

Short communication

## Least adder's-tongue (*Ophioglossum lusitanicum* L.) in Croatia – distribution, ecology and conservation

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**Abstract** – The presence of the least adder's tongue (*Ophioglossum lusitanicum*) in Croatia is sparsely documented in scientific literature, with only a few records to date. After findings from the 19<sup>th</sup> century, the species was not confirmed in the field for a whole century, and was consequently considered extinct in the Croatian flora. It has been recently confirmed in southern Istria (Croatia), in habitats with moderate anthropogenic impacts.

**Keywords:** Croatia, distribution, endangered species, flora, Istria, *Ophioglossum lusitanicum*

### Introduction

The genus *Ophioglossum* L. belongs to *Ophioglossaceae*, one of the most distinctive families among ferns, distinguished from 'modern' ferns by many atypical anatomical and morphological features (WAGNER 1990, PRYER et al. 2004). The genus comprises approximately 25–30 species distributed worldwide, but is most commonly found in warmer parts of the world. Altogether four species of *Ophioglossum* are registered in the European flora (DERRICK et al. 1987, AKEROYD 1993), three of them in Croatia and Istria: the adder's-tongue (*Ophioglossum vulgatum* L.), least adder's-tongue (*O. lusitanicum* L.) and small adder's-tongue (*O. azoricum* Presl) (HRŠAK 1994, NIKOLIĆ and TOPIĆ 2005, BRANA and REŠETNIK

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2005, BRANA 2010, NIKOLIĆ 2013). *O. lusitanicum* is a cosmopolite species, widely distributed in Europe in the Mediterranean/Western European part (CLAUSEN 1938, JALAS and SUOMINEN 1972, DERRICK et al. 1987, AKEROYD 1993).

The least adder's-tongue was first recorded in Croatia in the middle of the 19<sup>th</sup> century, during the earliest botanical studies of this area. According to WRABER (2001), the earliest note dates from January 1843, relating to a herbarium example collected in Istria (islet of Veruda) and deposited in the Natural History Museum of Trieste (TSM). The label on this example, written by Mutius (Muzio) Tommasini, indicates that it was collected by Checco, who was for 24 years collecting for Tommasini (WRABER 2001). The first published note on *Ophioglossum lusitanicum* in Istria is from Tommasini (1873), providing February 1<sup>st</sup> 1846 as the date of the record in the surroundings of Premantura. Later FREYN (1877) published several localities in the surroundings of Pula, while PETTER (1852) and VISIANI (1852) record *O. lusitanicum* in Hvar archipelago. The record from Hvar archipelago was quoted later by several botanists (SCHLOSSER and VUKOTINOVIĆ 1869, HIRC 1905, TRINAJSTIĆ 1993), but remains unconfirmed to date.

In spite of the many botanists who studied the flora of potential localities of *Ophioglossum lusitanicum* on the Eastern Adriatic coast (NEUGEBAUER 1875, ROSSI 1930, MEZZENA 1986, TOPIĆ and ŠEGULJA 2000) no additional localities of this species were discovered. Only some reviews of Yugoslavian flora contained notes on *O. lusitanicum*, presumably adopted from historical literature (JANCHEN 1952, MAYER and HORVATIĆ 1967). The first field confirmation, a hundred years after the historical data, was made by Slovenian and other botanists in 1974, who recorded *O. lusitanicum* several times between 1974 and 2001 in the surroundings of Ribnjak in southern Istria (Volme), but published it only in 2001 (WRABER 2001). For this reason, *O. lusitanicum* was overlooked and listed as extinct in the first complete index of the Croatian flora (HRŠAK 1994) and the first Red Book of plant species of the Republic of Croatia (MARKOVIĆ 1994). However, the species re-appeared in the new Red Book of vascular flora of Croatia as a critically endangered species (CR), partly supported by our personal observations and discovery of new localities (MARKOVIĆ 2005).

Regarding the Eastern Adriatic coast in Croatia and Slovenia, the least adder's-tongue was never recorded north of southern Istrian localities (POSPICHAL 1897, MARTINČIĆ 1999). Although the species is noted for the flora of Italy, there are no known localities in northern Italy (PIGNATTI 1982), except the records for Friuli (FIORI 1943), which have not been confirmed.

Ecologically, *Ophioglossum lusitanicum* prefers disturbed habitats in their early to middle successional stage, and its development strongly depends on mycorrhizal fungi (WAGNER 1990, MERCKX 2013). Overall, the species has a winter growing season, with above-ground parts disappearing in spring. Accordingly, the sporulation occurs during winter, although different authors provide different periods: December–April (FREYN 1877), October–February (MAYER and HORVATIĆ 1967), April–May (SCHLOSSER and VUKOTINOVIĆ 1869), and August–December (PIGNATTI 1982).

Taking into consideration the deficiency of data on *Ophioglossum lusitanicum* in Croatia, the aim of this paper is to: 1) provide details on historic and recent studies of this species and reveal its currently known distribution in Croatia, 2) elaborate in more detail the distributional/ecological information used for assessing its current threat status and 3) provide further considerations on its conservation.

## Materials and methods

Intensive field work started in the period from 2000 to 2002, as a part of the project »Endemic, rare & threatened taxa of Istrian vascular flora« and was continued throughout the following years. The search included Istrian localities from the literature, as well as many other sites where *Ophioglossum lusitanicum* might occur. In addition, the islands of Krk, Cres, Lošinj and partly Rab were thoroughly searched for this species. When found, the species was photographed, and in some cases herbarium specimens were collected and stored in Herbarium Croaticum (ZA). Since the discovery, Istrian localities have been visited yearly in the winter period, with the last visit in 2013.

We have also searched the Istrian peninsula for *Ophioglossum vulgatum* and *O. azoricum* to record the emergence of leaves and duration of the sporulation period and make comparisons with the phenology of *O. lusitanicum*. Since most localities of *O. lusitanicum* are situated in south western Istria, the observation of the phenology of all three species was carried out on the populations ranging mostly between Pula and Rovinj, and the average between those meteorological stations was used as a climatic data.

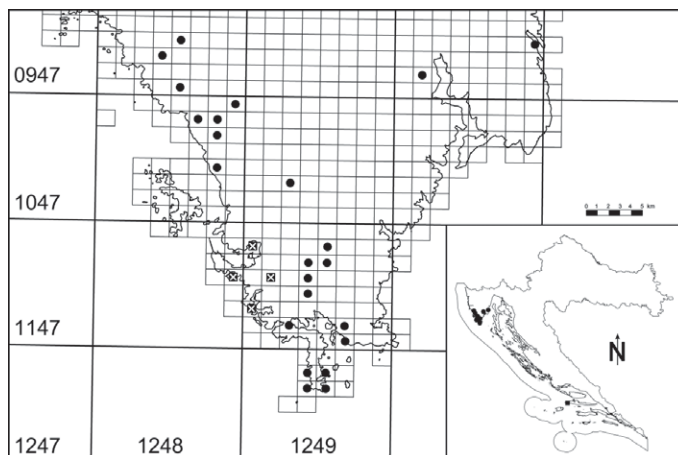
In 2005, co-occurring species were recorded at five localities where *O. lusitanicum* grows most abundantly (Sveta Foška, Kamenjak, Cintinere, Barbariga and Vela Gospa) and their abundance was estimated, to determine the species composition at sites where *O. lusitanicum* occurs in great numbers. The determination of species was carried out using standard determination keys and guides (JÁVORKA and CSAPODY 1975, TUTIN et al. 1968–1980, TUTIN et al. 1993, DOMAC 1994, ROTHMALER 1995) and the nomenclature is in accordance with NIKOLIĆ (2013).

All localities, except the unconfirmed historical records, were associated with corresponding Gauss-Krueger coordinates (geocoded with a GPS device Garmin e-Trex). The current known records on the distribution of the species in Istria are presented using the MTB 1/64 grid, according to the proposed national standard (NIKOLIĆ et al. 1998), and a distribution map is prepared with ESRI ArcGIS 9.2 software. Since we were not able to geocode two historical records (Štinjan and Mt. Pero) into MTB 1/64 fields, those records are not presented on the map.

## Results and discussion

### Distribution

Although many localities in the northern part of the Eastern Adriatic were examined, the species was confirmed only in Istria, with the first finding in 2000 on Cape Kamenjak. During the following years, several more spots with *Ophioglossum lusitanicum* were registered on Kamenjak, which nowadays represents one of the richest Croatian localities of this species. Altogether, we registered its occurrence in 23 localities (Fig. 1, Tab. 1). The number of individuals in most of the recorded populations is extremely small (sometimes only few individuals). The largest populations are recorded in Sveta Foška, Kamenjak and Cintinere, comprising several hundred (up to more than a thousand) specimens, while the populations in Barbariga and Vela Gospa are also among larger ones (tens of individuals, up to one hundred). Apart from the difficulties in the detection of the species, estimation of its population size is additionally problematic due to the vegetative reproduction by adventitious roots.



**Fig. 1.** Main map: distribution of *Ophioglossum lusitanicum* L. in Istria, presented in MTB 1/64 fields. Lower right corner: location of all known records of *O. lusitanicum* in Croatia. Black dots – confirmed and new findings, crosses – examined but unconfirmed historical findings, black square – unexamined historical finding from Pakleni otoci. For further explanation of the nomenclature of MTB fields see NIKOLIĆ et al. (1998).

Our study shows that the least adder's-tongue nowadays appears only in southern Istria, most abundantly in the area Kamenjak–Marlera. In spite of a very detailed search, we could not confirm most records from the wider surroundings of Pula from FREYN (1877). We conclude that the species probably disappeared from those localities due to habitat loss.

The record from Pakleni otoci (VISIANI 1852) remains unconfirmed. Considering the time elapsed, it is possible that the species is actually lost on this locality. However, *O. lusitanicum* is a very small, non-distinctive plant, easily overlooked in the grassland habitat. Its leaves appear above ground at the same time when young leaves of other plants start to emerge and develop and thus it could be easily mistaken for some other species. Most importantly, it grows in the winter period, i.e. outside the main vegetation season. Due to the above-mentioned difficulties, the species is rarely found during regular floristic studies. A detailed, specific search is necessary to be more certain about the loss from Pakleni otoci, but to our knowledge, no such search was ever undertaken.

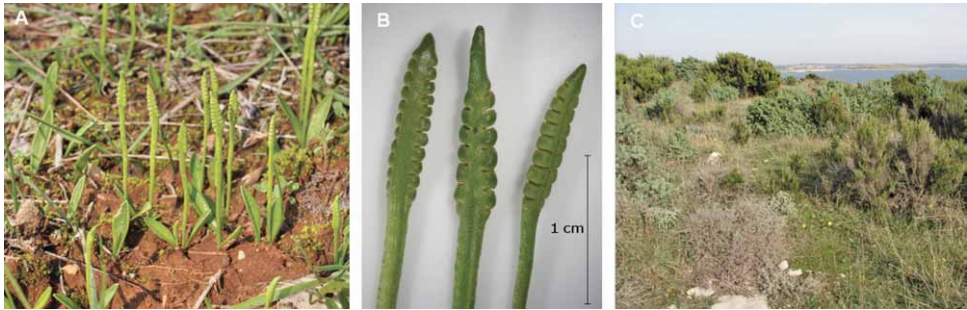
### Habitat and phenology

Regarding soil types, the eolian sands of southern Istria seem to be optimal for this species, especially on spots subjected to aerosol during late autumn. In our case, the least adder's-tongue mostly grows on disturbed habitats, e.g. pathways, passages and edges of paths in the anthropogenically influenced garrigue vegetation, where trampling, grazing and fire occur (Fig. 2C), confirming that this species is favoured by a certain level of disturbance, as previously indicated by WAGNER (1990). In addition, the composition of other species indicates that the largest populations of least adder's-tongue are found in anthropogenically influenced, heterogeneous habitats, mostly transitions between pasture and garrigue vegetation. Species such as *Eragrostis minor*, *Anagallis arvensis*, *Euphorbia cyparissias*, *Muscari comosum*, *Plantago* spp., *Trifolium angustifolium* and *Tragus racemosus* clearly show an-

**Tab. 1.** Records of *Ophioglossum lusitanicum* L. in Istria presented in MTB 1/64 fields. Unconfirmed records are in bold. For further explanation of the nomenclature of MTB fields see NIKOLIĆ et al. (1998).

MTB 1/64 code	MTB basic field name	Locality	Reference of previous findings
0948/324	Bale	Črnibek	
0948/411	Bale	Vela Gospa	
0948/433	Bale	Betiga	
0950/332	Labin	Rebići	
0950/422	Labin	Prklog	
1048/214	Fažana	Barbariga-Komunal	
1048/222	Fažana	Mednjan-Kanestrin	
1048/223	Fažana	Sveta Foška	
1048/241	Fažana	Sveta Foška	
1048/421	Fažana	Peroj	
1049/323	Vodnjan	Monteki	
<b>1148/2</b>	<b>Štinjan</b>	<b>Štinjan</b>	<b>Freyn (1877)</b>
<b>1148/244</b>	<b>Štinjan</b>	<b>Fort Max</b>	<b>Freyn (1877)</b>
<b>1149/1</b>	<b>Pula</b>	<b>Mt. Pero</b>	<b>Freyn (1877)</b>
<b>1149/113</b>	<b>Pula</b>	<b>Pta. Aguzzo</b>	<b>Freyn (1877)</b>
<b>1149/134</b>	<b>Pula</b>	<b>Bat. Corniale</b>	<b>Freyn (1877)</b>
1149/142	Pula	Jadreški	
1149/144	Pula	Šikići	
1149/213	Pula	Jadreški	
1149/231	Pula	Jadreški	
<b>1149/313</b>	<b>Pula</b>	<b>Veruda</b>	<b>Freyn (1877)</b>
1149/322	Pula	Čampanož	
<b>1149/341</b>	<b>Pula</b>	<b>Gospa od Volam</b>	<b>Wraber (2001)</b>
1149/341	Pula	Kunfini	
1149/432	Pula	Cintinere	
1149/434	Pula	Cintinere	
1249/124	Premantura	Kamenjak	Tommasini (1873)
1249/142	Premantura	Kamenjak	
1249/213	Premantura	Kamenjak	
1249/231	Premantura	Kamenjak	

thropogenic influence in the habitat (On-line Supplement Tab 1.). Presuming that the largest populations reflect the optimal environmental conditions for this species, these observations are also in agreement with WAGNER (1990), who associates all *Ophioglossaceae* with early to middle succession.

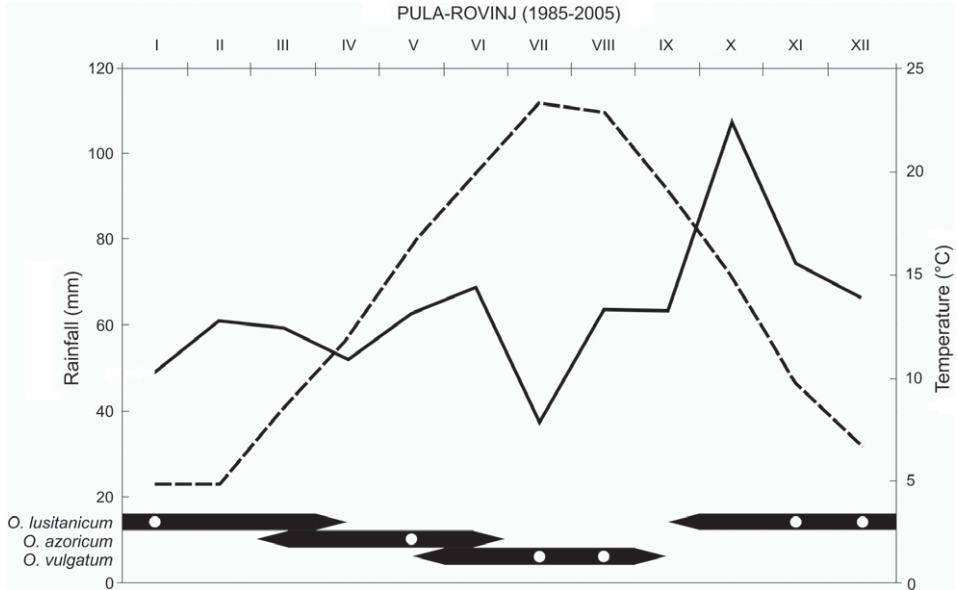


**Fig. 2.** Habitus (A), sporangia (B) and habitat of *Ophioglossum lusitanicum* L. on Kamenjak (C) (photo: S. Brana).

It is noteworthy that habitats of Cape Kamenjak, one of the richest localities of least adder's-tongue in Croatia, are regularly disturbed by grazing, tourist visits and even military exercises (performed yearly in autumn). A recent study on the effect of trampling on some rare species on the French island Quessant has revealed that *O. lusitanicum* favours low-medium intensity of trampling (KERBIRIOU et al. 2008). It has been found that for successful germination, spores of *O. lusitanicum* require variable periods of darkness (WHITTIER 1981). Therefore, pushing the spores deeper into the soil by trampling could account for the beneficial effects of this type of disturbance on this species.

All *Ophioglossum* species are believed to be dependent on mycorrhizal relationships in both gametophyte and sporophyte generations (WAGNER 1990). Due to the occurrence of heterotrophic life stages, the least adder's-tongue shares many of the habitat preferences with other myco-heterotrophic plants such as orchids, which are indeed abundantly present in the same localities (e.g. on Kamenjak, VUKOVIĆ et al. 2011, 2013). For the same reason, it is not rare to find several species of *Ophioglossum* growing in a mixed population, a so-called »genus community«, also usual for some other *Ophioglossaceae* (WAGNER and WAGNER 1983). In some cases, we have found *O. lusitanicum* and *O. azoricum* in such communities, where their different phenology served as a distinctive feature, along with some other (sometimes very subtle) differences. Regarding phenology, FREYN (1877) remarks that the least adder's-tongue appears in great numbers, but with a small number of fertile individuals.

Our observations of the phenology have revealed that the above-ground parts (Figs. 2A, B) emerge during winter and sporulation occurs during November-January, while the two other *Ophioglossum* species in Croatia show spring/summer reproductive seasons (Fig. 3). We have noticed that the number of fertile leaves can differ from year to year, sometimes even extremely, possibly due to the difference in the climate conditions. For example, we found approximately 1,000 fertile leaves in Sveta Foška in 2003, but only 30 fertile leaves in 2009. We observed the highest number of fertile leaves in winters with high moisture and mild temperatures, while during harsh winters fertile leaves appear in considerably smaller numbers. However, we did not compare exact climatic data with the number of fertile leaves. In our opinion, the appearance of fertile leaves should not be ascribed to one factor exclusively, and some other factors such as moisture in the soil and/or the condition of the mycorrhizal fungi could also be important in this context.



**Fig. 3.** Emergence of leaves (black arrows) and sporulation (white circles) of *Ophioglossum lusitanicum* L., *O. azoricum* Presl and *O. vulgatum* L. compared to climate in the period 1985–2005. The data for rainfall (solid line) and temperature (dashed line) throughout the year represent the average values between Pula and Rovinj meteorological stations.

### Conservation considerations

Cytological observations suggest that the genus *Ophioglossum* has undergone repeated cycles of polyploidy, and nowadays represents a group of species at the very edge of extinction, a possible evolutionary »dead end« (KHANDELWAL 1990). In Croatia, *O. lusitanicum* is estimated as critically endangered (MARKOVIĆ 2005) and accordingly legally strictly protected (ANONYMOUS 2013). The species is mainly threatened by anthropogenic influence in the habitat: pasture, tourism and infrastructure development (MARKOVIĆ 2005). However, our experience of more than 10 years of observations suggests that this is not the case. On the contrary, according to our observations, moderate trampling and grazing are in fact beneficial, and *O. lusitanicum* is favoured by such disturbance, which is in accordance with observations of WAGNER (1990) and KERBIRIOU et al. (2008).

In our opinion, active conservation measures are necessary to prevent the loss of this species from the national flora, and this especially applies to the conservation of habitats. *Ophioglossum lusitanicum* requires special conditions in the environment (disturbance and the presence of fungi), while at the same time its habitats are threatened by succession. Within this context, we recommend the development and implementation of an action plan for this species, which would include measures against natural succession, while keeping trampling at a medium level. In addition, we strongly support legal protection of the largest finding sites of *O. lusitanicum*.

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