International Scholarly Research Network ISRN Botany Volume 2012, Article ID 127250, 7 pages doi:10.5402/2012/127250

Research Article

A Comparative Study of Two Endemic *Isoëtes* Species from South Italy

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Received 9 August 2012; Accepted 2 October 2012

Academic Editors: M. Adrian, S. Rachmilevitch, S. Satoh, and N. Uehlein

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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.

1. 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 [1, 2], which are classified mainly on the basis of megaspore surface morphology [3–5]. In recent years, a number of investigations have sought to determine the phylogenetic relationships among *Isoëtes* species based on morphological, ecological [6–12], karyological [13, 14] and biomolecular [1, 10, 15, 16].

Macrospore surface morphology provides a ready means for species sorting and is of great importance in species identification [17–22]. 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 [23]. Together with other features and habitat preferences, macrospore characteristics are also useful for determining natural species alliances [4]. Microspores, on the other hand, have been largely neglected in taxonomic schemes until recently [24].

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 [24]. Plant morphology

is considered to be relevant to plant systematic, ecology, genetics, and physiology [25].

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 [26–28].

Two new taxa have been recently described in southern Italy. They appear morphologically similar [29] 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 [30] and *Isoëtes todaroana* Troia *et* Raimondo [31].

2. 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 Quèzel, [32]). Especially in terms of climate and geological characteristics, the area where *I. iapygia* is located is classified as "Hills of Murge and Salento" [33, 34]. 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% [34]. The main soil types are: shallow and eroded soils, soils with carbonates, clays, and sandy soils [34]. The area where *I. todaroana* is located is classified as "Hills of Sicily on Tertiary clayey flysch, limestone, sandstone and gypsum, and coastal plains" [33, 34]. 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% [34]. The main soils are characterised by accumulation of carbonates and more soluble salts, clay, and alluvial deposits [34].

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. [30]. All morphological and anatomical characteristics were observed by SEM, after being treated in accordance with protocols [30]. Sporal characteristics were defined in accordance with Ferrarini et al. [35], Musselmann [24], Prada [6], and Hickey [4].

3. 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 (Figures 1(a) and 1(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 (Figures 1(c) and 1(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 (Figures 2(a) and 2(b)) that differ from those described in the literature [6, 10, 24, 36, 37]; the perine of *I. todaroana* has elongated filaments, welded together, similar to what has been reported for *Isoëtes histrix* Bory (Figures 2(c) and 2(d)) [8, 37, 38].

The two taxa also differ in the size and ornamentation of microspores, ligule shape, and length, leaf shape and number, and corm structure (Table 1). The corm of *I. iapygia* is larger than that of *I. todaroana* and the shape of its cross-section is significantly different (Figure 3(a)): 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 (Figure 3(b)).

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 Hyckey, [39]). 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 [40]. Scales are complete leaf primordia which become arrested early in their development and are in general unsclerified [40]. By this definition, *I. iapygia* has a small number of minute, brown, translucent scales [30] at the base of the naked corm (Figure 4(a)) while *I. todaroana* has black, hardened scales that are similar to phyllopodia, with two rounded lateral lobes and one short central spine-like lobe [31] (Figure 4(b)).

The geographical range of *I. iapygia* is well defined, limited to 10 sites in the southern part of Puglia [29] 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 $200 \times 100 \text{ m}$ [31].

The habitat of both taxa is Mediterranean temporary ponds (3170*), considered an international priority [41]. 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. and Schult., Scirpus cernuus Vahl, Mentha pulegium L., Damasonium alisma subsp. bourgaei (Coss.), Oenanthe sp., Lythrum sp., Tamarix sp., and Romulea sp. [31, 42]. 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, and 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 (Table 1) (Troìa and Raimondo [43]).

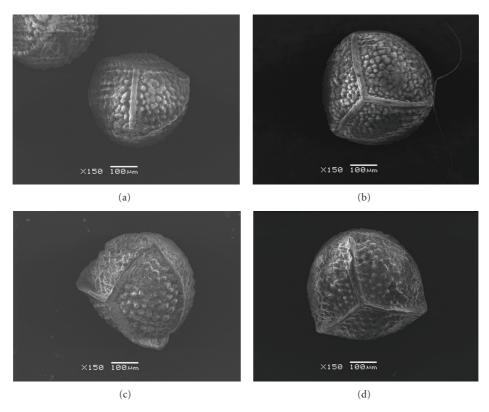


Figure 1: Macrospore of *I. iapygia* viewed by SEM ((a): lateral view, (b): proximal view) and *I. todaroana* ((c): lateral view, (d): proximal view). Scale bar: $100 \, \mu \text{m}$.

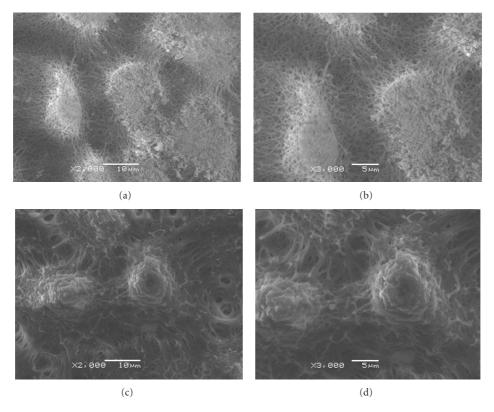


Figure 2: Macrospore microornamentation types viewed by SEM: *I. iapygia* ((a), scale bar: $10 \,\mu\text{m}$; (b), scale bar: $5 \,\mu\text{m}$) and *I. todaroana* ((c), scale bar: $10 \,\mu\text{m}$; d, scale bar: $5 \,\mu\text{m}$).

Table 1: Characters used to compare and describe individuals.

| Morphometric characteristics | Isoëtes iapygia | Isoëtes todaroana |
|-------------------------------|---|---|
| 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 |
| Macrospore macroornamentation | Tuberculate | Tuberculate |
| Microspore macroornamentation | Coniculate-echinate | Aculeate |
| Macrospore microornamentation | Densely fimbriate | Filamentous |
| Macrospore diameter (μm) | 450 | 440 |
| Macrospore profile | Rounded | Triangular |
| Microspore lenght (μ m) | 30 | 25 |
| Ligule shape | Ovate | Lanceolate |
| Ligule lenght (mm) | 1.2 | 1.0 |
| Laesura 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* |
| Characteristic species of | Pleurochaete squarrosa (Brid.) Lindb., Cheilotela chloropus (Brid.) Broth., Ranunculus sardous Crantz. | Bolboschoenus maritimus (L.) Palla, Eleocharis palustris (L.) Roem. and Schult., Scirpus cernuus Vahl, |
| community | Polypogon maritimus Willd., Romulea bulbocodium (L.) Sebast. et Mauri, Romulea columnae Sebast. et Mauri. | Mentha pulegium L., Oenanthe sp., Lythrum sp., Tamarix sp., Romulea sp., |
| Geographical range | | |
| Sites of presence | 10 | 1 |
| Chorology | Southern Puglia | Insufficient data |

In contrast, *I. iapygia* grows on a thin layer of soil or mosses on a limestone substrate with a neutral or subbasic pH (Table 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 [30]. 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 [30].

In contrast, the type locality of *I. todaroana* is a temporary wetland that dries out only in summer. It is a remnant

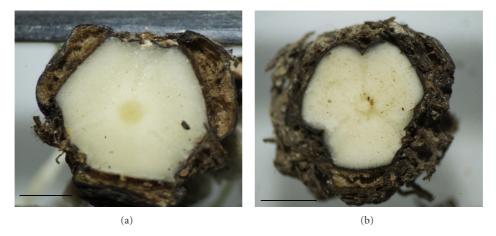


Figure 3: Cross section of corm ((a): I. iapygia, (b): I. todaroana). Scale bar: 0.25 cm.

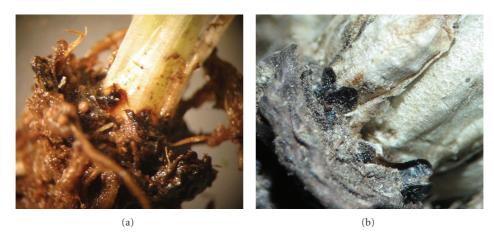


FIGURE 4: Enlargement of scales and phyllopodia of (a): *I. iapygia* and (b): *I. todaroana*.

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 [31].

4. 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* [40, 44–49].

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 [8, 37].

The formulation of hypotheses is impeded by the documented morphological convergence and phenotypic

plasticity of the genus [50]. *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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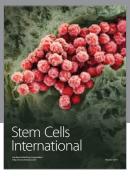
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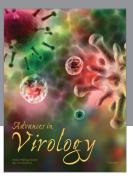
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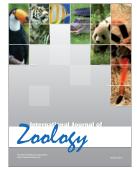


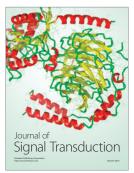














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