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# Orobanche lycoctoni Rhiner (Orobanchaceae), a poorly known species of the Central European flora

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## Abstract

SCHNEEWEISS, G. M., B. FRAJMAN & I. DAKSKOBLER (2009). Orobanche lycoctoni Rhiner (Orobanchaceae), a poorly known species of the Central European flora. *Candollea* 64: 91-99. In English, English and French abstracts.

*Orobanche lycoctoni* Rhiner *(Orobanchaceae)* is a poorly known species described from Eastern Switzerland, constituting a separate lineage with unclear relationships to other taxa of *Orobanche* L. s.l. In this paper, we report on its occurrence in the Julian Alps (Slovenia), significantly extending its known distribution area (Cantabrian mountains and Swiss Alps). The molecular phylogeny and phytocoenological characterization of this species has been inferred on specimens growing in the newly found localities. We conclude that this species differs from *Orobanche flava* F. W. Schultz in many morphological characters, as well as its host plant *Aconitum lycoctonum* L.

## Key-words

*OROBANCHACEAE* – *Orobanche* – Julian Alps – Molecular phylogeny – Taxonomy – Phytocoenology – Floristics

## Résumé

SCHNEEWEISS, G. M., B. FRAJMAN & I. DAKSKOBLER (2009). Orobanche lycoctoni Rhiner (Orobanchaceae), une espèce peu connue de la flore d'Europe Centrale. *Candollea* 64: 91-99. En anglais, résumés anglais et français.

*Orobanche lycoctoni* Rhiner *(Orobanchaceae)* est une espèce peu connue de l'est de la Suisse, constituant une lignée séparée sans relations claires avec d'autres taxons d'*Orobanche* L. s.l. Dans cet article, nous rendons compte de sa présence dans les Alpes Juliennes (Slovénie), ce qui agrandit de manière significative son aire de distribution connue (montagnes cantabriennes et Alpes Suisses). La phylogénie moléculaire et la caractérisation phytocoenologique de cette espèce a été établie sur la base des spécimens issus des nouvelles localités. Nous concluons que cette espèce diffère d'*Orobanche flava* F. W. Schultz par beaucoup de caractères morphologiques, de même que par sa plante-hôte *Aconitum lycoctonum* L.

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## Introduction

The holoparasitic broomrapes *(Orobanche* L. s.l., *Orobanchaceae)* are among the taxonomically most difficult non-apomictic groups within the European flora. This is due to the, compared to normal plants, strongly reduced number of potentially useful characters, especially vegetative ones; the high phenotypic variability; and the uniform darkening during desiccation resulting in the loss of potentially useful coloration characters in herbarium specimens (KREUTZ, 1995). Consequently, our knowledge on taxonomy and distribution of many broomrape species is still insufficient even in floristically well-explored regions. For example, since the treatment of *Orobanche* s.l. in 'Flora Iberica' (FOLEY, 2001), several new species of *Orobanche* s.str. and *Phelipanche* Pomel have been described from the Iberian Peninsula (PUJADAS-SALVÀ & CRESPO, 2004; CARLÓN & al., 2005, 2008a; PUJADAS-SALVÀ, 2008).

In recent years, the application of molecular data has greatly advanced our understanding of the relationship of genera within *Orobanchaceae* in general (BENNETT & MATHEWS, 2006) as well as those of *Orobanche* and related genera in particular (MANEN & al., 2004; SCHNEEWEISS & al., 2004a; PARK & al., 2007a, 2008). In the course of these studies, previous assertions on narrower generic concept based mainly on morphological data have been confirmed (HOLUB, 1977, 1990; TERYOKHIN & al., 1993), and have been further corroborated by karyological, cytological and retroelement data (SCHNEEWEISS & al., 2004b; WEISS-SCHNEEWEISS & al., 2006; PARK & al., 2007b). Although not the main focus of these studies, the used markers (nuclear ITS, plastid *rps2* and *rbcL*) have allowed to address also relationships at the specific level. Generally, the majority of subsections and sections in the classifications of BECK-MANNAGETTA (1890, 1930) and TERYOKIN & al. (1993), respectively, are not monophyletic. Specifically aimed towards species relationships are the studies by CARLÓN & al. (2005, 2008a). These successfully combined traditional morphological-systematic approaches supplemented by thorough photographic documentation with analyses of nuclear ITS data in a manner similar to DNA barcoding and allowed recognition of new species and clarification of the taxonomic status of poorly known and often neglected species, such as *O. serbica* Beck & Petrovi and *O. grenieri* F. W. Schultz.

One of these poorly understood entities is *O. lycoctoni* Rhiner (Fig. 1). Initially it was named as *O. flava* var. *albicans* Rhiner (*nomen nudum*: RHINER, 1870) from eastern Switzerland. Later Rhiner considered it to be distinct enough from *O. flava* F. W. Schultz to merit recognition on the species level, naming it *O. lycoctoni* after the host plant *Aconitum lycoctonum* L. (RHINER, 1892). This name appears to have been overlooked in the monograph of the genus by BECK-MANNAGETTA (1930), who actually also doubted any reports of *Orobanche flava* on *Aconitum lycoctonum*. In the following

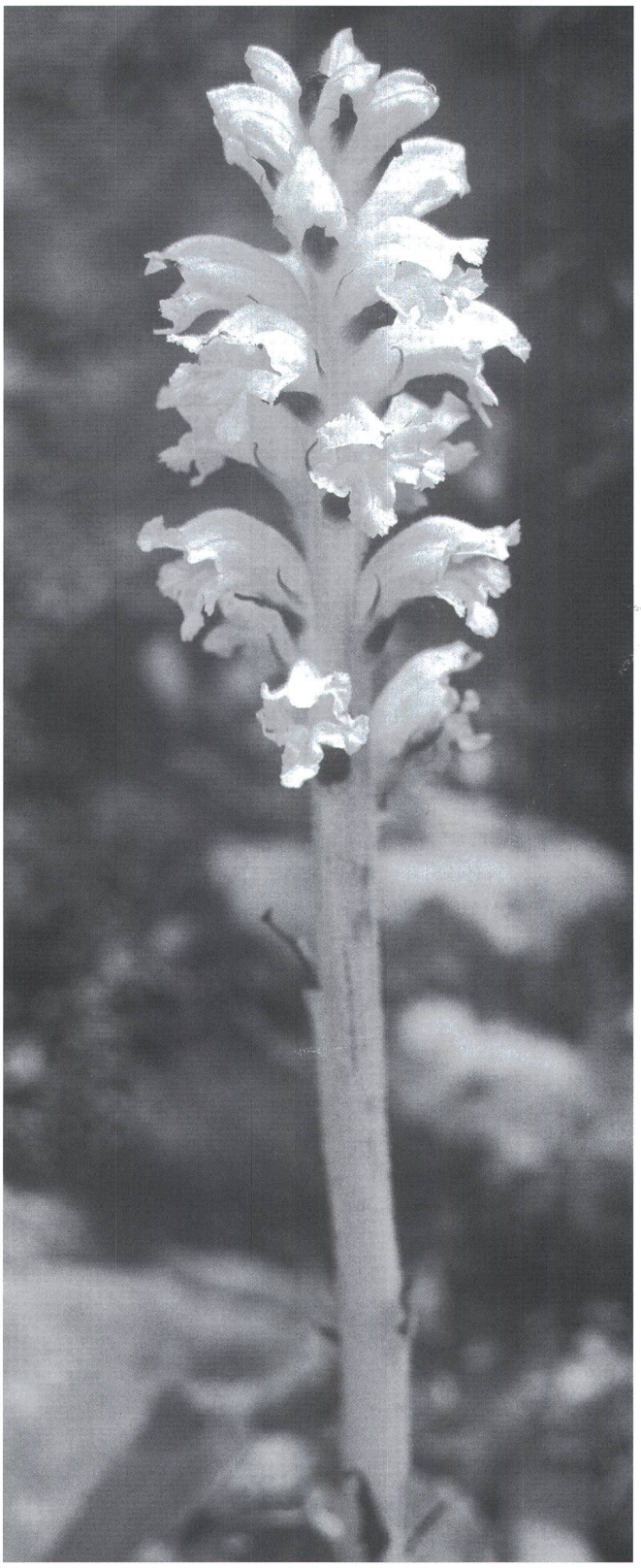

**Fig. 1.** – *Orobanche lycoctoni* Rhiner.
[Photo: Igor Dakskobler]

decades, only rarely observations of *Orobanche* on *Aconitum lycoctonum* have been mentioned (BONNIER & DOUIN, 1911-1935), and only in 2002 a species of *Orobanche* growing on this host was reported from the Picos de Europa in the Cantabrian mountains and described as *O. aconiti-lycoctoni* Carlón & al. (CARLÓN & al., 2002). Later, the authors became aware of Rhiner's observations and name and, as already suggested by PUJADAS SALVÀ (2003), suspected that the Swiss and the Cantabrian plants are conspecific, what was confirmed recently by molecular data (CARLÓN & al., 2008a). Furthermore, molecular data showed that *O. lycoctoni* is not even closely related to *O. flava*, but instead constitutes a separate lineage with unclear relationships to other *Orobanche* taxa (MANEN & al., 2004; CARLÓN & al., 2005, 2008a).

This prompted one of us (I. D.) to re-evaluate occurrences of *Orobanche* on *Aconitum lycoctonum* from the Slovenian Julian Alps. As already noted by RHINER (1892), the diagnostic characters of *O. lycoctoni* are hardly discernible on dried material, and we therefore used nuclear ITS data to test the identity of the Slovenian material. We summarize the currently known distribution of this species and list its diagnostic features to draw attention to this so far neglected taxon.

## Materials and methods

### *Molecular analysis*

Plant material from five localities in the Slovenian Julian Alps was investigated (Appendix 1). DNA was extracted from the herbarium material using the 2 × cetyl trimethyl ammonium bromide (CTAB) method of DOYLE & DOYLE (1987). Amplification and sequencing of the nuclear ITS region follows methods described in SCHNEEWEISS & al. (2004a). The newly obtained sequences (GenBank accession numbers EU817099–EU817103) were added to the data set of CARLÓN & al. (2008a). Species membership of the newly investigated accessions was determined by a neighbour-joining analysis using the maximum composite likelihood method (TAMURA & al., 2004) with pairwise deletion and with 1000 bootstrap replicates as implemented in MEGA 4 (TAMURA & al., 2007).

### *Phytocoenological analysis*

The vegetation in two relevés was recorded using the standard Central-European method (BRAUN-BLANQUET, 1964) and a characterized table (an ordered table including information on syntaxonomic units) was constructed manually. Taxa and syntaxa names follow the nomenclature used in MARTINČIČ & al. (2007) and AESCHIMANN & al. (2004), respectively.

## Results and discussion

### *Molecular phylogeny*

The newly analysed samples from different localities in the Julian Alps of Slovenia have essentially identical ITS sequences to *O. lycoctoni* from the Picos de Europa and from eastern Switzerland, thus confirming that the south-eastern Alpine accessions belong to *O. lycoctoni* (Fig. 2). It is worth re-emphasizing that against previous assertions *O. lycoctoni* is not a close relative of *O. flava*, but constitutes a strongly distinct and separate lineage with unclear relationships to other species.

This is a significant extension of the currently known distribution area, which encompasses four highly disjunct areas (Fig. 3): (i) Picos de Europa in the Cantabrian mountains of

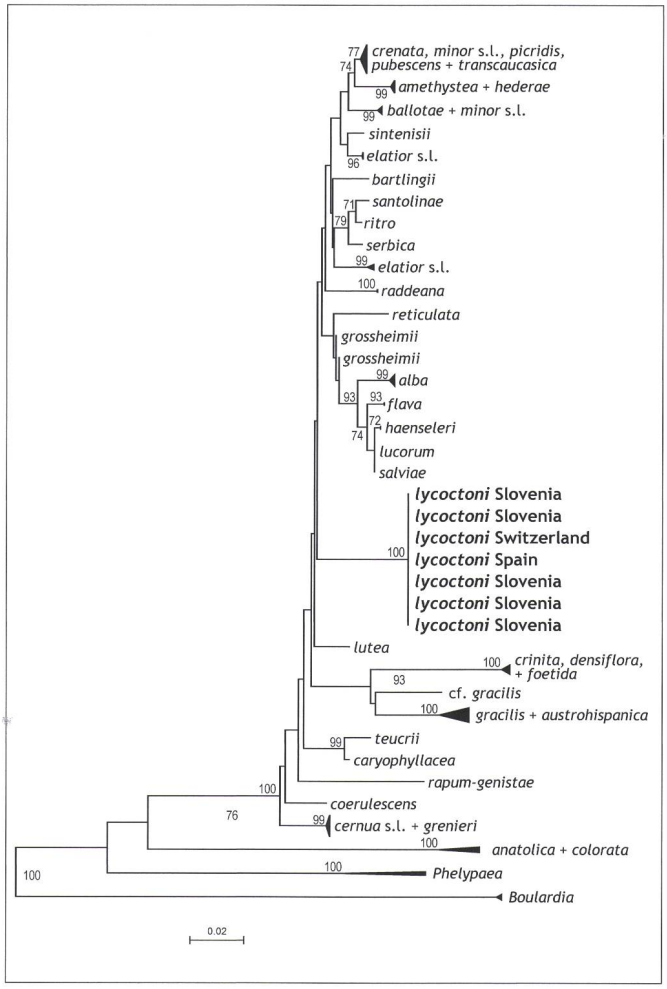


**Fig. 2.** – Neighbour joining tree of nuclear ITS sequences of *Orobanche* L., using the genera *Boulardia* F. W. Schultz and *Phelypaea* L. as outgroups. Some clades with more sequences per species and/or several closely related species are contracted to aid legibility (for a more detailed discussion of species relationships see SCHNEEWEISS & al., 2004a, and WEISS-SCHNEEWEISS & al., 2006). *Orobanche lycoctoni* Rhiner is indicated in bold larger font. Number at nodes are bootstrap support values.

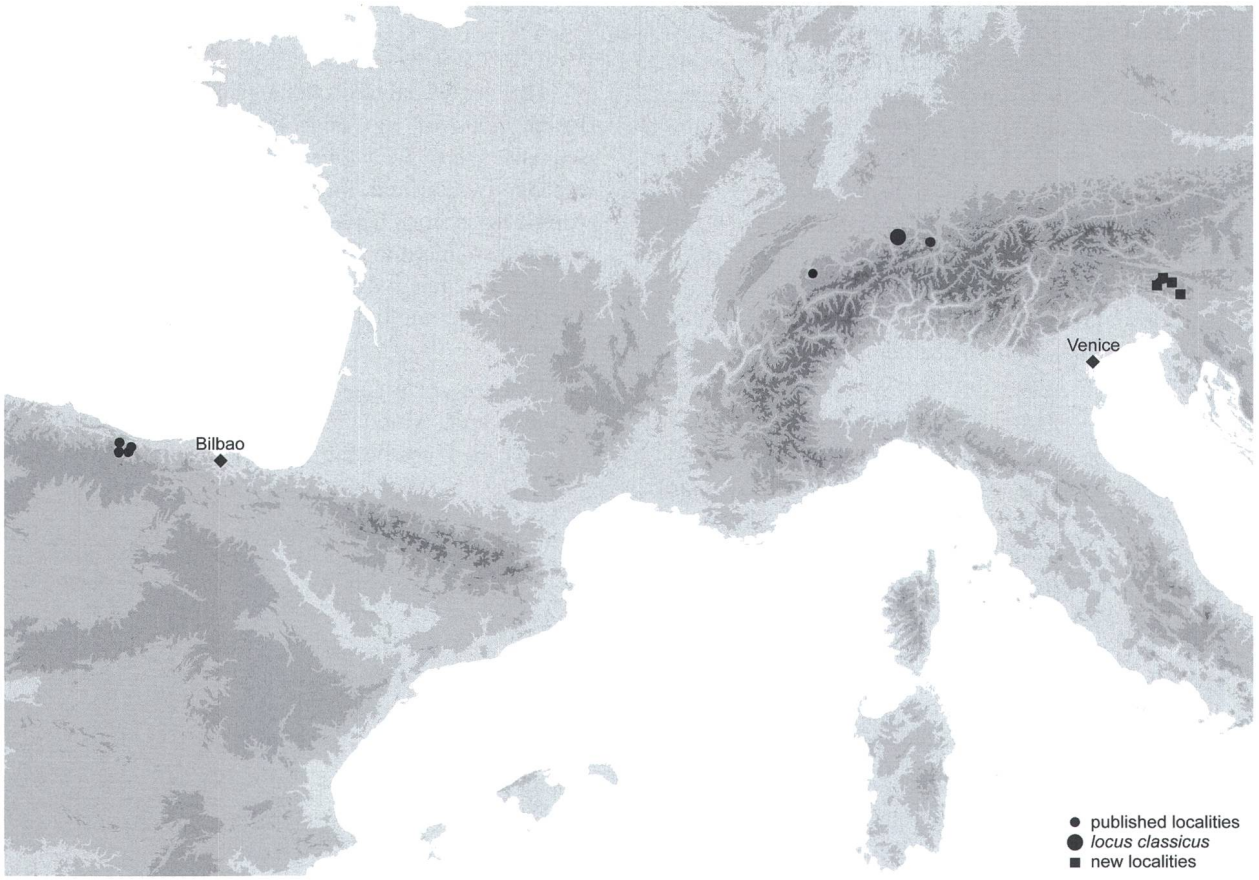


**Fig. 3.** – Distribution of *Orobanche lycoctoni* Rhiner (see Appendix for a detailed list of Slovenian localities). The type locality in the western Glarner Alps is highlighted.

N. Spain, (ii) the Préalpes Fribourgoises in western Switzerland (SONNBERGER, 2008), (iii) western and easternmost Glarner Alps in E Switzerland (which include the type locality) and (iv) the Julian Alps the Julian Alps in Slovenia. The actual distribution area of *O. lycoctoni* likely also includes other parts of the Alps (an occurrence in the French Alps is mentioned in CARLÓN & al., 2008a: 117) and the Pyrenees (see DIXON & al., 2008, for cases of connections between the Alps and the Cantabrian mountain ranges sparing the Pyrenees), and probably also extends to the Apennine, the Carpathians and the Balkan peninsula. Although it is possible that further localities of *O. lycoctoni* are documented in herbaria, but have remained undetected due to misidentifications (or lack of any identification), we expect that most gaps in the currently known distribution area will be filled in the course of future floristic research.

*Morphological data*

As indicated by CARLÓN & al. (2005) and confirmed on the Slovenian material (Fig. 1), *O. lycoctoni* is morphologically characterised by the overall pale yellow-whitish colour (yellow to reddish-brownish in *O. flava),* a thick stem, a dense inflorescence, corolla tubes with a rather straight lower outline nearly parallel to the stem and a distally abruptly curved upper outline (more regularly curved in *O. flava),* glabrous upper and lower lips (ciliate in *O. flava),* acuminate calyx teeth (often aristate in *O. flava),* a thick regularly curved style (more slender and irregularly curved in *O. flava),* and a deep yellow stigma, which is rather disc-like and not as distinctly bilobed as in most other species (see images in CARLÓN & al., 2008b). As far as we know this species is restricted to *Aconitum lycoctonum* as a host, which may thus act as an additional diagnostic feature. Many of these characters are either not at all or at least not unambiguously detectable on herbarium specimens, and therefore, as generally recommended for broomrape documentation, should be recorded on fresh material (including photographs).

*Phytocoenological characteristics*

A detailed characterization of the habitats of *O. lycoctoni* is available from the localities in the Julian Alps. These are in the subalpine to lower alpine belt (in Switzerland also in the

montane belt, in the Cantabrian mountains descending to 400 m or less with accordingly different surrounding vegetation: CARLÓN & al., 2005) over massive Triassic limestone and/or Triassic dolomite (BUSER, 1986, 1987; JURKOVŠEK, 1987a, b). The overall climate is humid with yearly precipitation of 2000-2500 mm and a mean annual temperature of 3-4° C. The vegetation period lasts from May (June) to October (for more details see MEKINDA-MAJARON, 1995; ZUPANČIČ, 1995; OGRIN, 1996). *Orobanche lycoctoni* was found mostly in tall herb communities dominated by *Aconitum lycoctonum* subsp. *ranunculifolium* (Rchb.) Finet & Gagnep. and *Adenostyles glabra* DC., which usually thrive on shady and moist screes with long lasting snow cover (Table 1). The first stand (relevé 1) belongs to the association *Aconito ranunculifolii-Adenostyletum glabrae* Surina 2005 of class *Thlaspietea* (see SURINA, 2005, for details) and is characterised by taxa of subalpine screes. The second stand (relevé 2) belongs to the association *Trollio europaei-Aconitetum ranunculifolii* Dakskobler 2008 (see DAKSKOBLER, 2008, for details), where due to the deeper soil (humus) tall herbs of the order *Adenostyletalia* and the class *Mulgedio-Adenostyletea* are prevailing.

**Table 1.** – Two tall herb stands with *Orobanche lycoctoni* Rhiner in the Julian Alps (in bold, name of the syntaxa).

| Quadrant | 9547/3 | 9647/1 |
|---|---|---|
| Locality | Nemške glave | Možnica-Čez Brežič |
| Number of relevé | 1 | 2 |
| Altitude [m] | 1430 | 1610 |
| Exposition | SE | SSE |
| Inclination [°] | 35 | 30 |
| Stone cover [%] | 30 | 10 |
| Cover of herbaceous layer [%] | 70 | 90 |
| Relevé area [m²] | 20 | 20 |
| Number of taxa | 24 | 34 |
| Date | 31.7.2007 | 4.9.2006 |
| ***Petasition paradoxi* W. Lippert 1966** | | |
| *Adenostyles glabra* DC. | 4 | 1 |
| *Aconitum lycoctonum* subsp. *ranunculifolium* (Rchb.) Finet & Gagnep. | 2 | 1 |
| *Orobanche lycoctoni* Rhiner | + | + |
| *Gymnocarpium robertianum* Newman | 2 | – |
| *Rumex scutatus* L. | + | – |
| *Silene vulgaris* subsp. *glareosa* (Jord.) Marsden-Jones & Turrill | + | – |
| *Dryopteris villarii* (Bellardi) Woyn. | + | – |
| *Aquilegia einseleana* F. W. Schultz | r | – |
| ***Arabidion caeruleae* Braun-Blanq. 1926** | | |
| *Festuca nitida* Kit. | + | – |
| ***Thlaspietea rotundifolii* Braun-Blanq. 1948** | | |
| *Biscutella laevigata* L. | + | – |
| *Campanula cochleariifolia* Lam. | + | – |
| ***Adenostyletalia* G. Braun-Blanq. & Braun-Blanq. 1931** | | |
| *Carduus carduelis* (L.) Gren. | – | 4 |
| *Crepis pyrenaica* (L.) Greuter | – | 1 |
| *Heracleum* cf. *montanum* Schleich. | – | 1 |
| *Thalictrum aquilegiifolium* L. | – | 1 |
| *Silene vulgaris* subsp. *antelopum* (Vest) Hayek | – | + |

| | | |
|---|---|---|
| ***Mulgedio-Aconitetea* Hadač & Klika 1948** | | |
| *Aconitum degenii* subsp. *paniculatum* (Arcang.) Mucher | + | – |
| *Veratrum album* L. s.l. | – | 2 |
| *Chaerophyllum villarsii* Koch | – | 2 |
| *Rumex alpestris* Jacq. | – | 1 |
| *Hypericum maculatum* Crantz | – | + |
| *Epilobium alpestre* (Jacq.) Krock. | – | + |
| ***Cystopteridion fragilis* J.-L. Rich. 1972** | | |
| *Cystopteris regia* (L.) Desv. | + | – |
| ***Asplenietea trichomanis* Braun-Blanq. 1934 corr. Oberd. 1977** | | |
| *Paederota lutea* Scop. | 3 | – |
| *Moehringia muscosa* L. | + | – |
| ***Elyno-Seslerietea* Braun-Blanq. 1948** | | |
| *Betonica alopecuros* L. | + | + |
| *Myosotis alpestris* F. W. Schmidt | + | + |
| *Heliosperma alpestre* Rchb. | + | – |
| *Knautia longifolia* Koch | – | 1 |
| *Poa alpina* L. | – | + |
| *Carex sempervirens* Vill. | – | + |
| *Phyteuma orbiculare* L. | – | + |
| *Carlina acaulis* L. | – | + |
| *Thesium pyrenaicum* Pourr. | – | + |
| *Campanula witasekiana* Vierh. | – | + |
| *Scabiosa lucida* Vill. subsp. *lucida* | – | + |
| ***Poo alpinae-Trisetalia* Ellmauer & Mucina 1993** | | |
| *Trollius europaeus* L. | – | 1 |
| *Ranunculus nemorosus* DC. | – | + |
| ***Fagetalia sylvaticae* Pawl. 1928** | | |
| *Lilium martagon* L. | + | + |
| *Galeobdolon flavidum* (F. Herm.) Holub | + | + |
| *Cyclamen purpurascens* Mill. | 1 | – |
| *Mercurialis perennis* L. | 1 | – |
| *Geranium robertianum* L. | r | – |
| *Daphne mezereum* L. | – | + |
| ***Epilobietea angustifolii* von Rochow 1951** | | |
| *Silene dioica* (L.) Clairv. | – | 1 |
| *Senecio fuchsii* C. C. Gmel. | – | + |
| ***Erico-Pinetea* Horvat 1959** | | |
| *Calamagrostis varia* (Schrad.) Host | 1 | – |
| *Buphthalmum salicifolium* L. | – | + |
| *Cirsium erisithales* Scop. | – | + |
| ***Molino-Arrhenatheretea* Tüxen 1937** | | |
| *Dactylis glomerata* L. | – | 1 |
| *Trifolium pratense* L. | – | + |

## Conclusions

*Orobanche lycoctoni* is a phylogenetically, morphologically and ecologically distinct species of the central and southern European mountain ranges. It clearly demonstrates that the broomrape flora is still comparatively poorly known even in floristically well investigated areas. Although traditional specimens will continue to be poor preservers of many important diagnostic features, this can easily be alleviated by proper (digital) photographic documentation and can be supplemented by molecular DNA barcoding approaches, as done successfully for *O. lycoctoni.*

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**Appendix 1.** – The new localities of *Orobanche lycoctoni* Rhiner in Slovenia.

**9547/3** (UTM UM93): Julian Alps, surroundings of Bovec: Nemške glave, V Podne, gravelly gully, 1430 m; tall herb community: *Aconito ranunculifolii-Adenostyletum glabrae* Surina 2005 (Table 1, relevé 1). Leg. I. Dakskobler, 31.VII. 2007, working herbarium ZRC SAZU. 46º24'16"N 13º34'39"E; GenBank EU817101.

**9547/4** (UTM UM 94): Julian Alps, Mt. Mangart, Rdeča skala, gravel under the wall, 1900 m; tall herb community with dominant *Aconitum lycoctonum* subsp. *ranunculifolium* (Rchb.) Finet & Gagnep. Leg. I. Dakskobler, 8.IX.2004, working herbarium ZRC SAZU. 46º26'2"N 13º38'52.5"E; GenBank EU817103.

**9548/4** (UTM VM03): Julian Alps, lake Spodnje Kriško jezero, 1890 m; tall herb community with dominant *Aconitum lycoctonum* subsp. *ranunculifolium* (Rchb.) Finet & Gagnep. Leg. I. Dakskobler, 10.IX.2004, working herbarium ZRC SAZU. 46º24'0.6"N 13º48'26.3"E; GenBank EU817102.

**9647/1** (UTM UM83): Julian Alps, the end of the Možnica valley, under the pass Čez Brežič, 1610 m; tall herb community with dominant *Aconitum lycoctonum* subsp. *ranunculifolium* (Rchb.) Finet & Gagnep. and *Carduus carduelis* (L.) Gren. (Table 1, relevé 2). Leg. I. Dakskobler, 4.IX.2006, working herbarium ZRC SAZU. 46º23'0.1"N 13º31'52"E; GenBank EU817099.

**9749/4** (UTM VM12): Julian Alps, Mt. Črna prst, above the alp Planina za Liscem, 1510 m; tall herb community with dominant *Adenostyles glabra* DC. and *Aconitum lycotonum* L. s.l. Leg. I. Dakskobler, 23.VIII.2001, working herbarium ZRC SAZU. 46º14'7"N 13º56'26"E; GenBank EU817100.