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## ***Isoëtes* (Lycophytina, Isoetaceae) with terrestrial habitat in Calabria (Italy). New karyological and taxonomical data**

### **Abstract**

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Three species belonging to the genus *Isoëtes* with terrestrial habitat occur in Calabria: *I. duriei* Bory ( $2n = 55$ ), *I. histrix* Bory ( $2n = 20$ ), *I. subinermis* (Durieu) Cesca & Peruzzi stat. nov. ( $2n = 22$ ). Karyological and morphological data are presented and discussed. Both *I. duriei* (pentaploid) and *I. histrix* (aneuploid) are probably derived from *I. subinermis* (diploid): the first because of autopoliploidy, the latter because of aneuploidy. *I. subinermis* is a neglected taxon, described in 1861, that acquires new taxonomic meaning under the data presented in this paper.

### **Introduction**

Only 3 species of *Isoëtes* with terrestrial habitat (sect. *Terrestres* Bory) are traditionally identified in the whole Europe: *I. histrix* Bory, *I. duriei* Bory and *I. chaeturetii* Mendès (Greuter & al., 1984; Jermy & Akeroyd, 1993). These 3 species are characterized by the presence of persisting sclerified phyllopodia and by a seasonal biologic cycle, which is linked to the occasional water pools typically forming in autumn-winter throughout Mediterranean regions. They are distinguished by dark and hardened persistent foliar bases, which provide of thorn-making horns. Bibliography do not furnish much karyological data:  $2n = 20$  for *I. histrix* (Manton, 1950);  $2n = \text{ca.}104\text{--}134, 110$  for *I. duriei* (Troia & Bellini 2000; Giovannini & al., 2001 respectively). *I. chaeturetii* is an entity between *I. histrix* and *I. duriei*, and it was described in Portugal in 1961; its chromosomal number is still unknown.

Some wild populations of *Isoëtes* have been studied in Calabria, with the aim of contributing to the karyological knowledge of this particular group, scarcely studied until now.

## Materials and methods

Morphological data have been obtained from living plants, which were at first picked up in many different stations of Calabria, where all the taxa investigated grow spontaneously: Molicelle (Rende, Cosenza, 200 m a.s.l.); Salierni (Montalto Uffugo Scalo, Cosenza, 243 m a.s.l.); S. Antonello (Montalto Uffugo, Cosenza, 180 m a.s.l.); Cozzo d'Orlando (Montalto Uffugo, Cosenza, 250 m a.s.l.); Piano Monello (Rende, Cosenza, 265 m a.s.l.); Failla (Rende, Cosenza, 180 m a.s.l.); Pallega (Rende, Cosenza, 174 m a.s.l.); Piano di Maio (Rende, Cosenza, 215 m a.s.l.); Tarsia (along the road by the dam, Cosenza, on the lake shore, 60 m a.s.l.); Rivalico di Rovito (Cosenza, 500 m a.s.l.); S. Pietro in Guarano (Lake Arente, Cosenza, 70 m a.s.l.); Serra Castello (Corigliano Calabro, Cosenza, 70 m a.s.l.); Cortale (by the river Pesipe, Maida, Catanzaro); S. Stefano di Lappano (Cosenza); Botanic Garden of Calabria University (Arcavacata, Cosenza, 196 m a.s.l.).

Herbarium specimens of each taxon are deposited in CLU.

*Karyological data were obtained from: I. duriei*- S. Stefano di Lappano, Cosenza; *I. histrix*: Failla, Rende; Botanic Garden of Calabria University; *I. subinermis*: Failla, Rende; Botanic Garden of Calabria University.

We used root tips for the counting and the observation of chromosomes. Root tips were pre-treated with a 0.3% solution of colchicine and fixed in Carnoy; afterwards they were hydrolyzed in 1N HCl solution and coloured with fuchsin; at the end, they were squashed in a 45% solution of acetic acid.

Mega- and microspores were metallized and examined by S. E. M. (Philips 501B) at 7.5 and 2 KV, respectively. The terminology used for the spore descriptions follows Ferrarini & al. (1986).

## Results and Discussion

Both morphological and karyological results (Figs. 1-3) are summarized in Table 1.

The genus *Isoëtes* is marked by the basic chromosome number  $x = 11$ . The only uncorresponding datum in the whole scientific literature is a  $2n = 20$  for *I. histrix*, as described by Manton (1950). According to her work, it was possible to confirm the aneuploid status of *I. histrix*; aneuploid individuals often grow together with the polyploid (*I. duriei*) and the diploid (*I. subinermis*) ones in Calabria. Therefore, we refute the hypothesis (Löve & al., 1977; Ferrarini & al., 1986) according to which the correct chromosome number of this species was  $2n = 22$ , as reported by Prada (1979). Indeed,  $2n = 22$  must be referred to *I. subinermis*, a species morphologically similar to *I. histrix*. Our observations on the morphology of this taxon confirm those listed in literature but for one exception: microspores, often described as "spinulose", result tuberculate-echinulate. Leaves are deep green and arched-shaped.

*I. subinermis* is an entity described as a form of *I. histrix* by Durieu in 1861, and it was successively neglected in the later Floras (Pignatti, 1982; Zangheri, 1976 Jermy & Akeroyd, 1993). It isn't surprisingly even mentioned as an *I. histrix* synonym in Med-Checklist, which is usually very precise in listing eventual synonyms (Greuter & al., 1984). Fiori (1926) reports an *Isoëtes histrix*  $\beta$  *subinermis* Dur. for Nice, Maremma loc. Capalbio, Latium loc. Nettuno, Sicily, Sardinia, Maddalena's archipelago and Corsica. He

Table 1. Morphological and karyological features of the three taxa in question.

<i>I. histrix</i>	<i>I. subinermis</i>	<i>I. duriei</i>
phyllopodia with lateral 0,5-3 mm long prickles, always bigger than central excrescence (Figs. 1a, 1b, 1c)	phyllopodia with irregular lateral prickles, long at least 1 mm. Lateral prickles often trunked at the base; phyllopodia sometimes with a third central prickle bigger than the lateral ones (Figs. 1d, 1e, 1f)	phyllopodia shortly tridented
deep green, arched leaves 5-10 cm x 0,5-1 mm.	green, rigid, a little arched leaves 4-8 cm x 0,5-1 mm.	green, linear, not arched leaves 8-12 cm x 0,5-1 mm.
roundish, tuberculate megaspores 320-560 i, smooth equatorial ridge, joined with laesura's arms. (Figs. 2a, 2b, 2c)	slightly roundish megaspores 320-560 i, with well evident, cristate, equatorial ridge, joined with laesura's arms. Tuberculate-coniculate surface. (Figs. 2d, 2e, 2f)	lofo-reticulate megaspores 600-800 i, with lack of equatorial ridge (Figs. 2g, 2h).
brown, tuberculate-echinulate microspores. (Fig. 2m)	brown, echinulate microspores (Fig. 2n)	pale brown and verrucate microspores (Fig. 2i, 2l)
chromosome complement 2n = 20 (Figs. 3d, 3e, 3f)	chromosome complement 2n = 22 (Fig. 3c)	chromosome complement 2n = 55 (Figs. 3a, 3b)

distinguishes  $\beta$  from typus variety, affirming that the latter is typically showing “squame attornianti il bulbo poche e colle corna quasi nulle”. He indicates this taxon as synonymic with *Cephaloceraton gymnocarpum* Gennari (1862) and *Isoëtes sicula* Tod. (1866).

Lojacono Pojero (1908) indicates *I. sicula* Tod. for Sicily (“Terreni arenosi, umidi, Ficuzza al Lupo Cit! Tod! Cefalù al finale, Tumminia, Ogliaastro, Castelbuono in Herb. Pan.”); the author affirms it to be synonymic with *I. histrix* var. *subinermis*. Moreover, he states that it: “differt a praecedente [*I. histrix*] phyllopodiiis paucis, cornua breviora v. nulla”.

Jermey (1965) indicates for Turkey *I. histrix* var. *subinermis* Durieu. He distinguishes it from *I. histrix* for the presence of “verrucose” microspores, instead of “spinulose”, straight leaves and lack of prickles in the phyllopodia.

Our specimens of *I. subinermis* totally correspond to the literature descriptions (Jermey, 1965; Fiori, 1943). This species shows, above the distinctive character of phyllopodia, megaspores with a cristate and more evident equatorial ridge than those of *I. histrix*, and their surface is tuberculate-coniculate rather than tuberculate. For what concerns microspores, either *I. histrix* and *I. subinermis* show echinulate appendixes (more relevant in *I. subinermis*, in contrast to what Jermey indicates) on the surface. Leaves are light green, hard and almost straight.

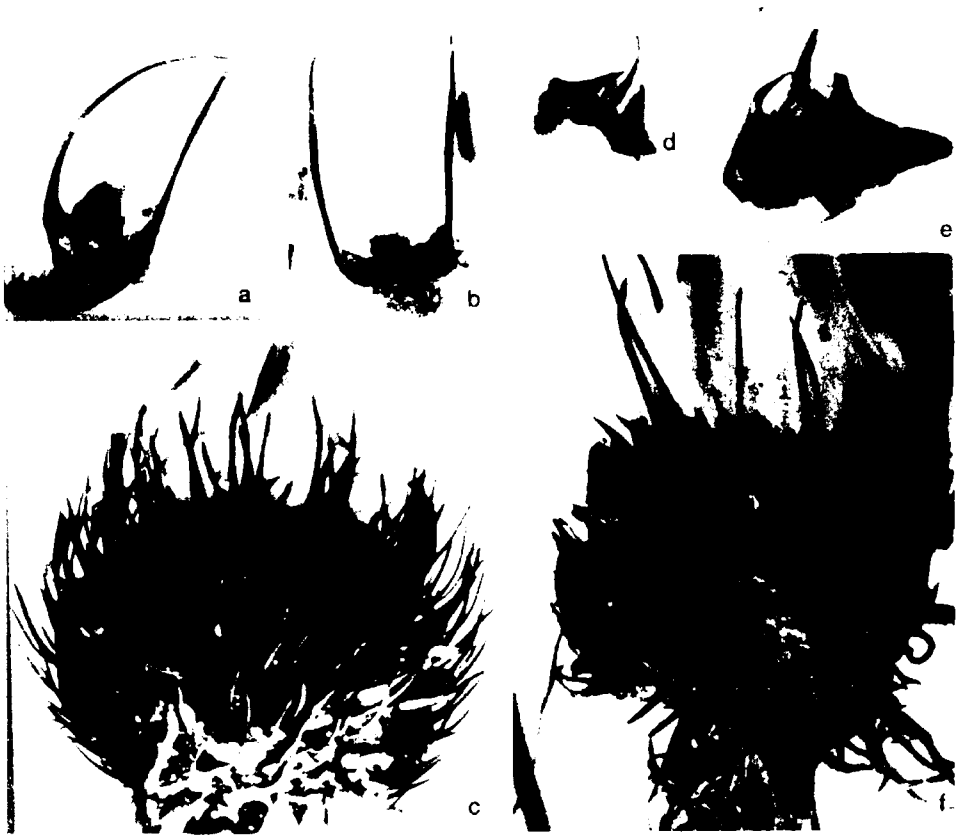


Fig. 1. *I. histrix*: phyllopodia (a, b), general view (c); *I. subinermis*: phyllopodia (d, e), general view (f).

*I. subinermis* is well distinguished from *I. histrix*, even by a karyological point of view: it shows a diploid chromosome complement  $2n = 22$ . Therefore, we deem it necessary to promote this taxon to a specific level.

*I. duriei* is characterized by the presence of lofo-reticulate megaspores, bigger than those of previously quoted species. Microspores are verrucose, phyllopodia are shortly tridentate. Under these accurate diagnostic features, it is not possible to muddle this species with others of terrestrial habitat. *I. duriei* was already studied by a karyological point of view; it shows a decaploid complement  $2n = 110$ , for Tuscan populations from Mount Pisano (Giovannini & al., 2001) and, for Sicilian populations, a complement  $2n = 104, 134$  ca., varying between decaploid and dodecaploid (Troia & Bellini, 2000). In contrast, our plants are characterized by a pentaploid ( $2n = 55$ ) complement: this result is new for this species.

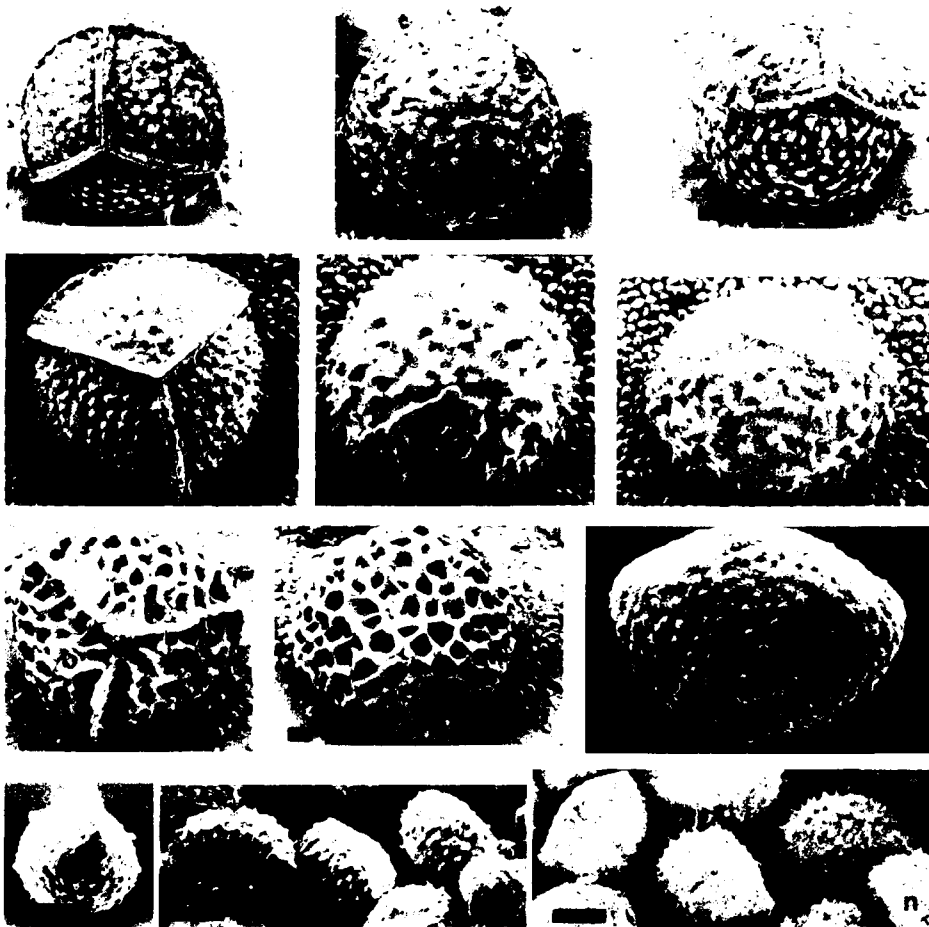


Fig. 2. *I. histrix*: megaspores (a, b, c), scale bars = 50  $\mu$ , microspores (m), scale bar = 10  $\mu$ ; *I. subinermis*: megaspores (d, e, f), scale bars = 150  $\mu$ , microspores (n), scale bar = 10  $\mu$ ; *I. duriei*: megaspores (g, h), scale bars = 50  $\mu$ , microspores (i, l), scale bars = 10  $\mu$ .

### Taxonomic Conspectus

*I. histrix* Bory, Compt. Rend. Hebd. Séances Acad. Sci. Paris 18: 1166. (1844)

Syn. (Greuter & al., 1984; Fiori, 1926, 1943): *I. delalandei* Lloyd, Notes Fl. Ouest France: 25 (1851); *Cephaloceraton hystrix* Gennari, Comm. Soc. Critt. It. 1 (3): 3 (1862); *I. histrix* var. *phrygia* Boiss., Fl. Or. 5: 748 (1884); *I. pseudohistrix* Levier, Barb. Fl. Sardoae Comp.: 74 (1885); *I. phrygia* Hausskn., Mitth. Thüring. Bot. Vereins 13: 72. (1899); *I. histrix* subsp. *delalandei* (Lloyd) P. Fourn., Quatre Fl. France: 6 (1934); *I. histrix* Durieu ex Bory var. *desquamata* A. Br. forma *longispina* (A. Br.) Fiori, Fl. It. Crypt. 5: 424-425 (1943).

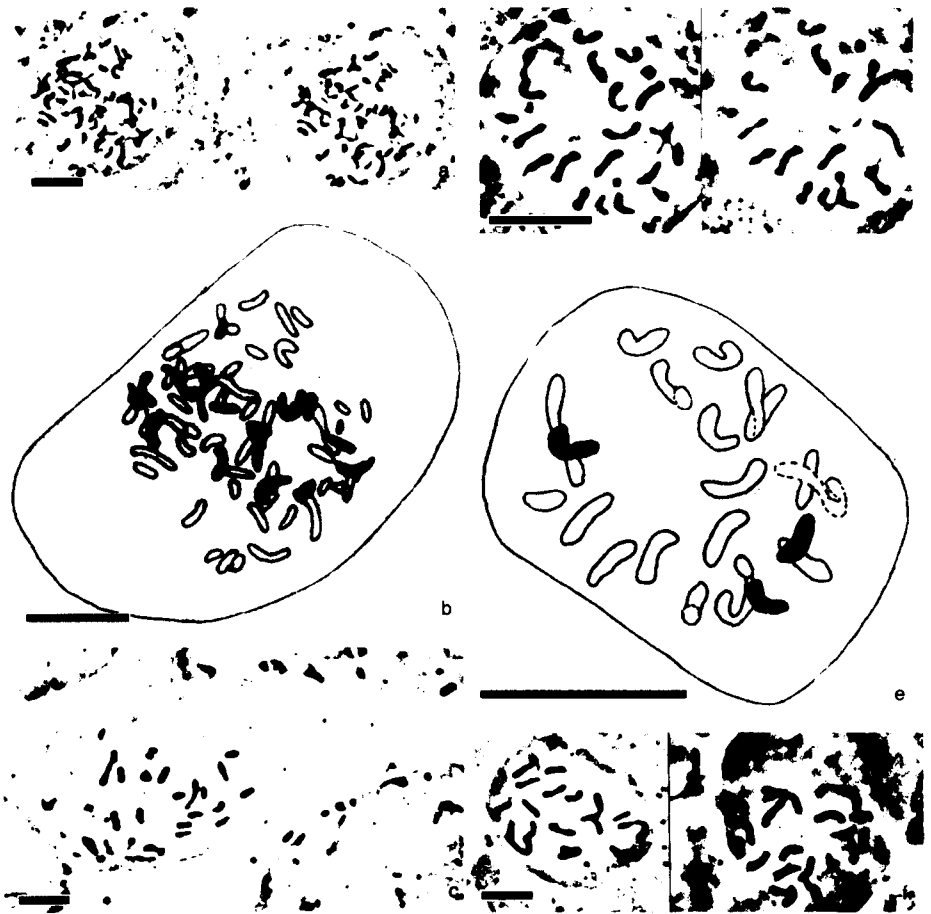


Fig. 3. *I. duriei*,  $2n = 55$ : one and the same metaphase plate viewed by two different focuses (a) and relative drawing (b); *I. histrix*,  $2n = 20$ : two metaphase plates (f), metaphase plate viewed by two different focuses (d) and relative drawing (e); *I. subinermis*,  $2n = 22$ : metaphase plate (c). Scale bars = 10  $\mu$ .

***I. subinermis* (Durieu) Cesca & Peruzzi, stat. nov.**

Basionym: *I. histrix* Bory forma *subinermis* Durieu, Bull. Soc. Bot. Fr. 8: 164 (1861).

Syn. (Fiori, 1926, 1943): *Cephaloceraton gymnocarpum* Gennari, Comm. Soc. Critt. It. 1 (3): 112 (1862); *I. sicula* Tod., Syn. Pl. Acot. Vasc. Sic.: 46 (1866); *I. histrix*  $\beta$  *subinermis* (Durieu) Fiori, Nuova Flora Analitica d'Italia 1: 44 (1926); *I. histrix* subsp. *sicula* (Tod.) P. Fourn., Quatre Fl. France: 6. (1934); *I. histrix* Durieu ex Bory var. *desquamata* A. Br. forma *subinermis* (Durieu) Fiori, Fl. It. Crypt. 5: 424-426 (1943).

***I. duriei* Bory**, Compt. Rend. Hebd. Séances Acad. Sci. Paris 18: 1166. (1844).

## Conclusions

Three species of *Isoëtes* with terrestrial habitat are present in Italy: *I. histrix*, *I. subinermis*, *I. duriei*. These 3 taxa can be easily distinguished by their morphology and karyology. *I. subinermis* is a species characterized by a diploid chromosomal number ( $2n = 22$ ); the two other species are very probably derived from *I. subinermis*: *I. histrix* through aneuploidy ( $2n = 20$ ) and *I. duriei* through autopolyploidy ( $2n = 55, 110, 121ca.$ ) phenomena.

According to Taylor & Hickey (1992), most of terrestrial species of genus *Isoëtes* would have developed through gradual speciation events due to geographical isolation of ancestral populations. We confute this speciation hypothesis, as it was previously supposed by Troia & Bellini (2000): it seems that genomic mutations have had a leading role, also considering that the 3 entities often cohabit in Calabria.

It would be interesting in the future to study karyologically *I. chauretii* with the aim of clarify its phylogenetic relations with the other 3 species.

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