First record of the fly orchid, *Ophrys insectifera* L. (*Orchidaceae*) for the flora of the Republic of North Macedonia

Slavčo Hristovski¹ and Slave Nakev²

¹Ss. Cyril and Methodius University, Faculty of Natural Sciences and Mathematics, Institute of Biology, Arhimedova 5, Skopje, Republic of North Macedonia, *slavco_h@pmf.ukim.mk

²Macedonian Ecological Society, Todor Skalovski 9A, Skopje, Republic of North Macedonia, nakev@mes.org.mk

Abstract

Ophrys insectifera L. (Fly Orchid) of the family Orchidaceae has European distribution although it is rarer towards south. The distribution and ecology in the central Balkans is little known. It was recorded in Jasen protected area at Kozjak locality (north-western parts of North Macedonia) in May 2021. This is the first record for the species in the flora of the Republic of North Macedonia. Data on the distribution, habitat preferences, and population size are presented in the article.

Key words: Ophrys insectifera L. (Fly Orchid), new record, North Macedonia, habitat, distribution

Introduction

Genus *Ophrys* L. is distributed from Macaronesia, Europe to Caucasus, and Mediterranean to South Turkmenistan (POWO 2024). Depending on the species concept genus *Ophrys* may contain nine phylogenetic species with numerous subspecies, 28 distinct species with fewer subspecies, or at least 350 "ethological" species (Delforge 2016; Bateman 2018; POWO 2024). The center of diversity of this genus is in the Mediterranean region (Pedersen and Faurholdt 2007; Delforge 2016). Based on the structural differences of the lip, i.e., according to the pollinating insect position on the lip during pseudocopulation, the genus is divided into two sections – *Pseudophrys* Godfery, with 12 species groups and *Euophrys* Godfery (nom. nud.), with 20 groups (Antonopoulos and Tsiftsis 2017).

The genus *Ophrys* in the Republic of North Macedonia is represented by five confirmed taxa: *Ophrys apifera* Huds., *Ophrys scolopax* subsp. *cornuta* (Steven) E. G. Camus, *Ophrys sphegodes* subsp. *sphegodes* Mill., *Ophrys sphegodes* subsp. *mammosa* (Desf.) Soó ex E. Nelson and *Ophrys helenae* Renz (Hristovski and Bouchet 2024).

Ophrys insectifera L. (Fly Orchid) of the family Orchidaceae (section *Euophrys,* species group *'insectifera'*) is a widespread but rare European species with mostly central European distribution which reaches central Scandinavia; it is considered very rare in the Mediterranean zone (Delforge 2016). Modelling of the species distribution suggests that it will disappear from South Europe and the Balkans, but it will gain areas in North Europe (Charitonidou et al. 2022). In May of 2021 a small population was found in the Jasen protected area. Thus, the aim of this paper is to present the first records of *Ophrys insectifera* in North Macedonia with notes on its habitat and threat status.

Materials and Methods

Fieldwork at Kozjak locality within Jasen protected area was part of the short botanical excursion of S. Hristovski (Skopje), G. Tomović (Belgrade) and M. Niketić (Belgrade) through different parts of North Macedonia conducted in May 2021. The species was identified already in the field according to the distinguishing features by Delforge (2016). No specimens were collected due to its small population size. The same population was observed by S. Nakev on 24.05.2021 and by S. Hristovski and S. Nakev on 19.05.2024. Photographs of the plants were taken by

digital camera.

The floristic composition of the habitat was analysed during the site visits. Nomenclature of the species follows Euro+Med PlantBase and Plants of the World Online database (Euro+Med 2006; POWO 2024). The floristic composition was used for identification of the plant community and habitat and based on published phytocenological data for the area.

Results and Discussion

Ophrys insectifera is a genetically isolated *Ophrys* species that belongs to the "*insectifera*" group. The species is sometimes considered as one taxon (Euro+Med 2006), or sometimes three subspecies are recognized (POWO 2024): nominotypical, *O. insectifera* subsp. *aymoninii* Breistr. an endemic of France and *O. insectifera* subsp. *subinsectifera* (C.E.Hermos. & Sabando) O.Bolòs & Vigo – endemic to Spanish and French Pyrenees. The population from North Macedonia shares the characteristics of nominal subspecies: *Ophrys insectifera* subsp. *insectifera*. Other

authors treat these three taxa as distinct species: *O. insectifera* L., *O. aymoninii* (Breistr.) Buttler and *O. subinsectifera* C.E.Hermos. & Sabando (Delforge 2016). The results of molecular analysis indicated a recent diversification in the three extant Fly Orchid taxa with presence of isolated populations on the Balkans (Triponez et al. 2013).

Ophrys insectifera is an easily recognizable species (Fig. 1). The following description including the dimensions is only from literature (Fay et al. 2015; Delforge 2016). It is perennial plant, 15-60(–80) cm tall. Tubers globose. Stem with 2-5 basal leaves, glabrous, and 1–2 small, sheathing (stem) leaves. Inflorescence lax, with 2-15(–20) flowers. Sepals subequal, pointed, yellow-green, hooded at tips, 6-9mm long, 3-4mm wide. Petals linear-filiform, purple-brown, velvety, 4–7mm long. Labellum rich brown, velvety, three-lobed, longer than wide, (8-)9-12mm long, (5-)6–10mm wide; lateral lobes elongated, rounded at tip; median lobe divided apically in two triangular lobes. Speculum is iridiscent blue. Chromosome number 2n =36. Flowers



Figure 1. Fly Orchid, Ophrys insectifera L., Kozjak, 22.05.2021 and 19.05.2024 (photo: S. Hristovski)



Figure 2. Distribution of *Ophrys insectifera* L. (Fly Orchid) in the Republic of North Macedonia.

from (April) May to July.

The pollination of flowers of *Ophrys insectifera* is achieved by sexual deception i.e. pseudocopulation like most of the species in genus *Ophrys*. However, *Ophrys insectifera* is one of the few *Ophrys* species which is pollinated by male wasps while others species are pollinated by solitary bees (Pridgeon et al. 2001). Primary pollinators are male digger wasps *Argogorytes* (Hymenoptera: Sphecidae): *A. mystaceus* (L.) and *A. fargei* (Schuckard) (Fay et al. 2015; Delforge 2016). The scent that is produced to attract pollinators differs among different populatios in the amounts of aliphatic hydrocarbons, methyl esters, short chain aliphatic 1alcohols and monoterpene alcohols (Borg-Karlson et al. 1993).

Distribution in North Macedonia

Ophrys insectifera was observed at the following locality (Fig. 2):

Karadzhica, Rudine, Kozjak, N41.889568°, E21.226139° (UTM EM13), 1060 m a.s.l., 22.05.2021, leg. S. Hristovski, G. Tomović, M. Niketić, (photographed, not collected)

The same population was observed by S. Nakev on 24.05.2021 and by S. Hristovski and S. Nakev on 19.05.2024 when 7 and 12 specimens were counted, respectively.

Habitat and ecology

The habitats of the species in its areal of distribution are usually grasslands, roadside verges, forest edges open forests but also wetlands, and dark forests on calcareous to neutral substrates, sometimes acidic, up to 1600 m a.s.l. (Fay et al. 2015; Delforge 2016). In the Balkan countries (Serbia and Bulgaria) it was mostly found on limestone substrates in communities of *Ostrya carpinifolia* and other low-growing deciduous woods and rarely in grasslands, forest clearings, etc. (Peev et al. 2015; Djordjević et al. 2017).

The specimens on Kozjak develop in the community Globulario-Centaureetum grbavacensis Matevski, Čarni, Ċušterevska, Kostadinovski et Mucina, 2015 (Matevski et al. 2015). Dominant plant species were Anthyllis aurea, Centaurea grbavacensis, Fumana procumbens, Juniperus oxycedrus, accompanied by: Globularia cordifolia, Ephedra major, Paronychia chionaea, Poa bulbosa, Matthiola fruticulosa subsp. vallesiaca, Helianthemum canum, Iris pumila, Thymus skopjensis., Muscari racemosum, Thalictrum minus, Carex liparocarpos, Genista januensis, Achillea ageratifolia subsp. serbica, Saxifraga federici-augusti subsp. grisebachii, Scorzonera austriaca, Viola herzogii, Haplophyllum albanicum, Achillea fraasii, Thesium linophyllon, Hypericum rumeliacum, Dactylis alomerata, Onosma heterophylla, Sesleria cf. commosa, Salvia ringens, Silene conica, Saponaria bellidifolia, Linum austriacum, Vincetoxicum hirundinaria, Globularia bisnagarica, Asphodeline lutea, A. taurica, Asphodelus albus, Papaver dubium, Ajuga Melampyrum arvense, etc. Scattered laxmannii, patches with trees are also present (Ostrya carpinifolia, Fraxinus ornus, Syringa vulgaris, Buxus sempervirens, Sorbus aria, Populus tremula, Quercus petraea, Cotoneaster nebrodensis, Pinus nigra, Pyrus communis subsp. pyraster, Cornus mas, Crataegus monogyna).

The following Orchid taxa were also present at the same locality: *Neotinea ustulata* var. *ustulata* (L.) R.M.Bateman, Pridgeon & M.W.Chase, *Neotinea tridentata* (Scop.) R.M.Bateman, Pridgeon & M.W.Chase, *Anacamptis coriophora* (L.) R.M.Bateman, Pridgeon & M.W.Chase, *Anacamptis pyramidalis* (L.) Rich., *Ophrys sphegodes* subsp. *mammosa* (Desf.) Soó ex E. Nelson, *Ophrys scolopax* subsp. *cornuta* (Steven) E. G. Camus, *Orchis purpurea* Huds. and *Orchis mascula*



Figure 3. Habitat of *Ophrys insectifera* L., Kozjak, 19.05.2024 (photo: S. Hristovski)

subsp. *mascula* (L.) L. *Platanthera chlorantha* (Custer) Rchb. and *Cephalanthera damasonium* (Mill.) Druce. were recorded in some of the small groups of trees. In total, 11 orchid species were recorded.

The habitat of *Ophrys insectifera* on Kozjak is actually calcareous grassland of Helleno-Balkanic *Satureja montana* steppes (code E1.21 according to the EUNIS classification, https://eunis.eea.europa.eu/ habitats/1845) that develops on dolomite bedrock (Fig. 3). According to the EU Habitats Directive this habitat is Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*). This habitat can be considered as prioriy (* important orchid sites) due to the presence of several Orchid species, including the single known locality of *Ophrys insectifera* in North Macedonia.

Distribution and threat status

Ophrys insectifera is rare species throughout its range in Europe with isolated populations on the Balkans. It is known from most of the Balkan countries: Bulgaria (Tsvetanov et al. 2005), Albania (Barina et al. 2018), Greece (Strid 2024), Serbia (Djordjević et al. 2021), Bosnia and Herzegovina (Šabanović et al. 2021), Croatia (Nikolić 2020) and Slovenia (Pezzetta 2018). So far, we are not aware of records from Kosovo and Montenegro.

It is included in the Appendix II of the CITES convention which covers species not necessarily threatened with extinction, but in which trade must be controlled in order to avoid utilization incompatible with their survival (CITES 2023).

The species is assessed as Least Concern according to the global assessment in the IUCN red list of Threatened species (Rankou 2011). However, it is declining in many countries. For example, the status of Least Concern was attributed to the species In the United Kingdom in 1999 but later on, due its population decline it received a status of a Vulnerable species (Fay et al. 2015).

In Bulgaria it is known from four locations, each of them with less than 25 individuals, hence assessing the species as Critically Endangered: CR [C2a(i); D] (Peev et al. 2015). *Ophrys insectifera* was not evaluated separately in the red list assessment of Albania but all of the *Ophrys* species were considered as Vulnerable – VU [A1b]. In Croatia it was also assessed as Vulnerable: VU (NT) [A4c] (Nikolić and Topić 2005). The status in Serbia was not presented in the Red Data Book of the Flora of Serbia (Stevanović 1999); but later on its status was assessed by Djordjević et al. (2017) as Endangered – EN B2ab(iii); D.

Ophrys insectifera in North Macedonia is confined to only one locality with less than 50 individuals. Consequently its status can be assessed as **Critically Endangered - B1a+2a; C2a(i,ii); D**.

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