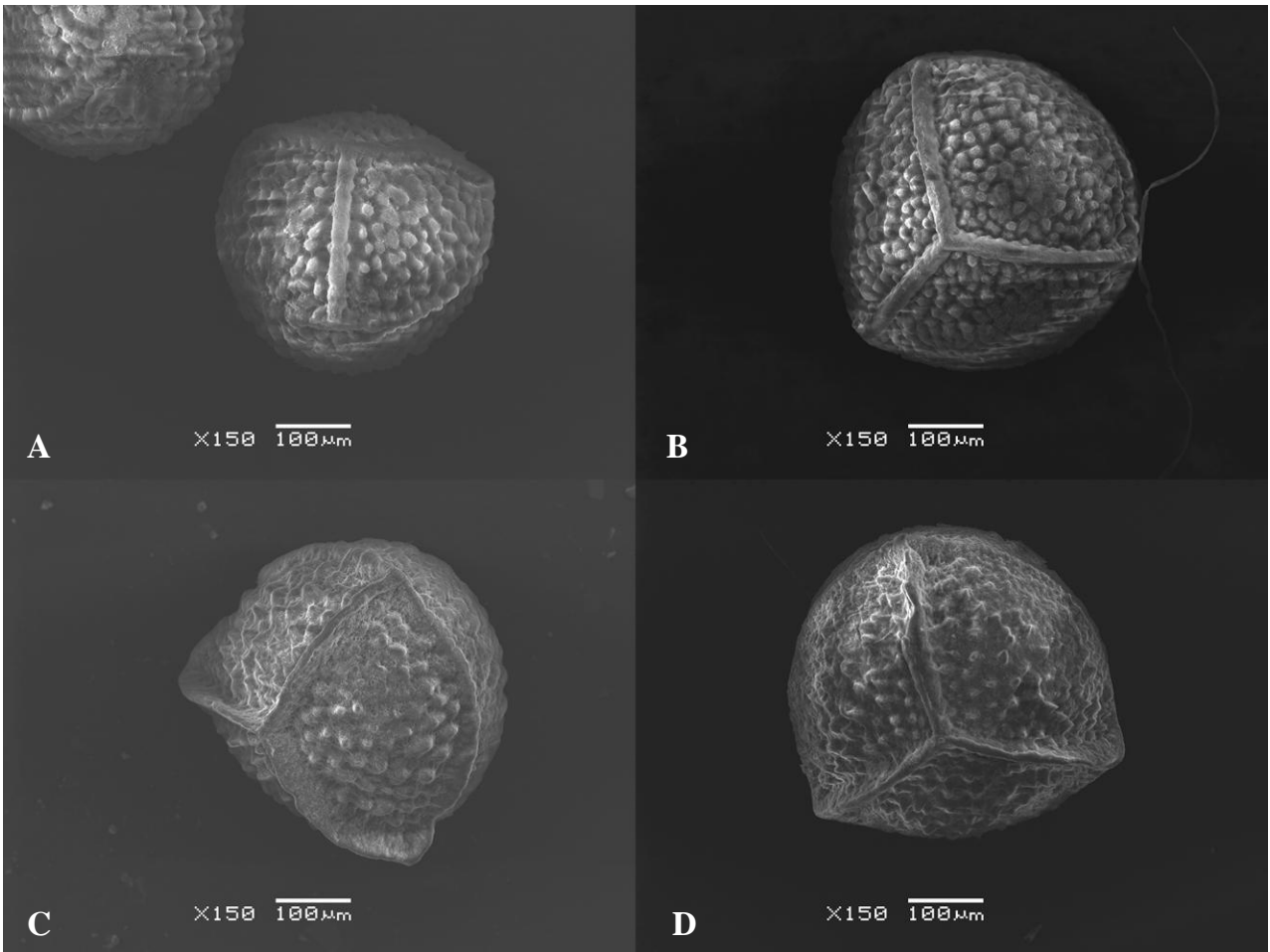


Fig. 1 Macrospore of *I. iapygia* viewed by SEM (A: lateral view, B: proximal view) and *I. todaroana* (C: lateral view, D: proximal view). Scale bar: 100µm.

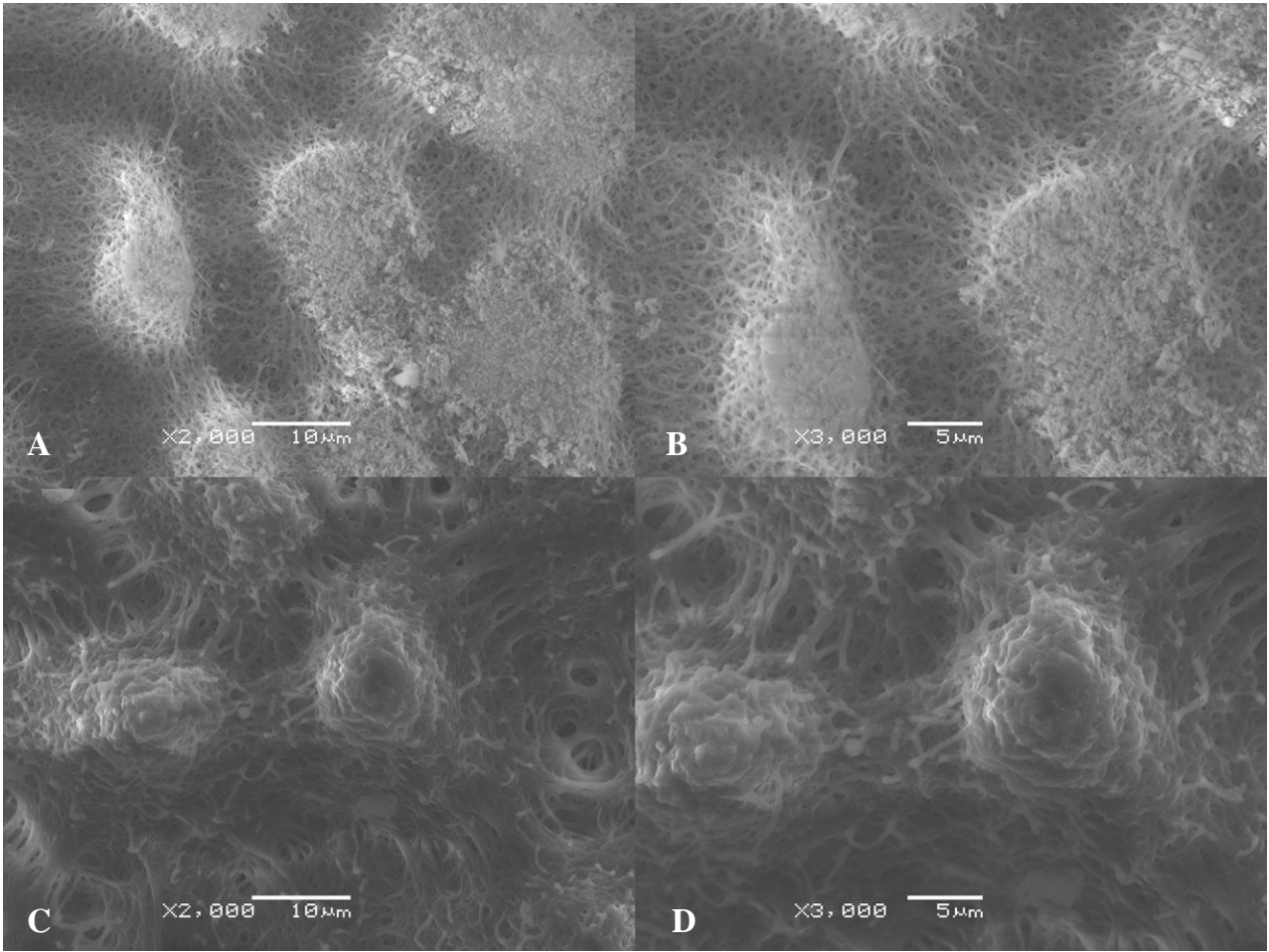


Fig. 2 Macrospore microornamentation types viewed by SEM: *I. iapygia* (A, scale bar: 10 μm; B, scale bar: 5 μm) and *I. todaroana* (C, scale bar: 10 μm; D, scale bar: 5 μm).

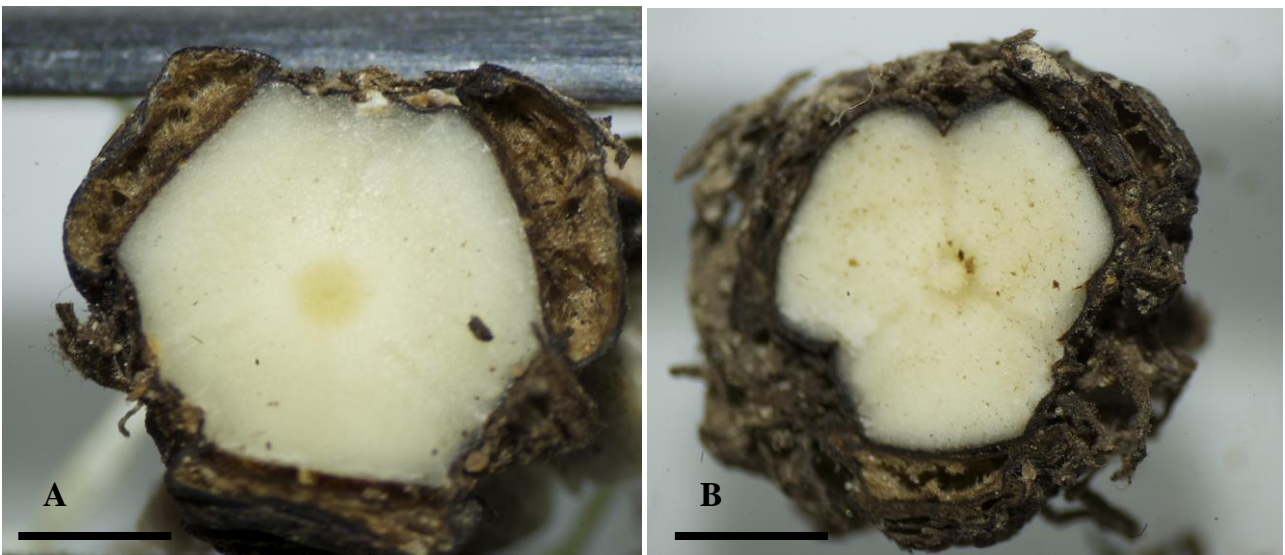


Fig. 3 Cross section of corm (A: *I. iapygia*, B: *I. todaroana*). Scale bar: 0.25 cm.

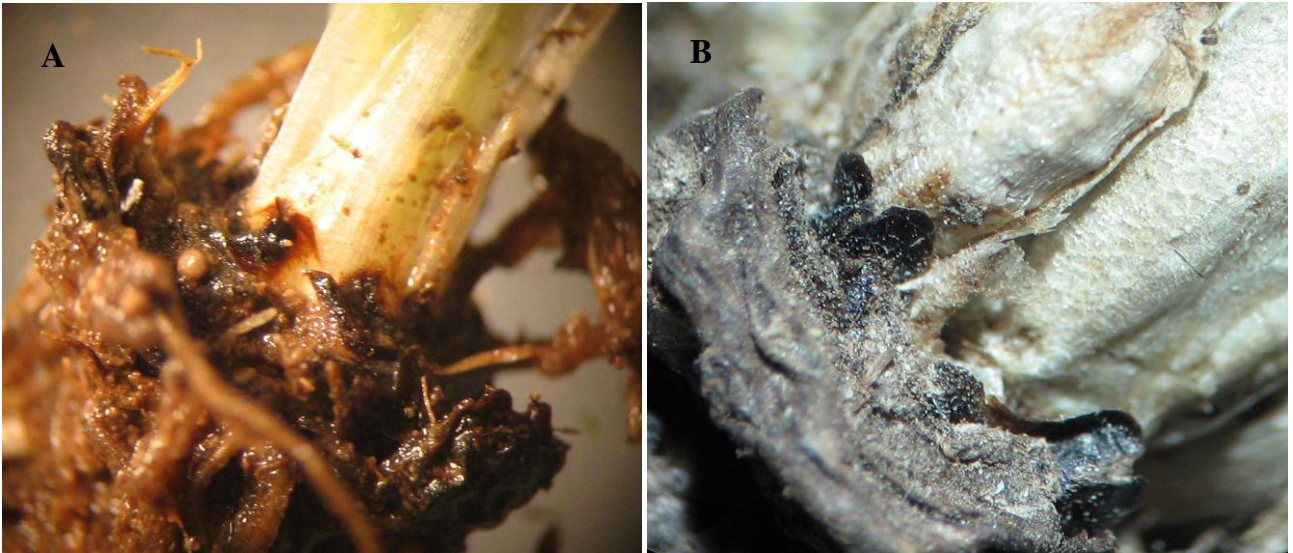


Fig. 4 Enlargement of scales and phyllopodia of A: *I. iapygia* and B: *I. todaroana*.