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THE FIRST RECORD OF *EUCERA TRICINCTA* ERICHSON, 1835 (HYMENOPTERA: APIDAE) AS A POLLINATOR OF AN ENDANGERED ORCHID *HIMANTOGLOSSUM FORMOSUM* (STEVEN) K. KOCH (ORCHIDACEAE)

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Summary. A male of *Eucera* (*Synhalonia*) *tricincta* Erichson, 1835 was recorded as the first known flower visitor and pollinator of *Himantoglossum formosum* (Steven) K. Koch. The bee specimen was carrying four pairs of the orchid pollinaria, three of which were with partially spent massulae. About a half (48.6%) of all flowers of *H. formosum* in the locality under study were observed to be visited by pollinators and 22.9% were pollinated. It is comparable to other species of *Himantoglossum* Spreng. except *H. caprinum* (M. Bieb.) Spreng., which has significantly lower pollination rate. *Himantoglossum formosum* is highly threatened in Russia, being estimated as endangered taxon, according to the IUCN categories and criteria. However, the rarity of the species is apparently not related to its pollination ecology.

Key words: bees, ecology, pollination, Dagestan, Caucasus, Russia.

С. П. Иванов, А. В. Фатерыга, С. А. Свирин, Р. А. Муртазалиев, В. В. Фатерыга. Первая находка *Eucera tricincta* Erichson, 1835 (Hymenoptera: Apidae) как опылителя находящейся под угрозой исчезновения орхидеи *Himantoglossum formosum* (Steven) K. Koch (Orchidaceae) // Дальневосточный энтомолог. 2024. N 506. С. 10-16.

Резюме. Впервые зарегистрирован посетитель цветков и опылитель *Himantoglossum formosum* (Steven) K. Koch – самец пчелы *Eucera* (*Synhalonia*) *tricincta* Erichson, 1835. Этот экземпляр нес на себе четыре пары поллинариев орхидеи, три из которых были с

частично израсходованными массулами. Около половины (48.6%) всех цветков *H. formosum* в изученном местонахождении оказались посещенными опылителями, а 22.9% – опыленными. Это сравнимо с другими видами *Himantoglossum* Spreng., за исключением *H. caprinum* (M. Bieb.) Spreng., уровень опыления которого значительно ниже. *Himantoglossum formosum* – крайне угрожаемый в России вид, оцененный как находящийся под угрозой исчезновения, согласно категориям и критериям МСОП. Однако редкость данного вида вряд ли связана с его экологией опыления.

INTRODUCTION

The bees (Hymenoptera: Apoidea: Anthophila) are the most important group of the orchid pollinators worldwide. Among about 2,900 orchid species studied so far, 57.5% are pollinated by various bees (Ackerman *et al.*, 2023). The total number of orchid species is, however, estimated to be almost ten times more (28,000 according to Christenhusz & Byng, 2016). Thus, the pollinators of the major diversity of orchids are still unknown. The orchid genus *Himantoglossum* Spreng. (Orchidaceae: Orchidoideae: Orchideae), distributed from Atlantic Europe through the Mediterranean to the Caucasus and Crimea, contains nine species and one additional subspecies (Bateman *et al.*, 2017; POWO, 2024). Representatives of the genus are very spectacular orchids, producing substantial spikes of showy flowers. Morphology, phylogeny, taxonomy, and distribution of *Himantoglossum* were recently studied in detail (Delforge, 1999; Sramkó *et al.*, 2014; Bateman *et al.*, 2017). However, few species were sufficiently studied in respect of their pollination ecology.

All representatives of the genus are cross-pollinating and nectarless orchids, exploring a food-deceptive attraction of the pollinators, which are always bees. All species except the basalmost *Himantoglossum comperianum* (Steven) P. Delforge have joint viscidia, so that both pollinaria can be removed only simultaneously (Claessens & Kleynen, 2011). Most studies of the pollination ecology (reviewed by Claessens & Kleynen, 2011) were dedicated to two species of *Himantoglossum*: *H. hircinum* (L.) Spreng. and *H. adriaticum* H. Baumann. These species are pollinated mainly by bees of the genus *Andrena* Fabricius, 1775 (Andrenidae), as well as the honeybee (*Apis mellifera* Linnaeus, 1758, Apidae) (Claessens & Kleynen, 2011, 2016; Biró *et al.*, 2015). The only known pollinator of *H. calcaratum* subsp. *rumelicum* (H. Baumann & R. Lorenz) Niketić & Djordjevic (erroneously reported as *H. caprinum* (M. Bieb.) Spreng.) is also the honeybee (Claessens & Kleynen, 2011). Basal species of the genus *Himantoglossum*, such as *H. comperianum*, as well as *H. robertianum* (Loisel.) P. Delforge and *H. metlesicsianum* (W. P. Teschner) P. Delforge, are reported to be pollinated mainly by bees of the genera *Bombus* Latreille, 1802 and *Eucera* Scopoli, 1770 (Apidae) (Ivanov *et al.*, 2009; Claessens & Kleynen, 2011). *Himantoglossum caprinum* is known to be pollinated mainly by bees of the genus *Megachile* Latreille, 1802 (Megachilidae) but also *Anthidium* Fabricius, 1804 (Megachilidae) and *Eucera* as well (Ivanov *et al.*, 2009, 2011).

Himantoglossum formosum (Steven) K. Koch is a Caucasian endemic, known from Dagestan (Russia), southernmost Armenia, Azerbaijan, and northwestern Iran (Grossheim, 1940; Renz, 1978; Averyanov, 2006; Vakhrameeva *et al.*, 2008; Delforge, 2016), being recently discovered also in easternmost Georgia (Lazarovič, 2019). It is the rarest and perhaps the least known orchid of the Caucasian region (Molnár *et al.*, 2021), as well as one of the rarest species of the genus *Himantoglossum*. There was a positive distributional change observed for *H. formosum* in Russia in the middle of the XX century, but after that it had neutral dynamics (Efimov, 2023).The estimated IUCN Red List category for *H. formosum* in Russia is EN B2ab(iii) (Murtazaliev, in preparation). Nothing was previously known on its pollination ecology. The purpose of the present study is to report preliminary data on the pollinators and the rate of visited and pollinated flowers of *H. formosum*.

MATERIAL AND METHODS

Field observations were carried out in the Republic of Dagestan on 11, 17, and 21 June, 2021. A population of *Himantoglossum formosum* was found on northeastern slopes of the Narat-Tyube Range in the vicinity of Makhachkala [42°56′20″N, 47°23′30″E]. The habitat was a forest consisted of oak (*Quercus petraea* (Matt.) Liebl.) and ash (*Fraxinus excelsior* L.), mixed with elm (*Ulmus pumila* L.) (Fig. 1). The herbaceous layer was predominated by *Elytrigia repens* (L.) Nevski (Poaceae).

There were 24 generative specimens of *H. formosum* observed: five solitary plants (Fig. 2) and two groups, of four and 15 (Fig. 3) specimens each. The distance between specimens was 12–46 cm in the small group and 7–26 cm in the large one; the distance between the groups was 68 m. Solitary specimens were in 8.1 and 9.8 m from the small group and in 0.9, 12.0, and 13.5 m from the large group.

Figs 1–4. *Himantoglossum formosum* (Steven) K. Koch in the vicinity of Makhachkala, Dagestan: 1 – habitat; 2 – specimen in full flower; 3 – group of flowering specimens; 4 – part of inflorescence.

Specimens of *H. formosum* were in full flower on 11 June (Figs 2–4) and finishing their flowering on 21 June. Possible pollinators (bees) were collected, with an entomological net, on all three days by three methods: observations at flowering individuals of *H. formosum*, observations at flowers of other plant species, and sweeping the surrounding vegetation.

Condition of flowers was studied at the end of the flowering period of *H. formosum* (on 21 June), with the help of a hand lens. Every flower of 22 specimens was examined (a total of 463 flowers); two remaining specimens were not studied because they had been damaged by a cow or a sheep. Six conditions of flowers were recorded (due to the joint viscidia of this species, there were no flowers in which one pollinarium was removed but another one still present): 0 – virgin flowers (not visited by a pollinator): pollinaria present and stigma without massulae; 1 – flowers of the first date (visited but not pollinated): pollinaria removed by a pollinator but stigma still without massulae (i.e., such a flower is the first flower of *H. formosum* visited by a certain specimen of the pollinator); 2 – pollinated flowers with removed pollinaria and massulae present on both lobes of stigma (since all other orchid species at this locality had been already faded, all these massulae were considered belonging to *H. formosum*); 3 – pollinated flowers with pollinaria still present but with massulae present on both lobes of stigma; 4 – pollinated flowers with removed pollinaria and massulae present on either lobe of stigma; 5 – pollinated flowers with pollinaria still present but with massulae present on either lobe of stigma. The pollination rate was calculated as the sum of the rates of flowers of the 2nd–5th conditions. The repetition index was calculated as the sum of the rates of flowers of the 1st–5th conditions dividing by the rate of flowers of the 1st condition. This index means how many orchid flowers are visited by a certain specimen of the pollinators on average (Svolynskiy *et al.*, 2014).

Photographs were made with a Canon EOS RP digital camera and a Sigma AF 105 mm f/2.8 macro lens with a Yongnuo YN-14EX macro flash. Focus stacking images of the pinned bee specimen were created with CombineZP software.

RESULTS

A male of *Eucera* (*Synhalonia*) *tricincta* Erichson, 1835 (Hymenoptera: Apidae) was recorded as the only pollinator of *Himantoglossum formosum* during three days of observations. It was collected on 17 June at the orchid itself, after visiting a flower. The male was with four pairs of pollinaria attached to the frons (Figs 5–6). The uppermost, freshly extracted pair contained complete amount of massulae. In three other pairs of the pollinaria, some massulae had been already spent. This means that the specimen had not only removed these three pairs of pollinaria before was captured but had already pollinated some flowers. No bee specimens with pollinaria of *H. formosum* were recorded both at flowers of other plant

Figs 5–6. Male of *Eucera tricincta* Erichson, 1835 with pollinaria of *Himantoglossum formosum* (Steven) K. Koch: 5 – lateral view; 6 – frontal view.

species and during a sweeping process. Other specimens of *E. tricincta* (males and females without orchid pollinaria) were recorded at flowers of *Phlomis herba-venti* subsp. *pungens* (Willd.) Maire ex DeFilipps (Lamiaceae); it was apparently the only forage plant for this bee species in this locality.

Inflorescences of *H. formosum* contained 8–33 flowers (mean = 21.0 ± 2.8; n = 22; p = 0.05). About a half (48.6%) of all flowers in the 22 examined inflorescences were visited by pollinators (Fig. 7). Of them, 25.7% of flowers were not pollinated (flowers of the first date) and 22.9% were pollinated. The repetition index amounted to 1.9. This means that each specimen of the pollinators visited about two flowers of *H. formosum* on average.

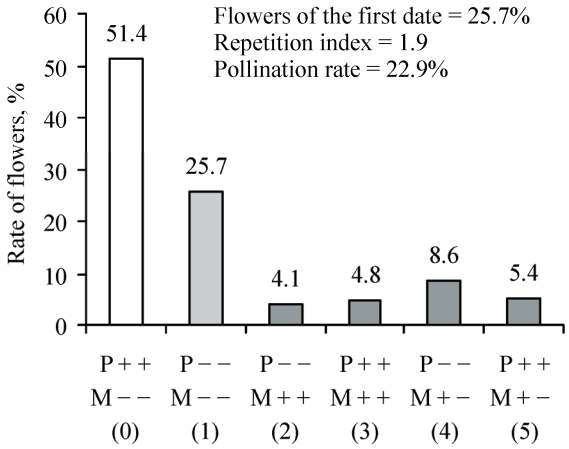

Fig. 7. Rate of virgin, visited, and pollinated flowers of *Himantoglossum formosum* (Steven) K. Koch in the vicinity of Makhachkala, Dagestan: 0 – virgin flowers: pollinaria (P) present, stigma without massulae (M); 1 – flowers of the first date (visited but not pollinated): pollinaria removed, stigma without massulae; 2–5 – pollinated flowers with either removed (2, 4) or present (3, 5) pollinaria and massulae present either on both lobes (2, 3) or either lobe (4, 5) of stigma.

DISCUSSION

Eucera tricincta is the first known flower visitor and pollinator of *Himantoglossum formosum*. It is not surprising that a species of the genus *Eucera* pollinates this orchid. Representatives of this bee genus were previously reported as pollinators of other species of *Himantoglossum*, such as *H. comperianum*, *H. caprinum*, and *H. robertianum* (Ivanov *et al.*, 2009, 2011; Claessens & Kleynen, 2011). In the two latter cases, even the same species, *E. tricincta*, was recorded among the pollinators. Preliminary data suggests that this bee species is an efficient pollinator of *H. formosum*. The rate of pollinated flowers observed (22.9%) is not very high but comparable to other species of the genus *Himantoglossum*, studied so far. The median pollination rate of *H. adriaticum* is 17.1% (based on Claessens & Kleynen, 2011 and Biró *et al.*, 2015), while it is 25.3% for *H. hircinum* and 35.9% for *H. robertianum* (Claessens & Kleynen, 2011). Much higher values are reported only for *H. comperianum* (48.2%) and *H. metlesicsianum* (54.8%) but these data are based on no more than two observations in each case (Ivanov *et al.*, 2009; Claessens & Kleynen, 2011).

The median rate of pollinated flowers is much lower in *H. caprinum* – 5.9% (Ivanov *et al.*, 2011). The median repetition index of *H. caprinum* (1.5) is also lower than that observed in *H. formosum*. At the same time, *H. caprinum* is much more common species than *H. formosum*, with sometimes very large populations (Ivanov *et al.*, 2011). Moreover, *H. caprinum* is distributed much wider, from the Peloponnese and the Aegean Islands through Asia Minor and the Levant to Crimea, the Black Sea coastal area of the Caucasus, Iraq, and Iran (Delforge, 2016; POWO, 2024), while *H. formosum* is a Caucasian endemic and one of the rarest Caucasian orchids. Despite the rarity of the latter species, it has not the lowest pollination rate among the relative species. Therefore, *H. formosum* is so rare and endangered due to apparently other, still unknown circumstances.

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