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

Federica Morretti¹, Giovanna Puppi², Claudia Giuliani³ & Fabio Conti⁴*

¹Strada Prati, 42/1, 65125 Pescara, Italy; federica.morretti@gmail.com
²Dipartimento BiGeA, Via Inerio, 42, University of Bologna, 40126 Bologna; giovanna.puppi@unibo.it
³Department of Biology, University of Florence, Via G. La Pira, 4, 50121 Florence, Italy
⁴Scuola di Bioscienze e Medicina Vetrinaria, Università di Camerino - Centro Ricerche Floristiche dell’Appennino (Parco Nazionale del Gran Sasso-Laga), S. Colombo, 67021 Barisciano (L’Aquila), Italy; fabio.conti@unicam.it

* Corresponding author.

Abstract


*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

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), on slopes near the plains, where the climatic conditions and the presence of rock outcrops limit the development of tree cover. 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).

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* (Pitroni & 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).

Heterostylos 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 selling and fertilizations among plants of the same morph. Moreover, heterostylos species...
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 heteromor-
phic 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 dimor-
phism. 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 vari-
ability in style morphology and compatibility systems; there-
fore, 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 mea-
sured (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 equa-
torial 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 micromorphology were analyzed using Scanning
Electron Microscope (SEM).

RESULTS AND DISCUSSION
The analysis of flower samples permitted the identifica-
tion 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 individu-
als 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 diam-
eter) and very small spinules (about 0.3 μm) (Fig. 3a).
– Type A (sensu Baker, 1966), occurring in the L-morph pres-
ents 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.

<table>
<thead>
<tr>
<th>Type of flowers</th>
<th>Type S</th>
<th>Type L</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of plants</td>
<td>20</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Calyx length (mm)</td>
<td>7.23±0.53 (6.0;8.1)</td>
<td>7.03±0.41 (6.1;7.8)</td>
<td>0.20</td>
</tr>
<tr>
<td>Corolla length (mm)</td>
<td>8.02±0.38 (7.2;8.6)</td>
<td>7.84±0.41 (7.2;8.7)</td>
<td>0.17</td>
</tr>
<tr>
<td>Filament length</td>
<td>6.98±0.40 (6.1;8.0)</td>
<td>6.16±0.56 (4.4;7.0)</td>
<td>0.81**</td>
</tr>
<tr>
<td>Stigma height (mm)</td>
<td>6.09±0.50 (5.2;7.2)</td>
<td>7.63±0.49 (5.9;8.3)</td>
<td>−1.55**</td>
</tr>
<tr>
<td>Filament l.–Stigma h. difference</td>
<td>0.89±0.43</td>
<td>−1.47±0.41</td>
<td>2.36**</td>
</tr>
<tr>
<td>number of pollen grains</td>
<td>80</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>Pollen size: polar axis (µm)</td>
<td>54.34±3.75</td>
<td>54.60±3.64</td>
<td>−0.25</td>
</tr>
<tr>
<td>Pollen size: equatorial diameter (µm)</td>
<td>41.47±3.70</td>
<td>42.10±3.47</td>
<td>−0.63</td>
</tr>
<tr>
<td>Pollen shape (polar/eq.diam.)</td>
<td>1.32±0.10</td>
<td>1.30±0.07</td>
<td>−0.01</td>
</tr>
</tbody>
</table>

**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.
**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 distyly in this species introduces a new factor in efforts for in-situ and eventual ex-situ preservation. We propose an immediate 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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**REFERENCES**


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