Pinguicula crystallina Sibth. et Smith subsp. *hirtiflora* (Ten.) Strid (Lentibulariaceae) in Calabria (Southern Italy). Cytotaxonomical study and *ex situ* conservation in the Botanic Garden of Calabria University

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Introduction

According to Casper (1966), *Pinguicula hirtiflora* Ten. (Lentibulariaceae) belongs to the subgenus *Isoloba* Barnhart, section *Cardiophyllum* Casper. It was originally described from plants found in the area of Castellammare di Stabia (Salerno, Southern Italy; Tenore, 1811).

Plants corresponding to this original description occur in Italy in Campania and Calabria (Casper, 1962; Casper, 1970; Pignatti, 1982; Moraldo *et al.*, 1986; Pinto *et al.*, 2000); the citation with doubts (Casper, 1962) for plants in Abruzzi (Mount Pelone) were later shown to be *P. vulgaris* L. (Tammaro & Pace, 1987). *Pinguicula hirtiflora* populations are found at locations from southeastern Italy to the southwestern part of the Balkan Peninsula (Casper, 1966). Moreover this unit was recently reported, with several doubts on its spontaneity, for France in the Roya river Valley (Joly et al., 2002).

It is the only Italian *Pinguicula* which has a tropical growth type (Casper, 1962; Casper, 1966; Legendre, 2000), i.e. it shows an overwintering basal rosette that makes it interpretable as a Thermophilous relic. In contrast, other *Pinguicula* species from Italy overwinter as buds (hibernacula), and they are all microtherm elements. *Pinguicula hirtiflora* has recently been reduced to a subspecies (Strid, 1991) of *P. crystallina* Sibth. et Smith, this latter taxon being a vicarious oriental unit that occurs from Cyprus to Anatolia (Casper, 1970; Greuter *et al.*, 1989).

Pinguicula hirtiflora is very rare and localized in Italy, justifying its IUCN listing as "Vulnerable" (VU; Conti *et al.*, 1997). In Calabria, where it is known from only one stand, it is assigned to the category "Critically Endangered" (CR). These plants are at approximately 200 m a.s.l. on stillicidious calcareous cliffs along the Celadi stream. The stand is subject to great risk because it is near a road, and the population is only a few hundred individuals in an area of around 50×10 meters. Recent road maintenance has destroyed part of the population. We have therefore placed plants into cultivation at the Botanical garden of the University of Calabria, with the goal of preserving the plant *ex situ*. The presence of *P. hirtiflora* in the Botanical Garden has allowed us to conduct a karyological study that has given interesting results.

Material and methods

Approximately 20 plants were collected in October 2000 and placed into greenhouse cultivation. The microhabitat built for them consists of a calcareous, friable substratum, over which water continuously drips. Plants that occur with *P. hirtiflora* (liverworts, Marcantiophyta; ferns, *Adiantum capillus-veneris* L.) are grown on the substratum so as to reproduce the conditions of the natural habitat as accurately as possible (see Figure 3).

Pinguicula hirtiflora herbarium specimens in Florence (FI), Naples (NAP), and Rome (RO) were examined in order to make morphological and chorological observations and comparisons to the plants from Calabria.

Specimina visa - Italy, Campania: Locis arenosis "aqua santa" in regione superiore M. S. Angelo prope Castellammare regionis neapolitanese, 3/VI/1874, *Henker* (FI); rupi umide all'Acquasanta di Monte Sant'Angelo di Stabbia, 22/VIII/1891, *Martelli* (FI); Amalfi: Valle delle Ferriere, 31/V/1952, *Moggi* (FI); ad fontium stillicidis Acqua Santa M.te S. Angelo di Castellammare, s.d., *Tenore* (FI); Castellammare Acqua Santa, IX/1843, *Avellino* (FI); adorna bellamente le stalattiti della Grotta Bonné, 10/V/1862, *Pasquale* (FI); Ad rupes humidas Aqua S.ta montis S. Angelo supra Castellammare, 2/VIII/1855, *Huet du Pavillon* (FI); Grotta dei Porci ossia Grotta dell'Acquasanta M. S. Angelo di Castellammare ossia dei Tre Pizzi 1200 m ca., 22/VIII/1891, *Sommier* (FI); M. S. Angelo di Castellammare rupi stillanti all'acquasanta 1400 m ca., VII/1911, *Guadagno* (FI); M. S. Angelo di Castellammare. Rupi calcaree acquitrinose 1250-1800 m, 27/VI/1909, *Pellanda* (FI); Vietri a mare (Salerno) nuova stazione sul livello del mare scoperta da Hoffman, su rocce calcaree del Trias stillanti, 4/X/1954, *Alexander* (FI); Napoli, 1943, *Alexander* (FI); in paludosi Amalfi, 1876, *Lacaita* (FI); Faggeti in uno stillicidio all'Acqua Santa circa 1300 m presso Vico equense (M. S. Angelo napoletano), 24/VIII/1891, *Biondi* (FI); Ravello (Salerno) calcare 350-400 m, 28-29/VII/1921, *Fiori* (FI); Valle della Ferriera (Amalfi), 31/V/1952, *Corradi, Bavazzano et Contardo* (FI); Castellammare, VI, *s.l.* (RO); Montibus Stabiarum, s.d., *s.l.* (RO); Monti Stabiani, VI/1879, *Pasquale* (RO); Sulla roccia per la quale gronda l'acqua così detta Santa alla base del pianalto primo di S. Michele a Castellammare, 1883, *Pedicino* (RO); In unico loco prope Neapolim all'Acqua Santa M. S. Angelo a Castellammare, s.d., *Pasquale* (RO); A aquae stillicidiis Acqua Santa supra M.te S. Angelo, 3/VII/1877, *Pasquale* (RO); All'Acqua Santa sul M.te S. Angelo di Castellammare, s.d., *Tenore* (RO); Monte S. Angelo di Castellammare, VII/1895, *Honig* (RO); Amalfi, s.d., *s.l.* (RO, sub *Pinguicula vulgaris*); Grotta di Bonnée, sotto la Trinità, VI/1864, *s.l.* (RO); Grotta di Bonnée a Cava, VI/1868, *Pedicino* (RO); Grotta di Bonnée sotto la Trinità di Cava ai Mulini, VI/1864, *Pedicino* (RO); Avvocatella sopra Cava, e presso il Mulino della Trinità, s.d., *Tenore* (NAP); Castellammare, s.d., *Tenore* (NAP); Calabria: Presso Rossano, Torrente Celadi, alt. ca. 200 m s.l.m., 20/IV/1979, *Del Prete, Garbari, Cesca* (CLU); Greece: In valle laterali sinistra fiuminis Achellòos vico Petrotòn 2 Km orientem versus (Grecia, Thessalia, Nomos et Eparchia karditsis) alt. 500 m. s.l.m., in rupibus schistosis scaturiginosis Adianto capillus veneris admixto, 19/IX/1980, *Binder, Hagemann, Hempel et Raus* (FI); In Monte Parnasso in scaturiginis reg. abietinae, VIII/1852, *Heldreich* (FI); In saxosis irriguis reg. super. M. Olympus Thessaliae, 21/VII/1857, *Heldreich* (FI); Karistae greola nächst Vucia (Flora for Hercegovina), 2/VI/1872, *Pantocsek* (FI, sub *Pinguicula laeta* mihi); Olympus Thessalia ad scaturigines prope Hogios Dionyous, 12/IX/1910, *Halàcsy* (FI); In Monte Korax Aetoliae objectae. In regione abietina superiori alt. 5500 fts., 24/VII/1879, *Heldreich* (FI); 5500 fts., west of Katara, north-facing slope in dense shade of beech-trees sopping-wet bank of stony alluvium and leaf mould by streams and with water percolating through it; exquisite lavender flowers; 3-4 m., 6/VIII/1964, *Archibald* (FI); Habitat in regione superiori montes Chelmos Peloponesi (Apzanua Opu) prope Stygem alt. 6000 fts., 6/VII/1851, *Orphanides* (FI, sub *Pinguicula megaspilaea* Boiss. et Orphanides).

For the karyological study, root tips were treated with a 0.3% solution of colchicine and fixed in Carnoy; afterwards they were hydrolyzed in 1N HCl solution and coloured with fuchsine; finally, they were squeezed in acetic orcein for counting and observation of chromosomes. In some cases we used phase contrast. At least five plants were used in order to establish the chromosome number.

Systematic study

Pinguicula crystallina Sibth. et Smith subsp. *hirtiflora* (Ten.) Strid, in Strid & Tan, Mountain Flora of Greece 2: 276 (1991).

Basionym: *Pinguicula hirtiflora* Ten., Fl. Napol. 1: 6 (1811).

Lectotypus (here designated, Figure 1): M.te della Cava di Castellammare, all'acqua Santa, s.d., *Tenore* (NAP!, erroneously designated as the holotype by Casper, 1966); other original material: Avvocatella sopra Cava, e presso il Mulino della Trinità, s.d., *Tenore* (NAP); Castellammare, s.d., *Tenore* (NAP); ad fontium stillicidis Acqua Santa M.te S. Angelo di Castellammare, s.d., *Tenore* (FI); All'Acqua Santa sul M.te S. Angelo di Castellammare, s.d., *Tenore* (RO).

Synonyms (Casper, 1966; Strid, 1991): *P. vulgaris* Petagna non L., Inst. Bot. 2: 38 (1787), nom. illeg., *P. albanica* Griseb., Spicil. Fl. Rumel. 2: 9 (1844); *P. laeta* Pant., Österr. Bot. Z. 23: 80 (1873); *P. megaspilaea* Boiss. et Orphanides in Boiss., Fl. Or. 4: 2 (1875), nom. inval.; *P. hirtiflora* Ten. var. *megaspilaea* (Boiss. et Heldr.) Schindler, Österr. Bot. Z. 58: 62 (1908); *P. vulgaris* L. var. *hirtiflora*

Figure 1. Lectotype of *Pinguicula hirtiflora* Ten., conserved in NAP.

(Ten.) Fiori, N. Fl. An. Ital. 2: 472 (1925); *P. louisii* Markgraf, Ber. Deutsch. Bot. Ges. 44: 430 (1926); *P. hirtiflora* Ten. var. *decipiens* Bornmüller, Ungar. Bot. Bl. 32: 129 (1933); *P. hirtiflora* Ten. var. *euboea* Beauverd et Topali, Bull. Soc. Bot. Genève sér. 2, 28: 155 (1938); *P. hirtiflora* Ten. var. *louisii* (Markgraf) Ernst, Bot. Jahrb. Syst. 80: 186 (1961).

In addition to the taxa listed above, we believe the following to by synonymic: *P. hirtiflora* Ten. f. *pallida* Casper, Biblioth. Bot. 127/128: 107 (1966); *P. hirtiflora* Ten. var. *gionae* Contandriopoulos et Quezel, Rev. Biol. Ecol. Medit. 1: 30 (1974).

The studied Calabrian plants have a peculiar chromosome number, $2n = 27$ (Figure 2), a number that was also reported for this unit in material from Thessalia, Greece (Strid & Franzen, 1981). For plants collected in the same Calabrian locality of our ones, the chromosome complement $2n = 28$ (Mikeladze & Casper, 1997) was already reported. Populations from the locus classicus in Campania have diploid chromosome complements $2n = 16$ (Honsell, 1959). Contandriopoulos & Quezel (1974) reported $2n = 16, 24, 32, 48$ for this unit in Greek material. They tried to correlate the ploidy level with some minor morphological differences, and identified four varieties for *P. hirtiflora*. We think the chromosome numbers $2n = 27, 28$ to be strictly correlated to triploids $2n = 24$ by ascending aneuploidy phenomena (or perhaps occurrence of B chromosomes, although it is very difficult to establish it for the extreme homogeneity of the chromosome size). A situation, in our opinion, to be interpreted in a similar way, occurs in *P. crystallina* subsp. *crystallina*: Contandriopoulos & Quezel (1974) counted $2n = 24$ chromosomes in material from Turkey, Mikeladze & Casper (1997) counted $2n = 28$ chromosomes in plants from Cyprus (where the species was described).

We do not pretend, in this work, to give an exhaustive survey of the variability of *P. crystallina* subsp. *hirtiflora*, since we have not been able to examine specimens from some places (i. e. Albania); however our plants are fully identifiable with typus material, and the quantitative differences observed, between Greek and Italian material are weak (Table 1); thus, to the actual state of knowledge, in our opinion the differences (both morphological and karyological) do not allow us to distinguish (for nomenclatural purposes) taxonomic categories within *P. crystallina* subsp. *hirtiflora*; although under this binomial probably more than one taxa are united.

Table 1. Morphological and karyological features of *Pinguicula crystallina* Sibth. et Smith subsp. *hirtiflora* (Ten.) Strid from different provenances. Data come from bibliography, herbarium specimens cited in the text and the present study. Values are in 10-90 percentiles with extreme values in brackets.

	Italy, Calabria	Italy, Campania	Greece
length of leaves	2-4 cm	2-4(6) cm	(3)5-6 cm
width of leaves	10-15 mm	10-18 mm	15-25 mm
length of scape	7-10 cm	(3)4-7(10) cm	10-12 cm
number of scapes	1-3	(1)2-4(7)	1-4
length of corolla	13-14 mm	10(15) mm	10-15 mm
length of upper lobes	5 mm	(4)5-6(7) mm	4-5 mm
length of lower lobes	8 mm	(4)7-8(12) mm	6 mm
length of spur	8-9 mm	6(10) mm	10-15 mm
length of calyx	3 mm	2-3(4) mm	3-4 mm
chromosome number	$2n$=27[a], 28[b]	$2n$=16[c]	$2n$=16, 24, 27, 32, 48[d]

[a]See Figure 2.
[b]Mikeladze & Casper, 1997.
[c]Honsell, 1959.
[d]Contandriopoulos & Quezel, 1974; Strid & Franzen, 1981.

Conservation *ex situ*

After almost two years the plants have fully adapted to artificial cultivation. Flowers are produced plentifully April-July (Figures 4,5), resulting in seedlings (Figure 6). Vegetative reproduction has not

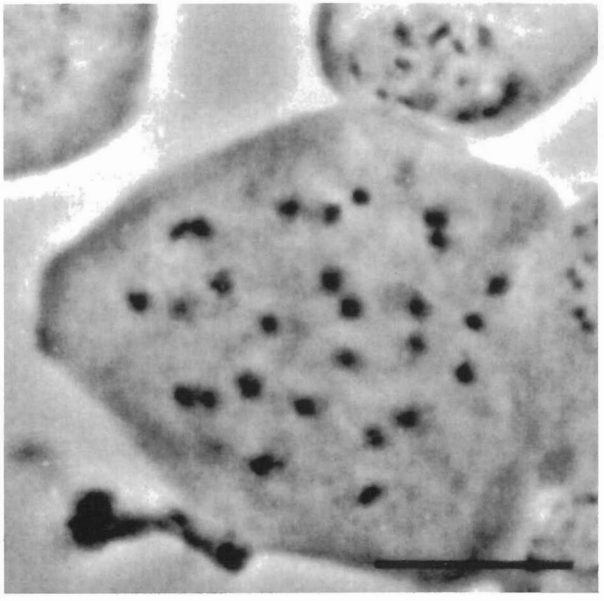

Figure 2. Metaphasic plate in root tip of *P. crystallina* subsp. *hirtiflora* from Rossano (Calabria, Southern Italy), showing $2n = 27$ chromosomes. Scale bar = 5μm

been observed for this plant. The reproduction by seed is unexpected because of the anomalous disploid chromosome number, so we think that the phenomenon of apomixy might be occurring. Further studies about embryology and biology of the reproduction of these plants are planned.

Conclusions

This study confirms the necessity to preserve the *P. crystallina* subsp. *hirtiflora* population from Rossano (Calabria, southern Italy) because it is the only Italian population which show $2n=27$, a cytological peculiarity known otherwise only from plants in Greece. The small habitat recreated in the Botanical Garden of Calabria University contributes to this aim and has also become a major point for guided visits, where the school students and visitors are spellbound and interested from this delicate and aesthetically attractive carnivorous plant.

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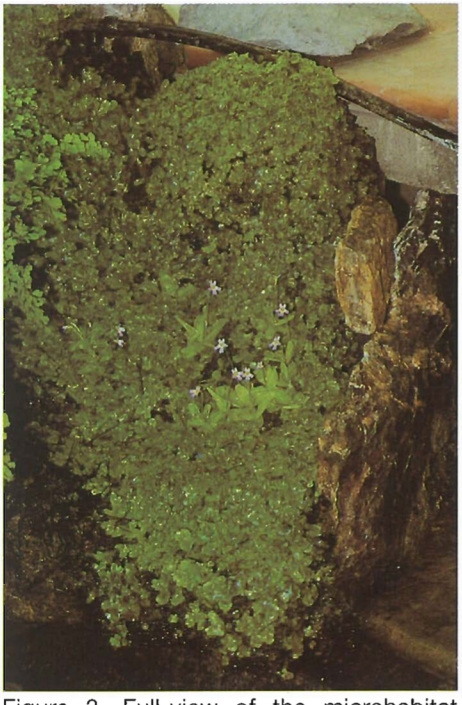

Figure 3. Full-view of the microhabitat where *P. crystallina* subsp. *hirtiflora* is cultivated in the Botanical Garden of Calabria University.

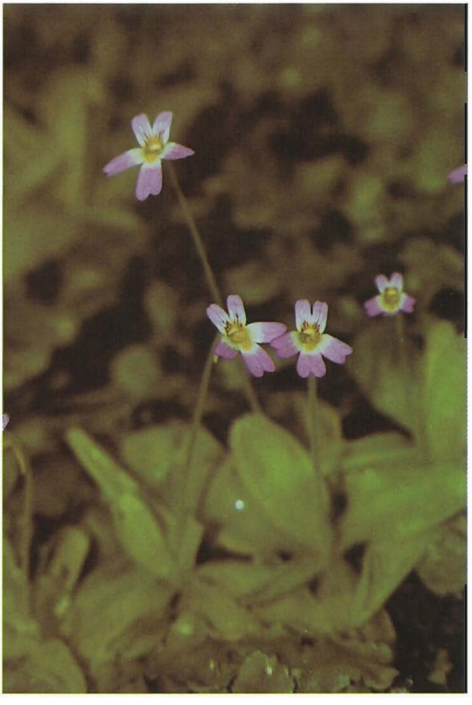

Figure 4. *P. crystallina* subsp. *hirtiflora* from Botanical Garden of Calabria University in full anthesis, general view.

Figure 5. *P. crystallina* subsp. *hirtiflora* from Botanical Garden of Calabria University in full anthesis, particular of the flower.

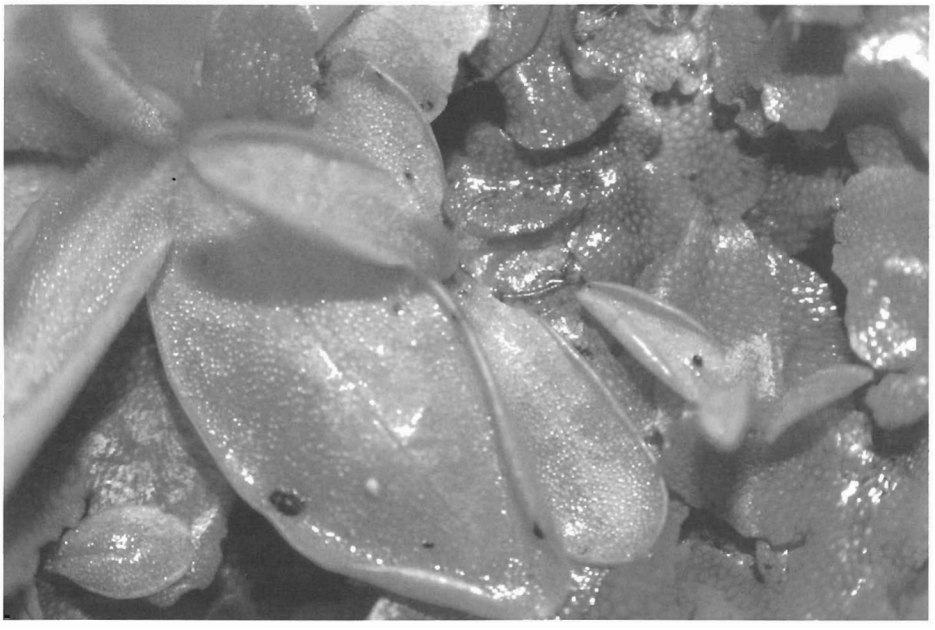

Figure 6. Seedlings of *P. crystallina* subsp. *hirtiflora* germinating in the Botanical Garden of Calabria University.

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