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Unraveling the taxonomy and nomenclature of the *Isoetes histrix* Bory species complex (Isoetaceae, Lycopodiidae)

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Abstract: Based on study of the lectotype of *Cephaloceraton gymnocarpum* Gennari, megaspore ornamentation, and chromosome number analysis of specimens from different Mediterranean areas, it is shown here that *Isoetes gymnocarpa* (Gennari) A.Braun is a species endemic to Sardinia, Central Italy, Corsica, and Baleares, different from the Mediterranean *I. histrix* Bory and from the mainly CE Mediterranean *I. sicula* Tod. (= *I. subinermis* (Gennari) Cesca & Peruzzi).

Key words: *Isoetes gymnocarpa*, *Isoetes sicula*, *Isoetes subinermis*, Mediterranean, Sardinia, spore ornamentation, terrestrial quillwort

1. Introduction

Cephaloceraton gymnocarpum, described for Caprera Island (Sardinia, Italy), was established by Gennari (1862) on the basis of the following putative distinctive characters compared with *C. histrix*: a) the phyllopodia shape, described as astatine-rhombic and tricuspidate for *C. gymnocarpum* and showing 2 long corns in *C. histrix*; b) the putative absence of a *velum* covering the sporangia, occurring instead in *C. histrix*. Concerning var. *subinerme*, indeed described for the first time by Gennari himself (1862), this author studied some specimens collected in Tuscany, identified by G. Savi (1769–1844) and O. Beccari (1843–1920), and indicated as a distinctive character compared with *C. histrix* the putative presence of shorter teeth in phyllopodia.

Just 2 years after the description of *C. gymnocarpum*, Braun (1864) validated the combination *Isoetes gymnocarpa* (Gennari) A.Braun, but considered it as falling within the variability of *I. histrix*.

Nearly in the same period Todaro (1866) described *Isoetes sicula*, putatively differing from *I. histrix* var. *subinermis* mainly in the shape of the phyllopodia. Just 2 years later, Cesati et al. (1868) considered *I. sicula* as a synonym of *I. histrix* var. *subinermis*.

Béguinot (1907) evidenced the polymorphic nature of Sardinian populations: of about 800 specimens collected in Caprera (*locus classicus* of *C. gymnocarpum*), he was able to recognize, according to the characters presented by Braun (1864), 2 main typologies attributable respectively to: a) a type described as var. *subinermis*, an epithet used also, in eventually different combinations, by Gennari (1862) and Braun (1864), and b) a type originally described as *C. gymnocarpum* (Gennari, 1862, 1865; Braun, 1864), but also intermediate forms. Possibly on this basis, Fiori (1923) recognized in the Italian flora 2 varietal taxa within *I. histrix*: "var. *typica*" (var. *histrix*, according to the current nomenclatural rules) and var. *subinermis* (= *C. gymnocarpum* and *I. sicula* Tod). Later, he reiterated the same interpretation (Fiori, 1943).

Thereafter, all these names were neglected for a long time (e.g., Pignatti, 1982; Jermy and Akeroyd, 1993), while the name *I. sicula* only was reported as a synonym of *I. histrix* by Greuter et al. (1984) and by Ferrarini et al. (1986).

Cesca and Peruzzi (2001) raised var. *subinermis* to species level. This choice was wrong on nomenclatural grounds, because a) the authors referred to an incorrect basionym (*I. histrix* f. *subinermis* Durieu *nomen nudum*), b) the authors listed among the synonyms other taxa originally described at species level (i.e. prioritary), such as, according to Fiori (1923, 1943), *Cephaloceraton gymnocarpum*.

This error was "corrected" by Arrigoni (2005) by proposing for this systematic unit the name *Isoetes gymnocarpa*. However, although he claimed to follow the work of Braun (1864), the latter author, as Gennari (1862), actually considered var. *subinermis* as distinct from *I. gymnocarpa*. Furthermore, he indicated among the synonyms of *I. gymnocarpa* also *I. sicula* Tod.

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Following the interpretation of Cesca and Peruzzi (2001), concerning taxonomy, and the proposal of Arrigoni (2005), concerning nomenclature, *I. gymnocarpa* was also recently recorded for Tuscany (Carta et al., 2009), Apulia (Ernandes, 2011), and for the recent synopsis of *Isoetes* in Italy (Troìa and Greuter, 2014). On the other hand, Peruzzi et al. (2003), Troìa (2005), and Troìa et al. (2011) favored for different reasons the use of the name *I. sicula*. Even more recently, Bolin et al. (2008), while approving the rank of species for *I. subinermis* as proposed by Cesca and Peruzzi (2001), remain doubtful on the synonymy between *I. gymnocarpa* and *I. subinermis* (under Art. 41.8(c) of the ICN, the correct authorship for this name is *I. subinermis* (Gennari) Cesca & Peruzzi; Troìa and Greuter, 2014).

Very recently, Troìa and Greuter (2014) selected a herbarium specimen in TO! (herbarium acronyms according to Thiers, 2014) as the lectotype of *C. gymnocarpum*. The specimen was collected in Caprera in 1861, i.e. 1 year before the publication of the species. As already stated by Braun (1864), Troìa and Greuter (2014) confirm that, contrary to what stated in the protologue, a *velum* is present and complete in the type specimen. Troìa and Greuter (2014) also considered *I. gymnocarpa* as a priority name vs. *C. histrix* var. *subinerme* and *I. sicula*.

Traditionally, patterns of megaspore ornamentation have provided the most relevant character for the taxonomy of quillworts at several systematic levels worldwide (Pfeiffer, 1922; Kott and Britton, 1983; Ferrarini et al., 1986). Microspore features have been largely neglected and the patterns of their surface ornamentation have not been standardized until recently (Musselman, 2003). Based on SEM images provided by different authors it is possible to distinguish different pattern of megaspore and microspore ornamentation and to evaluate if the combination of patterns in mega- and microspores is repetitive. Especially microornamentation data, certainly not available to earlier scholars, were recently shown to have good potentialities as taxonomic markers (Bagella et al., 2011). The latter authors reported the existence of 2 types of megaspores in specimens referable to *I. histrix* s.l. from Sardinia. The first type presented a macroornamentation smoothly tubercolate and an evident *laesura* on the radial ridges. To this macrospore type always corresponded a microspore echinate in the proximal face with no microornamentation. The specimens with this combination of mega- and microspores were referred as NF type (Not Fimbriate) and could be identified as *I. histrix* s.s. The second type of megaspore presented a macroornamentation markedly tuberculate with a densely fimbriate microornamentation. To this megaspore type always corresponded a psilate microspore finely filamentous with hooked apices. The specimens of this combination of macro- and microspores were referred to as F type (Fimbriate).

Concerning the chromosome number, *Isoetes histrix* is consistently reported as having $2n = 20$ ($x = 10$) chromosomes (Manton, 1950; Jermy and Akeroyd, 1993; Cesca and Peruzzi, 2001; Ernandes and Marchiori, 2013), although Prada (1986) reported both $2n = 22$ and $2n = 20$ for Iberian populations of *I. histrix* (perhaps erroneously, or not distinguishing among *I. histrix* and *I. subinermis*). Indeed, for *I. subinermis* the reported chromosome number is consistently $2n = 22$ ($x = 11$) in material from different areas of southern Italy: Calabria (Cesca and Peruzzi, 2001), Sicily (Troìa, 2005), and Apulia (Ernandes, 2011). In Sardinian Mediterranean temporary wet habitats, Bagella et al. (2011) reported the sympatric presence of individuals with 2 different combinations of mega- and microspores, suggesting the presence of 2 sibling species within *I. histrix*. The chromosome number of all these individuals was $2n = 20$.

Basing on the assumptions that i) Sardinia is the *locus classicus* of *Isoetes gymnocarpa* (Gennari, 1862), ii) the lectotype of this species was recently designated (Troia and Greuter, 2014), and iii) a single chromosome number (i.e. 2n = 20) but 2 different types of ornamentation were reported within the *I. histrix* species complex from Sardinia (Bagella et al., 2011) and chromosome number 2n = 22 was reported for other Mediterranean areas (Cesca and Peruzzi, 2001; Troìa, 2005; Ernandes, 2011), the aims of our research were: a) to clarify the application of the name *Cephaloceraton gymnocarpum*, b) to delimit the taxa within the *Isoetes histrix* species complex, and c) to provide a key for their identification.

2. Materials and methods

The application of the name *Cephaloceraton gymnocarpum* was clarified starting from the lectotypification proposed by Troia and Greuter (2014). Given the relevance of spore microoornamentation, SEM analysis was realized on material from the lectotype and on other specimens from different areas of the Mediterranean basin referred to as the *Isoetes histrix* species complex (see Appendix). In the same samples the presence/absence of spine-like teeth of phyllopodia and *velum* was also considered. Chromosome numbers were counted in material from the same areas following Bagella et al. (2011) and compared with data available in the literature.

Concatenating this information the taxa within the *Isoetes histrix* species complex were delimited and a key for their identification was provided.

3. Results

The presence of a complete *velum* reported for the *lectotypus* of *I. gymnocarpa* was confirmed by our analysis on abundant topotypical material collected in Caprera. Concerning the spine-like teeth of the phyllopodia, both

in type and topotypical material a large variability was observed, ranging from very short values (well below 3 mm) to ca. 10 mm. It was also possible to analyze with SEM the mega- and microspores' microornamentation from the lectotype and the other specimen of the sheet (Figure) and they all resulted in type F. The same result was obtained in all the material recently collected in Caprera. Further SEM analysis of macrospores from material from CLU, PI, and SASSA revealed the presence of specimens with type F macrospores from Elba island (Tuscan Archipelago) and continental Tuscany (Italy), Minorca (Baleares, Spain), and Corsica (France). In the same specimens, the fimbriate ornamentation in the microspores was not always observed and resulted hence in an inconstant character state.

Topotypical material of *Isoetes gymnocarpa* from Caprera Island resulted in $2n = 20$ chromosomes and the same result was obtained from many populations with F type spores from Sardinia, Corsica, and Minorca (Baleares) (Table).

4. Discussion

Troìa and Greuter (2014) state that the specimen designated as *lectotypus* of *Isoetes gymnocarpa* is different from the other specimen of the same herbarium sheet, basing this affirmation on the different length of the spine-like teeth of the phyllopodia. Nevertheless, the description of the specimen designated as *lectotypus* lacks any information concerning macrospore ornamentation.

While we showed here that spine-like teeth length is very variable in this species, both type specimens and all the analyzed topotypical material show mega- and microspores of type F (in the sense of Bagella et al., 2011). This kind of spores was never observed in the specimens from Calabria, S. Italy, studied by Cesca and Peruzzi (2001), and this was also confirmed here by a re-analysis of their materials in CLU. Hence the name *I. gymnocarpa* is applied to the plants with type F spores, irrespective of the length of spine-like teeth of the phyllopodia. These plants also consistently show $2n = 20$ chromosomes, a feature otherwise recorded only for *I. histrix* s.s. (see Introduction).

Isoetes gymnocarpa is an independent species, showing only a superficial phenotypic similarity with either *I. histrix* (if showing long teeth) or *I. subinermis* (if showing short teeth), but a completely different megaspore ornamentation and chromosome number with respect with the latter.

Comparing spore ornamentation and microornamentation and taking into account chromosome numbers, it is now possible to circumscribe 3 taxa within the *I. histrix* species complex:

I. histrix, $2n = 20$, spores of NF type. Originally described from Algeria (lectotype in MO, see Troìa and Greuter, 2014), but widespread in the Mediterranean. It should be emphasized that neither the spore microornamentation nor the chromosome number was analyzed in typical/topotypical material. This task will certainly need further studies.

I. gymnocarpa, $2n = 20$, spores of F type. Endemic to Sardinia, Minorca (Baleares), Corsica, and Central Italy (Tuscany). Often co-occurring with *I. histrix* in that area, we demonstrated here that this name applies to a different species in comparison with *I. sicula*, contrary to what was recently stated by Troìa and Greuter (2014).

I. sicula, $2n = 22$, spores of NF type. Originally described from Sicily (neotype in PAL designated by Troìa and Raimondo, 2014), but occurring elsewhere

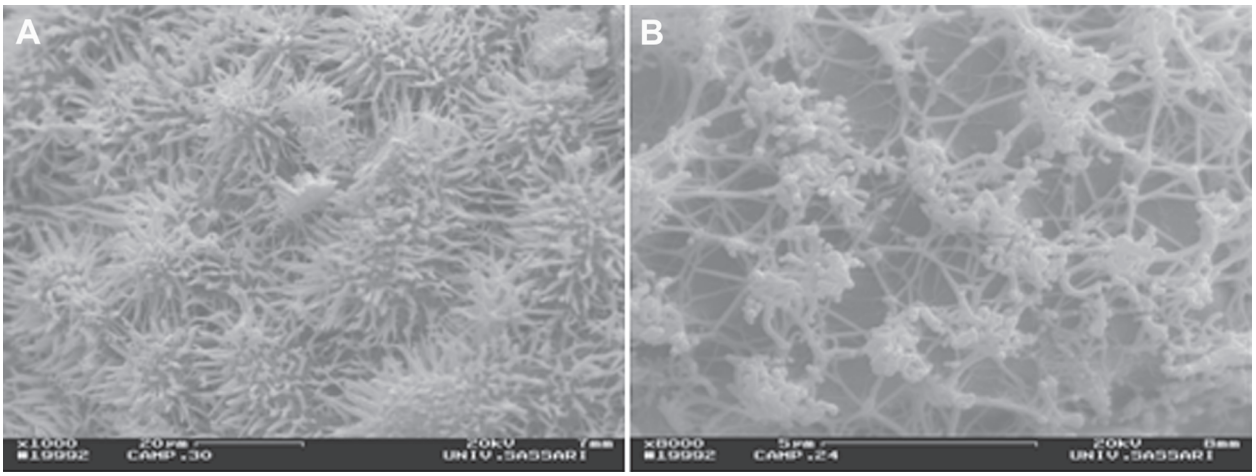

Figure. SEM photographs of microornamentation of megaspores (A) and microspores (B) from the lectotype of *Cephaloceraton gymnocarpum*.

Table. Chromosome numbers counted for each sampled locality of *Isoetes gymnocarpa*.

	Coordinates			
Locality	East	North	n. plates	2*n*
Sardinia (Paule Longa-Ghilarza)	8°50′22	40°02′27	10	20
Sardinia (Monte Rosso-Olmedo)	8°24′49	40°39′00	8	20
Sardinia (Vallicciola-Tempio)	9°09′18	40°51′06	7	20
Sardinia (Santa Maria-Bortigali)	8°47′43	40°21′21	10	20
Sardinia (Nuraghe Nuradeo-Suni)	8°34′07	40°20′31	8	20
Sardinia (Becco di Vela-Caprera)	9°28′19	41°13′01	10	20
Sardinia (Monte Minerva-Villanova Monteleone)	8°32′30	40°26′31	8	20
Sardinia (Pischina Ruja-Scano Montiferro)	8°41′08	40°13′19	10	20
Sardinia (Nuraghe Loelle-Buddusò)	9°19′01	40°34′05	10	20
Corsica (Capineru)	9°03′01	41°27′43	3	20
Corsica (Frasselli)	9°10′17	41°26′34	5	20
Minorca (S'Ermita - Marina de Son Morera-Ferreires)	3°52′23	40°03′05	3	20
Minorca (Ses planes de Son Arro-Ferreries)	4°03′02	39°59′09	2	20

in the Mediterranean, most seemingly in its central-eastern part. Its presence in Tuscany needs confirmation. Often co-occurring with *I. histrix*, from which it can be morphologically distinguished based on the absence of long (3–10 mm) lateral spine-like teeth in phyllopodia (Troìa and Greuter, 2014).

Key for the identification of the 3 species

1 Fimbriate megaspores ..... *Isoetes gymnocarpa*

1 Not fimbriate megaspores 2

2 Phyllopodia with 3–10-mm-long lateral spine-like teeth, 2n = 20 ..... *Isoetes histrix*

2 Phyllopodia with 0–3-mm-long lateral spine-like teeth, 2n = 22 ..... *Isoetes sicula*

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Appendix

Specimina visa

Isoetes gymnocarpa (Gennari) A.Braun

Paule Longa, Ghilarza (Oristano), Italia, pozze a mosaico con la macchia, 12.V.2010, M.C. Caria (SASSA); Monte Rosso, Olmedo (Sassari), Italia, fascia esterna di stagno temporaneo 11.V.2007, M.C. Caria (SASSA); Vallicciola, Tempio (Olbia-Tempio), Italia, zone umide temporaneamente inondate, 22.V.2012, M.C. Caria (SASSA); Santa Maria, Bortigali (Nuoro), Italia, zona umida temporaneamente inondata 05.V.2008, M.C. Caria (SASSA); Nuraghe Nuradeo, Suni (Nuoro), Italia, pozze a mosaico con la macchia, 30.V.2007, M.C. Caria (SASSA); Becco di Vela, Caprera (Olbia-Tempio), Italia, pozze a mosaico con la macchia, 05.V.2011, M.C. Caria (SASSA); Monte Minerva, Villanova Monteleone (Sassari), Italia, fascia esterna stagno temporaneo, 23.V.2011, M.C. Caria (SASSA); Pischina Ruja, Scano Montiferro (Nuoro), Italia, fascia esterna stagno temporaneo, 23.V.2007, M.C. Caria (SASSA); Nuraghe Loelle, Buddusò (Sassari), Italia, fascia esterna stagno temporaneo, 31.V.2007, M.C. Caria (SASSA); Capineru, Corsica, zona umida temporaneamente inondata, 2.IV.2012, M.C. Caria (SASSA); Frasselli, Corsica, fascia esterna stagno temporaneo, 3.IV.2012, M.C. Caria (SASSA); S'Ermita - Marina de Son Morera-Ferreires, Minorca, fascia esterna stagno temporaneo, 8.V.2009, *Legit*: S. Bagella, *Determinavit*: M.C. Caria (SASSA); Ses planes de Son Arro-Ferreries, Minorca, fascia esterna stagno temporaneo, 8.V.2009, *Legit*: S. Bagella, *Determinavit*: M.C. Caria (SASSA); Isola d'Elba (Livorno), presso la Serra (Buraccio), (UTM: 32T PN 12.35), pozzette effimere su quarzomonzoniti, 115 m., 6.IV.2008, *Legit*: A. Carta, *Determinavit*: L. Peruzzi, A. Carta sub *Isoetes histrix* Bory; M.C. Caria, 17.I.2014 (PI); Caprera, V.1961, P. Gennari sub *Cephaloceraton gymnocarpum* Genn. (TO, lectotype).

Isoetes histrix Bory

Paule Longa, Ghilarza (Oristano), Italia, pozze a mosaico con la macchia, 12.V.2010, M.C. Caria (SASSA); Monte Rosso, Olmedo (Sassari), Italia, fascia esterna di stagno temporaneo 11.V.2007, M.C. Caria (SASSA); Vallicciola, Tempio (Olbia-Tempio), Italia, zone umide temporaneamente inondate, 22.V.2012, M.C. Caria (SASSA); Santa Maria, Bortigali (NU), Italia, zona umida temporaneamente inondata 5.V.2008, M.C. Caria (SASSA); Nuraghe Nuradeo, Suni (NU), Italia, pozze a mosaico con la macchia, 30.V.2007, M.C. Caria (SASSA); Becco di Vela, Caprera (Olbia-Tempio), Italia, pozze a mosaico con la macchia, 5.V.2011, M.C. Caria (SASSA); Monte Minerva, Villanova Monteleone (Sassari), Italia, fascia esterna stagno temporaneo, 23.V.2011, M.C. Caria (SASSA); Pischina Ruja, Scano Montiferro (NU), Italia, fascia esterna stagno temporaneo, 23.V.2007, M.C. Caria (SASSA); Nuraghe Loelle, Buddusò (Sassari), Italia, fascia esterna stagno temporaneo, 31.V.2007, M.C. Caria (SASSA); Capineru, Corsica, zona umida temporaneamente inondata, 2.IV.2012, M.C. Caria (SASSA); Frasselli, Corsica, fascia esterna stagno temporaneo, 3.IV.2012, M.C. Caria (SASSA); S'Ermita - Marina de Son Morera-Ferreires, Minorca, fascia esterna stagno temporaneo, 8.V.2009, M.C. Caria (SASSA); Ses planes de Son Arro-Ferreries, Minorca, fascia esterna stagno temporaneo, 8.V.2009, M.C. Caria (SASSA); San Vincenzo (Livorno), Valle delle Rozze, versante meridionale Monte Montali (UTM: 32T PN 28.73), 87 m. s.l.m., pianori di un affioramento di vulcaniti (rioliti), 4.IV.2008, *Legit*: A. Carta, B. Pierini, *Determinavit*: L. Peruzzi, sub *Isoetes gymnocarpa* (Gennari) Braun; M.C. Caria, 14.I.2014 (PI); Isola d'Elba (Livorno), tra Cavoli e Castancoli (Campo nell'Elba), (UTM: 32T NN 97.33), 220 m, pratello umido su granito, 8.IV.2008, *Legit*: A. Carta, *Determinavit*: A. Carta, L. Peruzzi sub *Isoetes gymnocarpa* (Gennari) A.Braun; 17.I.2014 (PI).

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