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Heterostyly in *Goniolimon italicum* (Plumbaginaceae), endemic to Abruzzo (central Apennines, Italy)

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Abstract

Morretti, F., Puppi, G., Giuliani, C. & Conti, F. 2015. Heterostyly in *Goniolimon italicum* (Plumbaginaceae), endemic to Abruzzo (central Apennines, Italy). *Anales Jard. Bot. Madrid* 72(1): e014

Goniolimon italicum is an endemic species to central Apennines (Italy). Here we provide the first report of heterostyly in this species. Two morphological types were identified: an S-morph with stamen filaments longer than gynoecia, stigmas with a papillate surface and finely reticulated pollen grains with very small spinules (Type B); and an L-morph with stamen filaments shorter than gynoecia, stigmas with a cob pattern and coarsely reticulated pollen grains with small spinules (Type A). Two new locations of *G. italicum* were found and are here reported.

Key words: *Goniolimon*, heterostyly, Apennine, Italy

Resumen

Morretti, F., Puppi, G., Giuliani, C. & Conti, F. 2015. Heterostilia en *Goniolimon italicum* (Plumbaginaceae), un endemismo de los Abruzzos (Apeninos centrales, Italia). *Anales Jard. Bot. Madrid* 72(1): e014

Goniolimon italicum es una especie endémica del centro de los Apeninos (Italia) de la cual se da a conocer por primera vez la existencia de heterostilia. Se reconocen dos tipos mofológicos: el morfo S, con filamentos estaminales más largos que el gineceo, estigmas papilosos y granos de polen finamente reticulados con espínulas muy pequeñas (Type B); y el morfo L con filamentos estaminales más cortos que elgineceo, estigmas tipo mazorca ("cob") y granos de polen provistos de retículos laxos con espínulas pequeñas (Type A). Se aporta, además, el hallazgo de *G. italicum* en dos nuevas localidades.

Palabras clave: *Goniolimon*, heterostilia, Apeninos, Italia

INTRODUCTION

Goniolimon italicum Tammaro, Frizzi et Pignatti (Plumbaginaceae) is an endemic species of Abruzzo (Tammaro & al., 1982; Peruzzi & al., 2014). Species of the genus *Goniolimon* Boiss. are distributed from Central Asia, which is the center of diversity, to the Balkan Peninsula, where six species have been recorded. *Goniolimon tataricum* (L.) Boiss., occurs from Asia to the Balkan Peninsula and reaches the western boundary of the genus range in Tunisia and Algeria (Greuter & al., 1989; Domina, 2011).

G. italicum marks the western European boundary of the genus in central Italy (Abruzzo) and it is the only one species of this genus occurring in Italy. A description of the species *G. italicum* can be found in Tammaro & al. (1982), Frizzi (1986) and Conti & al. (2008). It occurs in L'Aquila's internal basins in dry grasslands with abundant limestone outcrops from 350 m up to 900 m asl. Eight locations have been recorded with certainty: Fossa Raganesca (Ocre), between Fossa Raganesca and S. Felice d'Ocre, Forca del Casale (Ofena), Capestrano (Collelungo and Poggio della Cisterna), St. Silvestro (Ofena), Le Pagliare (Ofena), Colle St. Eugenia (Navelli). Only one of them, which happens to be the smallest (5 individuals), near "Le Pagliare", is included in the National Park of Gran Sasso and Laga Mountains area (Conti & al., 2008). Among these locations, two represent new reports. One of the authors (FC) recently found a new population of 10 individuals within the National Park area, close to Forca del Casale (Ofena) and another previously unknown was found between Fossa Raganesca and S. Felice d'Ocre (100 individuals) by F. Bartolucci.

In Fossa Raganesca *G. italicum* occurs in pastures with rendzina soils of the *Asperulo purpureae - Brometum erecti* subass. *centauretosum ambigui*, where together with *Centaurea ambigua* it is a differential species (Frattaroli, 1988). In the Basin of Capestrano it occurs in the *Phleion ambigui-Bromion erecti* grasslands*: Globulario meridionalis-Stipetum capillatae* and *Lino tommasinii-Stipetum apenninicolae* (Pirone & al., 2001).

L'Aquila's internal basins are characterized by their drought (this is the driest area of Abruzzo: the average annual rainfall in Capestrano is about 550 mm) and marked temperature ranges. At local scales, *G. italicum* occurs mostly on slopes near the plains, where the climatic conditions and the presence of rock outcrops limit the development of tree cover. The ancient human presence at several sites (archaeological area of Capestrano, St. Silvestro, Colle St. Eugenia, Le Pagliare) may have favoured the grassland spread, thus facilitating the occurrence of *Goniolimon*.

In the red data book of Italy it was considered vulnerable (VU) (Conti & al., 1992, 1997) and more recently, according the IUCN criteria (2001), endangered (Conti & al., 2008).

Heterostylous plants are usually characterized by the presence of two morphs that differ in the position of their stigmas and anthers, reciprocally. This reciprocal herkogamy is sometimes associated with a heteromorphic incompatibility system that prevents from selfing and fertilizations among plants of the same morph. Moreover, heterostylous species

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may also present differences in ancillary characters between the morphs, which are mostly related to pollen and stigma features (Dulberger, 1992).

In the Plumbaginaceae heterostyly and the heteromorphic incompatibility system were extensively studied by Baker (1966) and afterwards several works have been carried out in several species of this family (e.g., Ferrero & al., 2009). According to Baker, the ultimate expression of evolution of these systems is represented by *Limonium vulgare*, which presents reciprocal herkogamy and pollen and stigma dimorphism. In this species it is conceivable that, according to the reactions of heteromorphic incompatibility system typical of Plumbaginaceae, pollen of type A manage to complete the fertilization only when placed on papillate stigmas, while pollen of type B only do when in contact with cob stigmas.

Plumbaginaceae family appears to present a wide variability in style morphology and compatibility systems; therefore, description of members of this family can be very useful for future studies dealing with evolutionary questions. For this reason, here we provide the first report of heterostyly in *G. italicum* and describe some characteristics of the pollen and stigmas on both morphs.

MATERIAL AND METHODS

The collection of samples was performed in August 2011 on three populations: Fossa Raganesca (*locus classicus*) (13 individuals), Conca di Capestrano (26 individuals) and Barisciano in the botanical garden of Floristic Research Center of the Apennine (8 individuals). The low number of individuals sampled is due to the fact that it is a very rare species of which only c. 400 individuals are known and not all are flowering the same year.

For each individual the following parameters were measured (1 flower per plant): calyx length, corolla length, one filament length, one stigma height (i.e. distance from base of ovary to tip of stigma), pollen size (polar and equatorial diameters). We chose to measure the length of the single filament because the stamen length is highly variable depending on the horizontal or vertical placement of the anther. Pollen size was measured under light microscope on 186 pollen grains from 46 individuals. Stigma surface and pollen grain micromophology were analyzed using Scanning Electron Microscope (SEM).

RESULTS AND DISCUSSION

The analysis of flower samples permitted the identification of two morphological types (Fig. 1, Table 1). They were designated S-morph and L-morph (Fig. 1). At the Fossa Raganesca population 4 individuals of S-morph and 9 individuals of L-morph were recorded; at the Conca di Capestrano population 11 individuals of S-morph and 15 individuals of L-morph were recorded; at Barisciano Botanical Garden 5 individuals of S-morph and 3 individuals of L-morph were recorded.

The two types differ in gynoecium length and stigma type, stamen filament length and pollen type. S-morph individuals have an average gynoecium length of 6.1 mm and filament length of 7.0 mm, whereas L-morph individuals have an average gynoecium length of 7.6 mm and filament length of 6.2 mm (Fig. 2). Only one individual had gynoecium and filament of the same length.

The pollen grains are oval, subprolate (polar diameter/equatorial diameter 1.14-1.33), tricolpate, reticulate, and two main types were observed (Fig. 3):

– Type B (sensu Baker, 1966), occurring in the S-morph, presents fine meshes (mesh size of about 1 µm in diameter) and very small spinules (about 0.3 µm) (Fig. 3a).
– Type A (sensu Baker, 1966), occurring in the L-morph presents large meshes (mesh size of about 7 µm in diameter) and small spinules (spinule length about 0.7 µm) (Fig. 3b). The stigmas are capitate in both morphs: S-morph shows a papillate surface (Fig. 3c) whereas L-morph presents a "cob" surface (Fig. 3d).

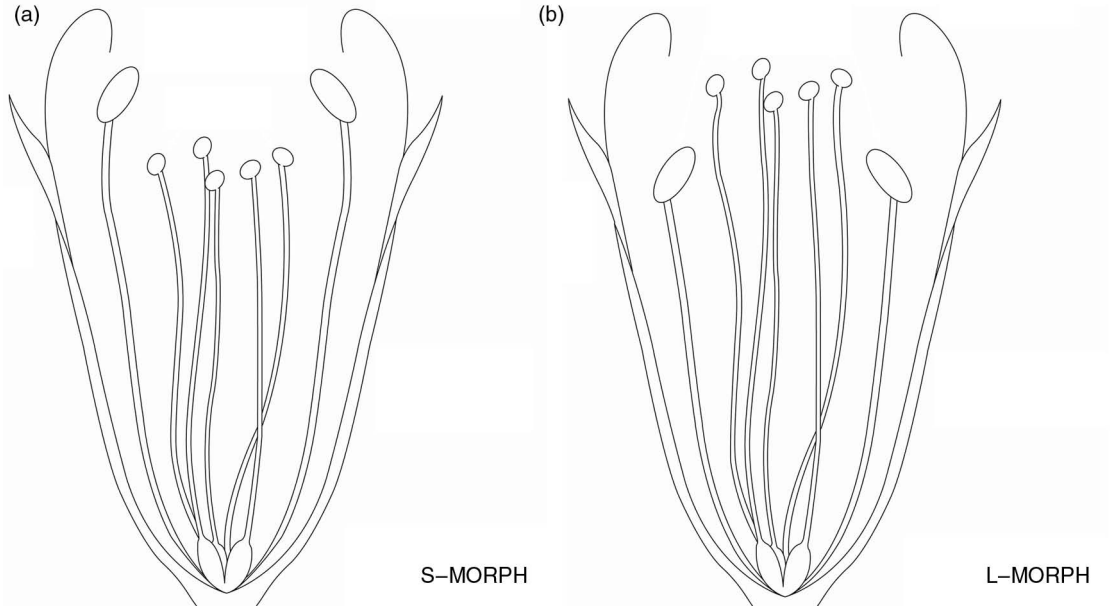

Fig. 1. S-morph and L-morph flowers of *Goniolimon italicum*.

Table 1. Floral characters of *Goniolimon italicum* measured in morphotypes S and L (means, standard deviations and range of values for each morphotype are reported. The last two columns show the results of a t-test comparing the means of those characters in the two morphotypes. Differences in "Filament length", "Stigma height" and "Filament l.-Stigma h." are all highly significant

Type of flowers	**Type S**	**Type L**	
Number of plants	20	27	*t*-test
Calyx length (mm)	7.23±0.53 (6.0;8.1)	7.03±0.41 (6.1;7.8)	0.20
Corolla length (mm)	8.02±0.38 (7.2;8.6)	7.84±0.41 (7.2;8.7)	0.17
Filament length	6.98±0.40 (6.1;8.0)	6.16±0.56 (4,4;7.0)	0.81**
Stigma height (mm)	6.09±0.50 (5.2;7.2)	7.63±0.49 (5.9;8.3)	–1.55**
Filament l.-Stigma h. difference	0.89±0.43	–1.47±0.41	2.36**
number of pollen grains	80	106	
Pollen size: polar axis (µm)	54.34±3.75	54.60±3.64	–0.25
Pollen size: equatorial diameter (µm)	41.47±3.70	42.10±3,47	–0.63
Pollen shape (polar/eq.diam.)	1.32±0.10	1.30±0.07	–0.01

**$p<0.001$.

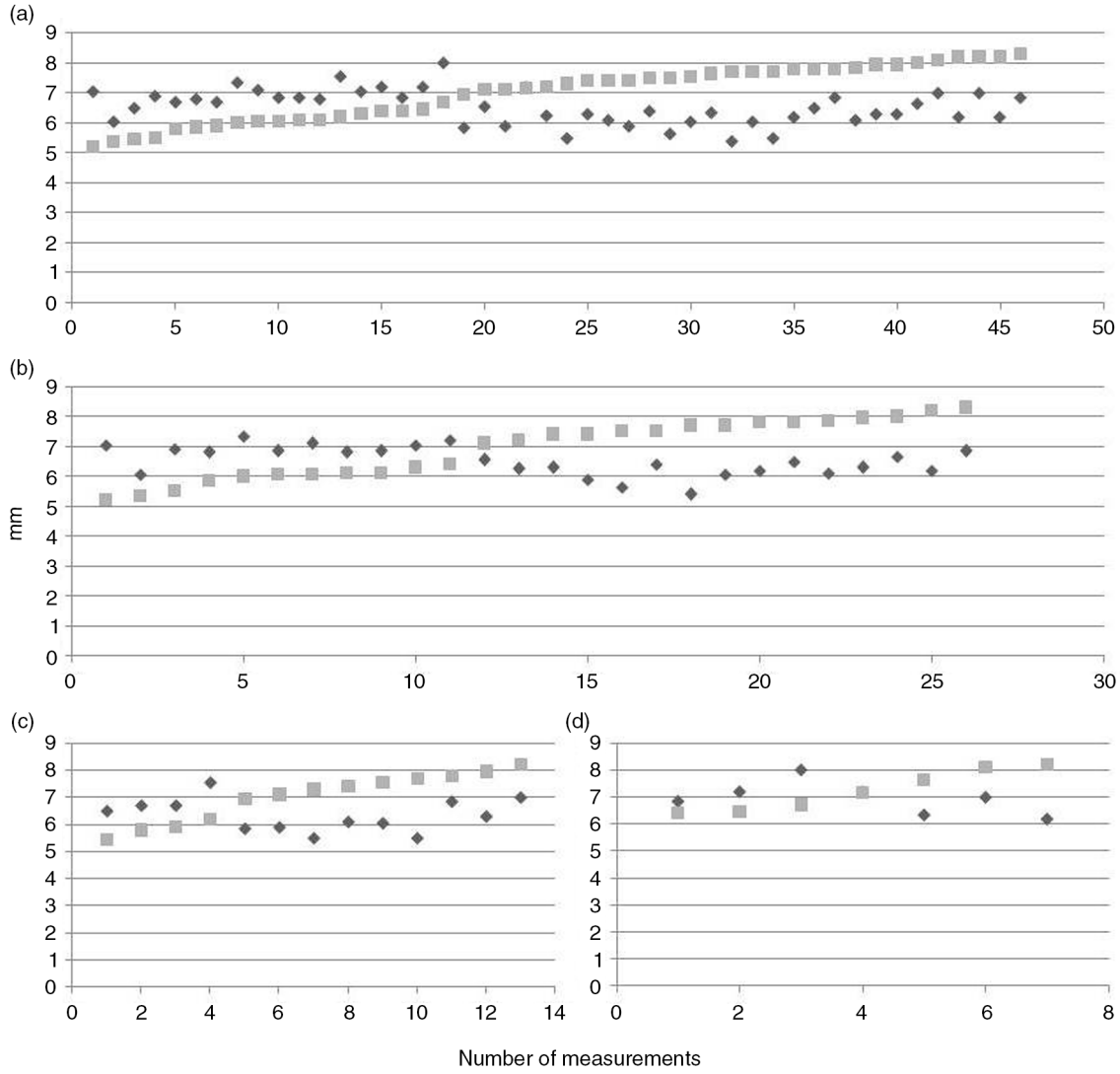

Fig. 2. Variation in the standardized height of style (squares) and stamen length (diamonds) in *Goniolimon italicum*. Individuals in each plot are ordered by increasing style length: (a) all measurements; (b) individuals of Fossa Raganesca; (c) individuals of Capestrano; (d) individuals of Barisciano Botanical Garden.

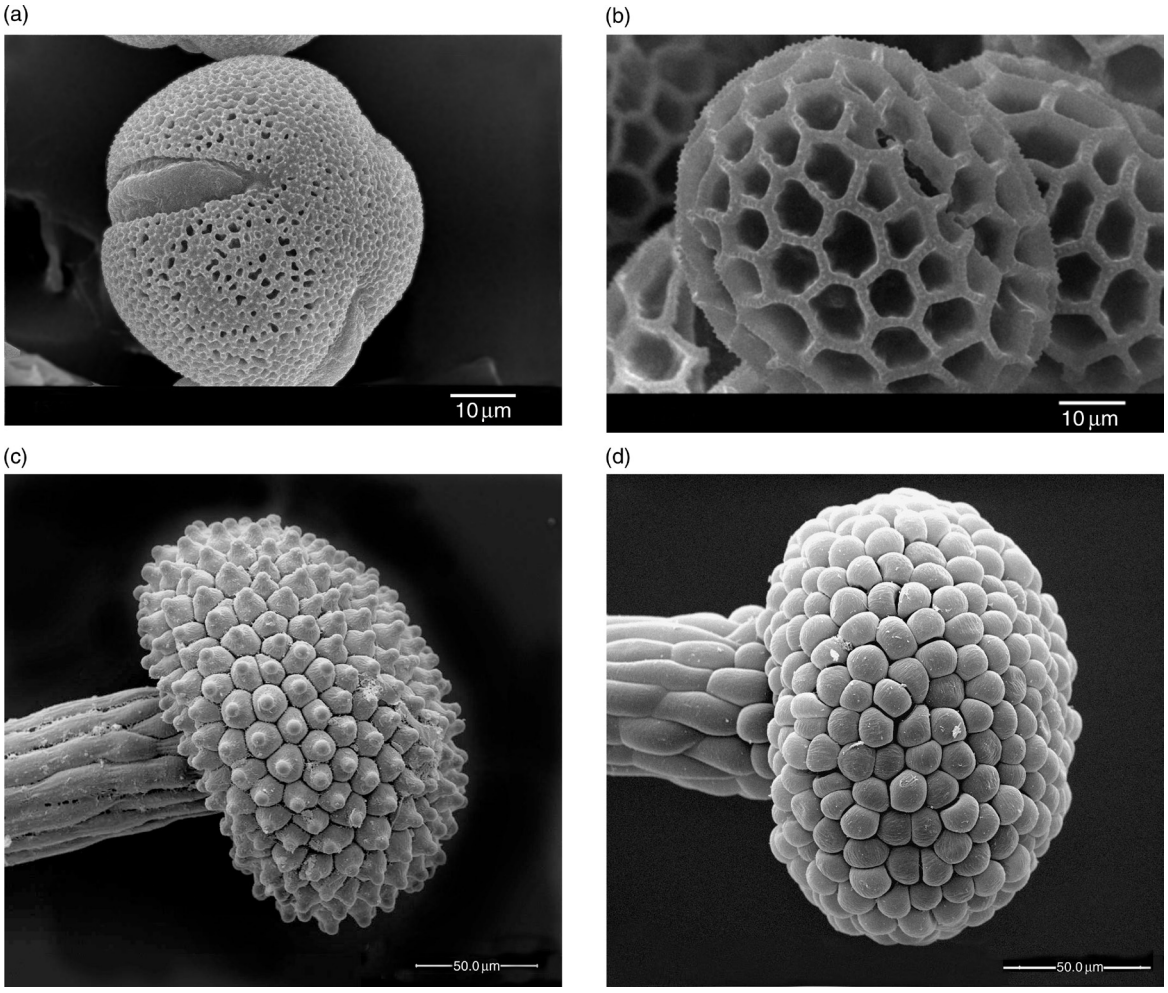

Fig. 3. SEM microphotographs of pollen grains and stigmas. (a) pollen grain, S-morph/Type B (sensu Baker, 1966); (b) pollen grain, L-morph/Type A (sensu Baker, 1966); (c) stigma, S-morph; (d) stigma, L-morph.

Goniolimon is phylogenetically related to the Australian *Muellerolimon* and placed in a clade that includes other eastern hemisphere genera with capitate/cylindrical stigmas (Lledó & al., 2005).

The observations made by Baker (1966) on *Goniolimon* showed heteromorphism only in the pollen grains, while the stigmas were reported to be monomorphic both in height and papillae). Subsequent studies carried out in *Goniolimon tataricum* with SEM reported heterostyly in flower, as well as dimorphism in pollen and stigma (height and shape of papillae) (Schill & al., 1985). In this study we confirm the distyly together with pollen-stigma dimorphism also in *G. italicum*.

As regards the geographic distribution, the discovery of two new populations does not change significantly the extent of occurrence and the area of occupancy of the species (the number of individuals known increases from about 300 to just over 400). The IUCN conservation status is confirmed. Recently, the species seems to have disappeared from "Le Pagliare", maybe due to uncontrolled collections. The implications of the presence of distily in this species introduces a new factor in efforts for in-situ and eventual ex-situ preservation. We propose an inmediate monitoring measure to survey the number of individuals of L and S-morph within smaller populations to find out if they are still able to produce new recruits to ensure their short-term viability.

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