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THE FIRST DISCOVERY OF THE POLLINATORS AND VISITORS OF *GYMNADENIA FRIVALDII* (ORCHIDACEAE) ON THE BALKAN PENINSULA

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Abstract

Gymnadenia frivaldii (Orchidaceae) is a subendemic taxon of the Carpathians and the Balkans, growing mainly in fen habitats of the high mountain areas. Although it is known that this is a nectar-producing species which attracts insects, the pollinators of this species have not been known and sufficiently researched so far. The study was conducted in July 2023 in the Kopaonik Mountains (Serbia) and the Rila Mountains (Bulgaria). A total of five pollinators and three visiting insects were recorded. In the Kopaonik Mountains, representatives of the order Diptera (Empididae: *Empis tessellata*, *E. dimidiata*, *E. discolor* and *Rhamphomyia magellensis*) were pollinators, while in the Rila Mountains, the orchid pollinator was *Coenonympha rhodopensis* (ordo Lepidoptera). Additionally, one genus (*Botanophila*) and five species (ordo Diptera) were found for the fauna of Serbia for the first time during this study.

Key words: orchid, pollinators, Diptera, Lepidoptera, Bulgaria, Serbia

Introduction. *Gymnadenia frivaldii* (Orchidaceae) was often assigned to the genus *Pseudorchis* (syn. *Leucorchis*) [1]. However, recent molecular phylogenetic analyses have shown that this taxon is well embedded in the genus *Gymnadenia* [1]. It is a subendemic taxon of the Carpathians and the Balkans,

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with a relatively small distribution range in Europe, restricted to the high mountain belts of the Carpathians and the central and eastern Balkans [2]. It has been recorded in northern Greece, North Macedonia, eastern Albania, Transylvanian Romania, southwestern Bulgaria, Montenegro, Bosnia and Herzegovina and Serbia [1–4]. This species inhabits mainly fen communities preferring siliceous bedrock types [5]. A recent study showed that this plant in western Serbia is an indicator of the vegetation class *Scheuchzerio-Caricetea fuscae* as well as of the schists-gneiss-phyllites type of geological substrates [5]. The species has been recorded in the following regions and localities in Serbia: Western Serbia (Golija Mtn), Central Serbia (Kopaonik and Željin Mts), Eastern Serbia (Stara planina Mtn), Southeastern Serbia (Vlasina, Besna Kobila and Dukat Mts) and Kosovo and Metohija (Prokletije and Šar planina Mts) [3, and the references therein], [6, and the references therein]. In Bulgaria, *G. frivaldii* is found on high mountains (Stara planina Mts, Vitosha Mtn, Rila Mts, Pirin Mts, Rhodope Mts, Osogovo Mtn) between 1200 m and 2400 m [7]. It is a strictly protected species with VU status in Serbia [3] and as such urgently requires research about its distribution, population ecology, threatening factors as well as pollination biology.

Gymnadenia frivaldii is a nectar-producing species that is rewarding for its pollinators [2]. According to some authors, the pollinators of this species are small moths and butterflies [8, 9]. However, detailed studies on the pollinators and visitors of this species have not been sufficiently carried out. Therefore, the aim of this study was to determine the pollinator diversity of this species and identify the insects that visit its flowers.

Material and methods. The study was conducted in July 2023 in the Kopaonik Mountains (Serbia) and the Rila Mountains (Bulgaria). The plant communities of the sites with *G. frivaldii* were determined according to Braun-Blanquet [10] methodology, whereas the habitat types were determined according to the EUNIS habitat classification (`http://eunis.eea.europa.eu/`). The bedrock types were determined using the geological map of the study area on a scale of 1:100 000. The population size of *G. frivaldii* at the studied localities was determined on the basis of counting the total number of flowering specimens. Only diurnal insects were investigated. Observations were done from 9 to 17 h. The time spent at each of the localities lasted from 20 min to 7 h. The examinations of morphological characteristics important for determination of Diptera, as well as the photographs of Diptera and pollinaria were done using Leica® Ivesta 3 stereomicroscope with mounted camera Leica® Flexcam C5. Photos of insects in the field were done using Nikon Coolpix L100. The collected insects are kept in the collection of Ana Nahirnić-Beshkova in National Museum of Natural History in Sofia (Bulgaria).

Results and discussion. During the studies in 2023 two localities in Serbia and seven localities in Bulgaria have been visited. All the localities with habitats and dates of visits are given below:

Serbia, Kopaonik Mts:

1. Kopaonik Mts, Jaram, 1793 m, N 43.301664°, E 20.823859°, 5–7 July 2023, exp. NW, incl. 15°, *Carici-Eriophoretum latifoliae* prov., EUNIS: D2.3 Transition mires and quaking bogs, HD 92/43: 7140 Transition mires and quaking bogs, porphyroid quartz monzonites and granites, 59 specimens within an area of ca. 30 m^2.

2. Kopaonik Mts, Sunčana Dolina, 1690 m, N 43.282500°, E 20.800325°, 6–7 July 2023, exp. W, incl. 25°, *Carici-Sphagno-Eriophoretum* R. Jovanović 1978, D2.3 – Transition mires and quaking bogs, porphyroid granodiorite with transitions to quartz monconites, 42 specimens within an area of ca. 200 m^2.

Bulgaria Rila Mts:

3. Rila Mts, Golyamo Pazardere, near "Ivan Vazov" mountain hut, 2310 m, N 42.190787°, E 23.285982°, 26 July 2023, almost all at the end of blooming period.

4. Rila Mts, Golyamo Pazardere, 2519 m, N 42.191361°, E 23.309722°, 26 July 2023, 180 specimens of *G. frivaldii* at 5 m^2 in a full bloom.

5. Rila Mts, Golyamo Pazardere, near stream, 2418 m, N 42.187674°, E 23.303356°, 26 July 2023, 400 specimens of *G. frivaldii* at 10–15 m^2 in a full bloom.

6. Rila Mts, Bistritsa river valley near "Macedonia" mountain hut, 2210 m, N 42.049531°, E 23.437852°, 25 July 2023, 40 specimens of *G. frivaldii*, second half of blooming period.

7. Rila Mts, Bistritsa river valley near "Macedonia" mountain hut, 2222 m, N 42.049947°, E 23.437838°, 25 July 2023, 190 specimens of *G. frivaldii*, second half of blooming period.

8. Rila Mts, Bistritsa river valley near "Macedonia" mountain hut, 2268 m, N 42.051586°, E 23.438240°, 25 July 2023, EUNIS: D2.3 Transition mires and quaking bogs, HD 92/43: 7140 Transition mires and quaking bogs, 520 specimens of *G. frivaldii*, second half of blooming period. This habitat type is considered EN in Red Data Book of Bulgarian habitats [11] and is protected by national law.

9. Rila Mts, river valley E of "Macedonia" mountain hut, 2344 m, N 42.058812°, E 23.452643°, 24 July 2023.

A total of five pollinators and three flower visiting insects were recorded. In the Kopaonik Mountains at Jaram, representatives of the order Diptera of the family Empididae (dance flies) – *Empis* (*Euempis*) *tessellata* Fabricius, 1794 males and females (Fig. 1a, b), *Empis* (*Leptempis*) *dimidiata* Meigen, 1835 males, *Empis* (*Leptempis*) *discolor* Loew, 1856 males and *Rhamphomyia* (*Lundstroemiella*) *magellensis* Frey, 1922 males – were identified as orchid pollinators (Fig. 1c, d), whereas the visiting insects were *Melanthia procellata* ([Denis & Schiffermüller], 1775) (Lepidoptera: Geometridae), *E. dimidiata* females, *Botanophila fugax* (Meigen,

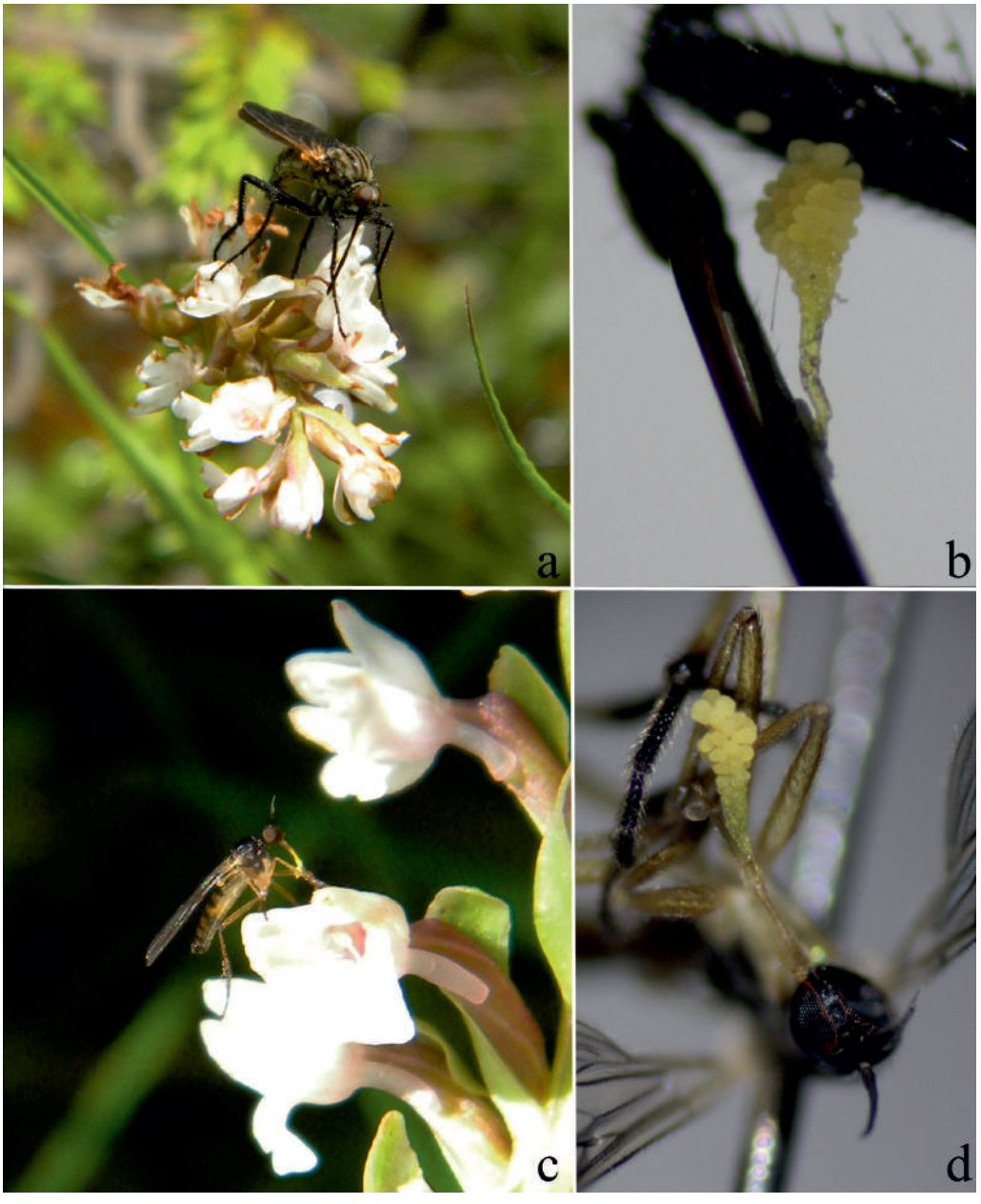

Fig. 1. a – *Empis tessellata* nectaring on *Gymnadenia frivaldii* while having pollinarium on its proboscis; b – pollinarium of *G. frivaldii* on its proboscis; c – *Rhamphomyia magellensis* nectaring on *G. frivaldii*; d – pollinarium of *G. frivaldii* on its proboscis. Kopaonik Mts, Jaram, 07 July 2023. (Photos: Ana Nahirnić-Beshkova)

1826) (Diptera: Anthomyiidae) and *Parydra littoralis* (Meigen, 1830) (Diptera: Ephydridae).

Empis tessellata has trans-Palaearctic distribution. The adults are nectar feeders and are commonly found on flowers. However, the males of *E. tessellata* are also predators [12]. *Empis dimidiata* and *Empis discolor* are known only from mountains of Central and South Europe [13]. Both males and females frequently visit flowers.

Rhamphomyia magellensis is a mountain species distributed in mountains of Central and South Europe: Pyrenees [14], and Austria, Czechia, Slovakia, Poland, Italy, Slovenia and Switzerland [15]. Both males and females are exclusively flower visitors, observed sucking nectar of *Valeriana* and *Geranium* [14].

Rhamphomyia magellensis has shorter proboscis than species of *Empis* (ca. 1 mm (Fig. 1d)), however, it has tiny body and can easily get inside the flower (Fig. 1c). Females of *E. dimidata* are potential pollinators as males and females of other species of the same genus are also pollinators. *Melanthia procellata* is potential pollinator as well as it has long enough proboscis and it was noticed to visit flowers of *G. frivaldii* on two occasions. *Parydra littoralis* is rather incidental visitor. Pseudotracheae of the genus *Parydra* is adapted to scraping, not sucking or licking.

Coenonympha rhodopensis Elwes, 1900 (Lepidoptera: Nymphalidae) (the pollinator on Rila Mts) was also present at Sunčana Dolina (Kopaonik Mts) but none of 10 caught specimens carried pollinaria. *Coenonympha rhodopensis* and *Erebia* spp. are potential pollinators at Sunčana Dolina because of variety of habitats and nectaring plants, while at Jaram they are not expected because of not suitable environmental conditions as shade most part of the day and poor nectar resources.

On the Rila Mountains, the orchid pollinator was *Coenonympha rhodopensis*, while the insect visitor was *E. oeme* (Hübner, [1804]) (Lepidoptera: Nymphalidae). At Golyamo Pazardere, near stream numerous pollinators were determined as *Coenonympha rhodopensis* males and females. When disturbed *C. rhodopensis* was also visiting small white *Trifolium* sp. Near "Macedonia" mountain hut at 2210 m pollinators were *C. rhodopensis* males and females, visitor *E. oeme*. At the same locality, there were also several *Gymnadenia rhellicani* (Teppner & E. Klein) Teppner & E. Klein, but it was visited by *E. oeme* and not by *C. rhodopensis*. Near "Macedonia" mountain hut at 2222 m, pollinators were *Coenonympha rhodopensis*, and the visitor was *E. oeme*. There were also several *G. rhellicani* there but it was visited by *E. oeme* and not by *C. rhodopensis*, males and females. *Erebia euryale* (Esper, 1805) male was observed to carry pollinaria of *G. rhellicani*. Near "Macedonia" mountain hut at 2268 m, pollinators were *C. rhodopensis* males and females and visitor was *E. oeme* male. There were also several *G. rhellicani* there but it was visited by *E. oeme* and not by *C. rhodopensis*. *Coenonymha rhodopensis* is a common species in high mountains in Bulgaria, while *E. oeme* is common and widespread species in Bulgaria [16].

In addition, a genus (*Botanophila*) and five species of Diptera (Empididae) were discovered for the fauna of Serbia for the first time during this study: *E. tessellata*, *E. dimidiata*, *E. discolor*, *Rh. magellensis* [17] and *B. fugax*.

The fact that five species of pollinators recorded in Serbia were not known for the country so far, shows that its pollinators are among poorly studied groups.

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