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***Ophrys sphegodes* subsp. *helenae* (Renz) Soó & D.M.Moore (Orchidacea): a new subspecies for the Bulgarian flora**

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**Abstract:** *Ophrys sphegodes subsp. helenae* (Renz) Soó & D.M.Moore is a Balkan endemic, for which so far there are data for distribution only on the territory of 2 countries: Greece and Albania. During a study of the flora of the Voyvodsko plateau, located in the northeastern part of the Republic of Bulgaria, in April 2019 a locality of this species was discovered. The development of the population in the period April 2019 - May 2021 was monitored. The population consists of 3 individuals located on an area of 0.5 m<sup>2</sup>. Of these, 2 individuals are in a generative state and 1 individual is in a vegetative state. The abiotic and biotic conditions in the habitat are described. It is characterized by a rich floristic diversity: the associated species are 60 in number. Anthropogenic impact has been reported. The prospects for the existence of the population are indicated. It is proposed to declare the territory around the locality a protected area in order to preserve the habitat from negative anthropogenic impacts.

**Keywords:** Voyvodsko Plateau, Balkan endemic species, habitat characteristics, conservation measures

## Introduction

The genus *Ophrys* L. is a principally Mediterranean genus, ranging from the Canaries to the Caspian Sea and from Southern Scandinavia to the Northern Maghreb. It includes 252 species distributed worldwide [1]. Of these, 20 species are distributed in Europe [2], and 5 species are distributed on the territory of the Republic of Bulgaria: *O. apifera* Huds., *O. scolopax* subsp. *cornuta* (Steven) E.G.Camus (syn. *O. cornuta* Steven), *O. insectifera* L., *O. sphegodes* subsp. *mammosa* (Desf.) Soó ex E.Nelson (syn. *O. mammosa* Desf.), *O. reinholdii* Spruner ex Fleishm. [3].

The species of the genus have the following characteristics: 2 (-3) root tubers, entire, ovoid to ellipsoid; bracts leaf-like; lateral sepals spreading; petals elongated, very different to sepals; lip without a spur, orientated downwards, entire or lobed, prominently hairy, hairs short, straight, in uniform layers, and a glossy, often hairless zone (= speculum), with a central symmetry, the ensemble, without any equivalent in Orchidoidae, resembling the body of an insect; rostellum 3-lobed, median lobe short, lamellar, separating the loculi at its base; 2 retinacles enclosed by 2 separate bursicles; ovary sessile, twisted; a chromosome count of  $2n = 36, 72$ . In case of *O. helenae* lip entirely cherry-red, apparently without a speculum.

The spectacular and varied flowers mimic both the body and the scent (pheromones) of female insects and this provokes the males to attempt to mate with the flowers (pseudocopulation). This highly specialised adaptation is directed towards a few genera of bees and wasps. It has produced intense selection pressures and an exceptional adaptive radiation, probably accentuated by the small number of effective pollinations, which are rare due to the complexity of the mechanisms for attracting pollinators.

It is very isolated and it does not form intergeneric hybrids. Hybrids between *Ophrys* species are rather frequent; there are more than 450 hybrids which have already been described. *Ophrys* hybrids are not generally too difficult to identify if they originate from parents belonging to unconnected groups or with different coloration or morphology (green sepals x pink sepals for example).

The genus is traditionally divided into two monophyletic sister sections, separated by the position of the pollinating insect on the lip during pseudocopulation, which determines whether the head or the body pick up the pollinia. Section Pseudophrys Godfery, in which the abdomen picks up the pollinia (the fusca-lutea-omegaifera complex), and section Euophrys Godfery (nom. nud.), in which the pollinia are attached to the insect's head (all other species).

Within **section Euophrys**, 6 major radiations are rather clearly defined and these have been ± confirmed by molecular analyses. One of these groups is ***Ophrys sphegodes* complex**. The characteristic of

this group is those petals elongated and hairless, margins undulate; marginal band of hairs on lip prominent and often complete; appendage triangular,  $\pm$  reduced, with a clean texture, inserted into a notch. Here they refer 45 species in 6 groups, mostly associated with bees in the families Andrenidae and Megachilidae [1].

One of these 6 groups is *Ophrys mammosa* group. The characteristics of the *O. mammosa* group are the following: Lateral sepals  $\pm$  clearly bicoloured; petals elongated, villous to finely velvety, rarely hairless; lip with a reduced marginal band of hairs, visible primarily on shoulders, clearly attenuated distally; micro-hairs of lip give a uniformly velvety appearance to centre and distal half; pattern of base of speculum often H shaped or forming 2  $\pm$  parallel vertical lines, joined to walls of stigmatic cavity; appendage with the same texture as sides of lip, usually not inserted into a notch but imperceptibly extending, sides; stigmatic cavity cup-shaped, rounded, I strongly constricted at base, with a pale specular stage; pseudo-eyes present, colour varied, located at junction of internal and external lips.

**The key to the species in the *O. mammosa* group is the following (In this key *O. sphegodes* subsp. *helenae* (Renz) Soó & D.M.Moore is represented by its synonym *O. helenae* Renz) [1]:**

- |   |   |
|---|---|
| 1 lip with a speculum.....2   | 5 basal field greenish, much paler than centre of lip..... <i>O. montenegrina</i> |
| 1* lip entirely cherry-red, apparently without a speculum..... <i>O. helenae</i>                                | 5* basal field concolourous with centre of lip..... <i>O. negadensis</i>          |
| 2 speculum central, no extension towards stigmatic cavity.....3   | 6 basal field pale brown, orange or rusty, lighter than centre of lip.....7       |
| 2* speculum with extensions reaching stigmatic cavity.....4   | 6* basal field blackish to pale brown, concolourous with centre of lip.....10     |
| 3 distal half of lip slightly convex transversally, sides often reflexed forwards..... <i>O. ferrum-equinum</i> | 7 early flowering, I-III (-IV) .....8   |
| 3* distal half of lip very convex, sides strongly turned under..... <i>O. gottfriediana</i>                     | 7* late flowering, (IV-) V-VI.....9   |
| 4 lip with an almost complete submarginal band of hairs.....5   | 8 pseudo-eyes dull, greenish..... <i>O. herae</i>                                 |
| 4* lip velvety on distal half.....6   | 8* pseudo-eyes shiny, centre blackish..... <i>O. alasiatica</i>                   |
|   | 9 plant robust; lateral sepals 12-18 mm long.....                                 |

..... <i>O. caucasica</i>	appearing 3-lobed..... <i>O. morio</i>
9* plant spindly; lateral sepals 9-12 mm long .....	17 lip 9-12 mm long..... <i>O. iceliensis</i>
..... <i>O. grammica</i>	17* lip 12-18 mm long.....18
10 pseudo-eyes pale, linked to the specular stage	18 petals 5-7 mm long..... <i>O. antalyensis</i>
on the floor of the stigmatic cavity.....11	18* petals 7-14 mm long..... <i>O. amanensis</i>
10* pseudo-eyes dark or pale but not linked to	19 lip with a wedge-shaped elongated base.....
specular stage.....12	..... <i>O. gortynia</i>
11 lateral sepals dark, olive-green, ± bicoloured	19* lip with a rounded base.....20
..... <i>O. leucophthalma</i>	20 lip 7-12 mm long, edged yellow; speculum
11* lateral sepals pale, uniform or faintly blotched	complex, extensive, often with 1-3 central ocelli
Reddish..... <i>O. aesculapii</i>	..... <i>O. hebes</i>
12 tip of column rather elongated, tending to	20* lip otherwise; speculum simpler, H-shape or 2
curve upwards.....13	longitudinal parallel lines.....21
12* tip of column short, straight.....19	21 main flowering season II-IV.....22
13 tip of column very elongated, (1.5-) 2-4 mm	21* main flowering season V-VI.....25
long.....14	22 lip 6-11 mm long..... <i>O. cretensis</i>
13* tip of column slightly elongated, 0.8-1.5 (-2)	22* lip (10-) 11-30 mm long.....23
mm long..... <i>O. cyclocheila</i>	23 lip 18-35 mm long..... <i>O. sphaciotica</i>
14 main flowering season II-IV.....15	23* lip (10-) 11-17 mm long.....24
14* main flowering season V-VI.....17	24 dorsal sepal pink..... <i>O. spruneri</i>
15 lip 8-11 mm long..... <i>O. hittitica</i>	24* dorsal sepal green..... <i>O. mammosa</i>
15* lip 12-19 mm long.....16	25 lip 7-10 (-11) mm long..... <i>O. epirotica</i>
16 lip globular in centre, optically oval.....	25* lip 10-20 mm long.....26
..... <i>O. transhyrcana</i>	26 lip 10-14 mm long..... <i>O. macedonica</i>
16* lip very convex transversally on distal half,	26* lip (14-) 16-20 mm long..... <i>O. hystera</i>

***O. sphegodes* subsp. *helenae* (Renz) Soó & D.M.Moore**

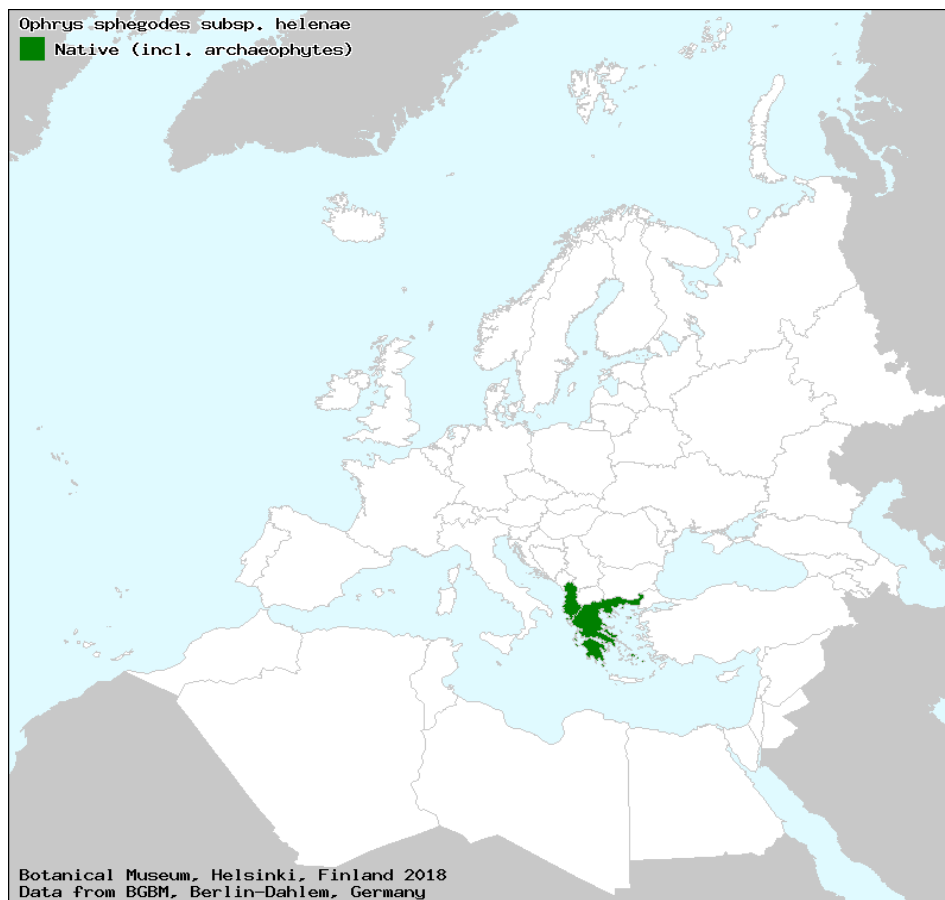
**Synonyms:** *Ophrys helenae* Renz, *Ophrys sphegodes* var. *helenae* (Renz) H.Sund., *Ophrys plakotiana* C.Thiele, G.Thiele, U.Thiele & W.Thiele [4], *Ophrys mammosa* subsp. *helenae* (Renz) Soó [5].

**Description:** Plant robust and slender, 15-40 cm tall; inflorescence dense to near lax; 2-8 flowers; sepals oval, 10-16 mm x 5-8 mm, rather pale, whitishgreen or yellowish, dorsal sepal sometimes curved forwards, lateral sepals spreading, sometimes washed pale violet; petals pale green to ochre, hairless to velvety, narrowly triangular, sometimes auriculate, 6-13 mm x 2-4 mm, margins straight to undulate; lip entire, orbicular to broadly oboval, 11-18 mm x 15-24 mm (when spread), globular, usually without basal swellings, cherry red to pale reddish-brown, velvety, submarginal band of hairs very attenuated and sometimes ochre (short and whitish on shoulders), bordered by a thin, paler, sometimes yellow, hairless margin; speculum lacking or concealed under the micro-hairs, sometimes visible, but then reduced to 2 unobtrusive bluish streaks at base of stigmatic cavity; appendage very small to absent, triangular, greenish-yellow to crimson-brown, prolonging margins of lip; basal field sometimes slightly darker than centre of lip; stigmatic cavity concolourous with basal field, lacking a distinct specular stage, relatively small, slightly constricted at base; pseudo-eyes reduced, whitish, hardly visible; column short [1]. Albifloric forms of this subspecies were also found in 2016 in Dodoni, Greece [6].

**Flowering season:** In Europe, this period is relatively long, lasting from late March to early June. Its main month of flowering is usually May [7].

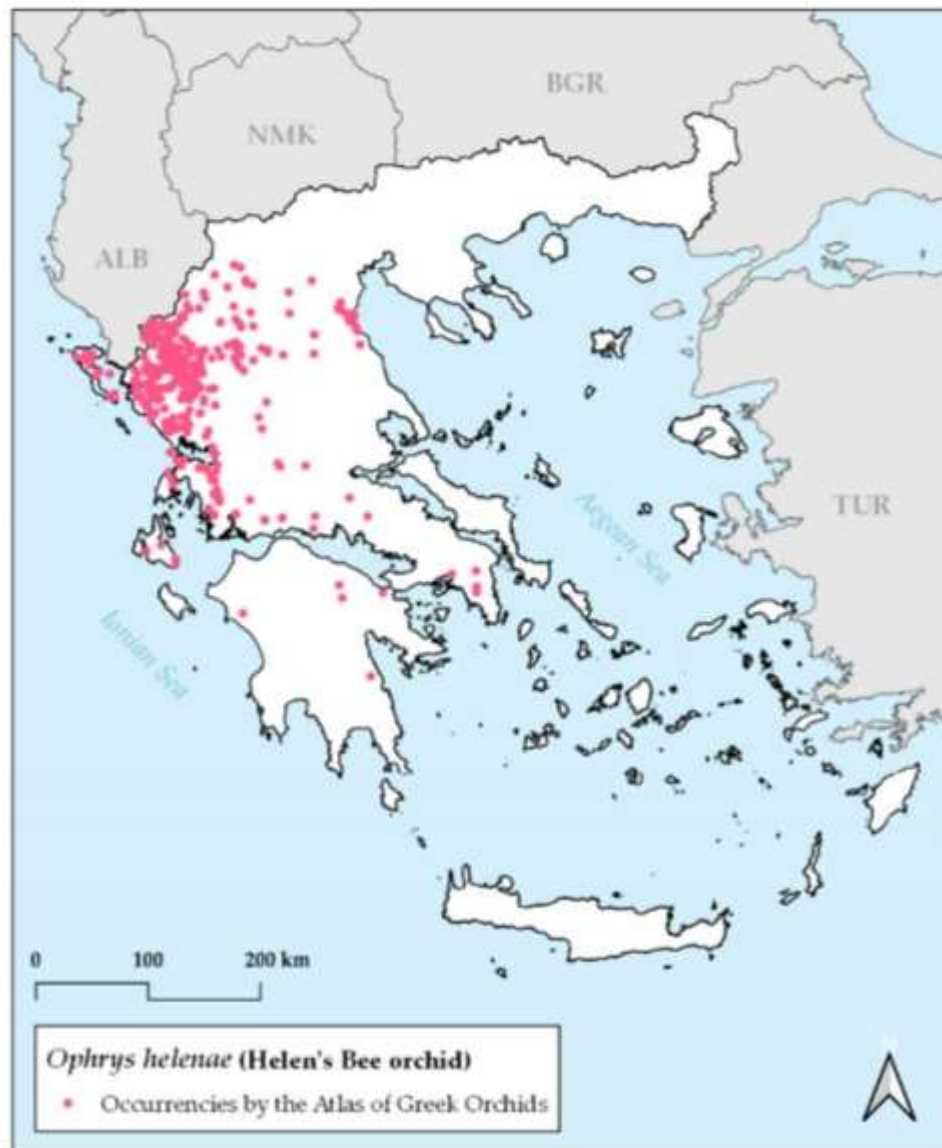
**Countries:** Greece, Albania (Fig. 1).

**Detailed distribution:** Northwest Greece, Southern Albania; from Corfu and Cephalonia east to Thessalia, south to the Gulf of Corinth and north to Kastoria; centre of range in Epirus. Also reported from a station in the Peloponnese (Ilia). Rather local but sometimes abundant [1, 4].



**Figure 1.** Map of world distribution of *O. sphegodes* subsp. *helenae*  
(The map is from The Euro+Med PlantBase [8])

*O. sphegodes* subsp. *helenae* was first described in 1928 on the island of Corfu by Dr. Jany Renz as *O. helenae* [9]. Its known distribution on the territory of Greece until the 80s of the 20th century was limited to Epirus (Northwestern Greece) and the island of Corfu [10, 11]. However, in the last few decades, its spread has expanded, with new populations being established in eastern and southern Greece (Fig. 2) [12, 13]. This observation is intriguing because it seems to contradict biological models, according to which climate change can be expected to expand the area to the north. Chen et al. [14] indicate that the average rate of increase in the area to the north by about 17 km per decade and the increase in altitude by 11 meters per decade. Although most species follow this trend, there are species (mainly taxa from forest plants) that have moved in the opposite direction: to the south or at lower altitudes [14, 15].



**Figure 2.** Map of distribution of *O. sphegodes* subsp. *helenae* in Greece, according to the Atlas of Flora of Greek Orchid [12]

**Habitats:** The species can be found in a variety of habitats and given that in some areas it can often be the sole orchid species present, appears to tolerate conditions unsuited to others [7]. It prefers full sun to mid-shade on dry to moist, alkaline substrates. Short grassland, garrigue, scrub, open woodland, up to 1000 m asl. [1].



**Ecology:** *Ophrys* species are characterized by sexual deception as a pollination mechanism. By capturing visual and olfactory signals, they deceive male individuals from Hymenoptera to pseudocopulation and through this procedure they achieve pollination and thus, reproduction [16]. *O. sphegodes* subsp. *helenae* is the only known exception to this rule. This species does not rely on sexual pollination fraud, but on imitation shelter [16, 17].

**Pollinator:** Probably *Eucera longicornis* (Linnaeus, 1758) (Hymenoptera: Anthophoridae) [1].

**Conservation status:** *O. sphegodes* subsp. *helenae* is included in Annex 2 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora [18] and in The IUCN Red List of Threatened Species in the category Least Concern (LC) [19].

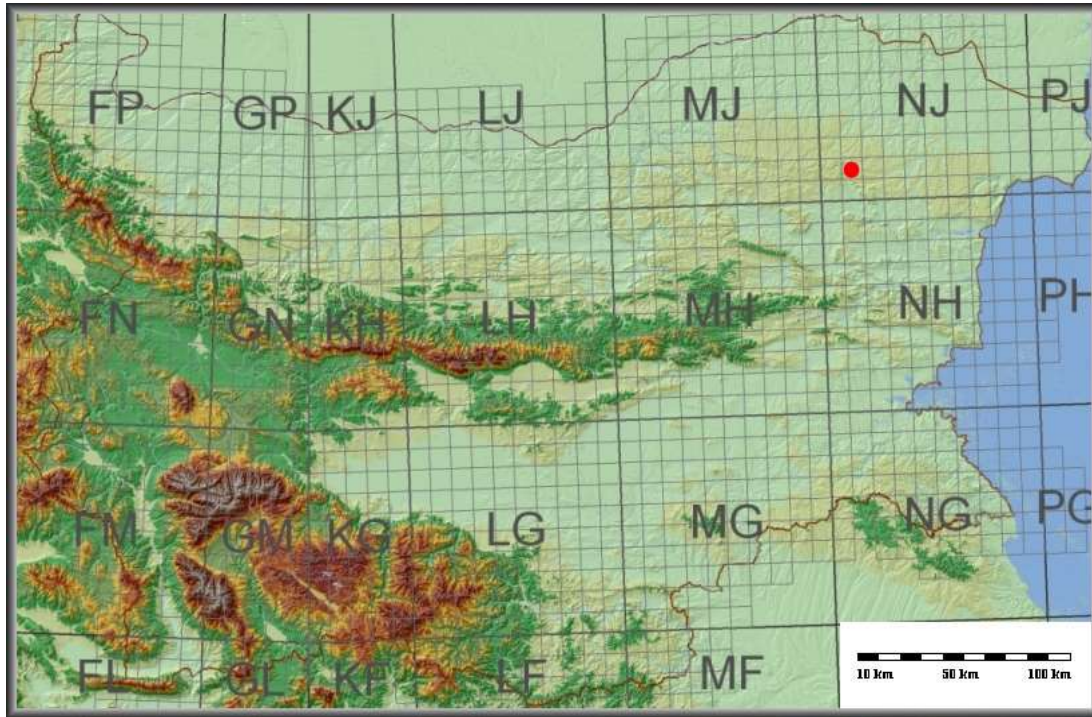
## Materials and methods

The present study was conducted by the route method in the period April 2019 - May 2021. In determining the associated species with *O. sphegodes* subsp. *helenae*, was used Plant Determinant in Bulgaria [20]. The names of the taxa are according to The Plant List [21]. Life forms are represented by the Raunkiaer system [22]. For their determination was used Flora of the People's Republic of Bulgaria, volumes from 1 to 9 [23, 24] and Flora of the Republic of Bulgaria, volumes 10 and 11 were used for their determination [25, 26]. The floristic elements are according to Assyov et al. [3]. The cover abundance of the accompanying species is according to Braun-Blanquet [27]. The marking of the population of the species was done with the help of a GPS receiver Garmin Oregon 450. The WGS 84 UTM 35N coordinate system was used. The distances from the locality to neighboring sites were measured over the air using software Google Earth Pro Portable ver. 7.1.5.

## Results and discussion

To date, *Ophrys sphegodes* subsp. *helenae* (Renz) Soó & D.M.Moore has not been reported for the flora of Bulgaria. During a study of the flora of the Voyvodsko plateau, located in the northeastern part of the Republic of Bulgaria, in April 2019 a locality of this species was discovered. It is located in quadrant NJ11 of the UTM network of Bulgaria (Fig. 3). The coordinates of the locality are: 43.451724 N and 27.135963 E. The altitude is 295 m above sea level.





**Figure 3.** An UTM grid map of Bulgaria showing the new locality of *O. sphegodes* subsp. *helenae* (the locality of the subspecies is marked with a red circle) (Scale 1:1500000)

The locality of *O. sphegodes* subsp. *helenae* was found about 670 m southeast of the Voyvoda village, Shumen district (Fig. 4). It is located in the eastern part of a natural mound and the exposure of the locality is east. The slope is variable and varies from 5 to 40°. The individuals of the species are located on the terrain with the smallest slope: 5°. The main rock is sandstone. The soil is shallow and is represented by carbonate chernozem [28]. The vegetation cover is 90%. The habitat of the species is dominated by deciduous shrub species (Fig. 5).



**Figure 4.** The location of the population



**Figure 5.** The habitat of the species



The population consists of 3 individuals located on an area of 0.5 m<sup>2</sup>. In 2019 and 2020, 1 individual in the generative state and 2 individuals in the vegetative state were identified. In 2021 the ratio changes to 2 individuals in the generative state (Fig. 6) and 1 individual in the vegetative state.

The flowering period established by us coincides with the descriptions of other authors for Europe [1, 7]: from the second half of April to the first half of May.

**A**



**B**



C



D



**Figure 6.** A – Habitus (photo by D. Zahariev, May 10, 2021); B – Inflorescence; C and D – A flower from *O. sphegodes* subsp. *helenae* (photo by D. Zahariev, April 26, 2019)

The discovery of new species north of the hitherto known locations is due to the conduct of systematic floristic surveys in Northeastern Bulgaria - an area that is not well studied in terms of flora of the individual geographical objects located in it. This is evidenced both by the discovery of the first for Northern Bulgaria locality of *Ophrys insectifera* L. in our studies in 2015 [29] and the discovery of the first for Bulgaria locality of *O. sphegodes* subsp. *helenae* in the present case. The reasons for the increase in the areal of *O. sphegodes* subsp. *helenae* remain unclear for now. We would not like to speculate, citing climate change. It is a possible reason, but the distance between the known localities and the newly established locality is very large - about 530 km by air. So far, there are no data on the distribution of the species in Southern Bulgaria, which can confirm a gradual rather than abrupt expansion of the species' range. Another possible reason is that in the

past the areal of the species was larger and it included the territory of Bulgaria, and subsequently suffered a rupture, the remnant of which is the currently isolated locality in Northeastern Bulgaria. This is possible because the floristic research in Bulgaria began very late: at the end of the 19th century after the proclamation of Bulgaria as an independent state, outside the Ottoman Empire. We hope that future discoveries of new localities of the species will clarify this case.

Species that accompany *O. sphegodes* subsp. *helenae*, were studied in the period April - August 2019. No change in the species composition in the following years was found. A complete list of these species is presented in Table 1.

**Table 1. Associated species of *O. sphegodes* subsp. *helenae* (Renz) Soó & D.M.Moore in the population southeast of the Voyvoda village**

**Braun-Blanquet Cover Abundance Scale: 2a – from 5 to 12.5%, 2m – projective cover less than 5% and number over 50 individuals, 1 – projective cover less than 5% and number among 6 and 50 individuals, + – projective cover less than 5% and number among 2 and 5 individuals, r – projective cover less than 5% and only one individual**

No.	Plant Name	Family	Life Form	Floristic Elements	Cover Abundance
1	<i>Ligustrum vulgare</i> L.	Oleaceae	Ph	subMed	2a
2	<i>Paliurus spina-christi</i> Mill.	Rhamnaceae	Ph	Eur-As	2a
3	<i>Plantago media</i> L.	Plantaginaceae	H	Boreal	2a
4	<i>Teucrium chamaedrys</i> L.	Lamiaceae	Ch	subMed	2m
5	<i>Thymus pulegioides</i> subsp. <i>pannonicus</i> (All.) Kergu��len	Lamiaceae	Ch	Eur	2m
6	<i>Achillea millefolium</i> L.	Asteraceae	H	Eur-Sib	2m
7	<i>Carex caryophyllea</i> Latourr.	Cyperaceae	H	Boreal	2m
8	<i>Medicago falcata</i> L.	Fabaceae	H	Eur-As	2m
9	<i>Pilosella piloselloides</i> subsp. <i>praealta</i> (Gochnat) S.Br��ut. & Greuter (syn. <i>Hieracium praealtum</i> Gochnat)	Asteraceae	H	Eur-Med	2m

No.	Plant Name	Family	Life Form	Floristic Elements	Cover Abundance
10	<i>Plantago lanceolata</i> L.	Plantaginaceae	H	Kos	2m
11	<i>Securigera varia</i> (L.) Lassen (syn. <i>Coronilla varia</i> L.)	Fabaceae	H	Eur-Med	2m
12	<i>Clematis vitalba</i> L.	Ranunculaceae	Ph	Eur	1
13	<i>Cytisus hirsutus</i> L. (syn. <i>Chamaecytisus supinus</i> (L.) Link)	Fabaceae	Ph	Eur-Med	1
14	<i>Ulmus minor</i> Mill.	Ulmaceae	Ph	Eur-Med	1
15	<i>Ononis pusilla</i> L.	Fabaceae	Ch	subMed	1
16	<i>Satureja coerulea</i> Janka	Lamiaceae	Ch	subMed	1
17	<i>Achillea clypeolata</i> Sm.	Asteraceae	H	Bal	1
18	<i>Anthyllis vulneraria</i> L.	Fabaceae	H	Eur-Med	1
19	<i>Astragalus monspessulanus</i> L.	Fabaceae	H	Pont-Med	1
20	<i>Astragalus onobrychis</i> L.	Fabaceae	H	Eur-As	1
21	<i>Bothriochloa ischaemum</i> (L.) Keng	Poaceae	H	SMed-As	1
22	<i>Clinopodium vulgare</i> L.	Lamiaceae	H	subBoreal	1
23	<i>Cota tinctoria</i> (L.) J.Gay.	Asteraceae	H	Eur-Sib	1
24	<i>Dactylis glomerata</i> L.	Poaceae	H	Eur-As	1
25	<i>Elymus caninus</i> (L.) L.	Poaceae	Cr	Boreal	1
26	<i>Elymus hispidus</i> (Opiz) Melderis	Poaceae	Cr	Pont-CAs	1
27	<i>Euphorbia agraria</i> M.Bieb.	Euphorbiaceae	H	subMed	1
28	<i>Fragaria viridis</i> Weston	Rosaceae	H	Eur-Sib	1
29	<i>Galium verum</i> L.	Rubiaceae	H	Eur-As	1
30	<i>Hypericum elegans</i> Stephan ex Willd.	Hypericaceae	H	Eur-Sib	1
31	<i>Leontodon crispus</i> DC. ex Nyman	Asteraceae	H	Pont-Med	1
32	<i>Lotus corniculatus</i> L.	Fabaceae	H	Eur-Med	1
33	<i>Poa compressa</i> L.	Poaceae	H	Eur-subMed	1
34	<i>Potentilla pedata</i> Willd. ex Hornem.	Rosaceae	H	Med	1



No.	Plant Name	Family	Life Form	Floristic Elements	Cover Abundance
35	<i>Ruta suaveolens</i> DC. (syn. <i>Haplophyllum suaveolens</i> G.Don.)	Rutaceae	H	Med	1
36	<i>Salvia nemorosa</i> L.	Lamiaceae	H	Eur-OT	1
37	<i>Sanguisorba minor</i> Scop.	Rosaceae	H	subBoreal	1
38	<i>Stipa capillata</i> L.	Poaceae	H	Pont-Med	1
39	<i>Veronica austriaca</i> L.	Scrophulariaceae	H	Eur-Med	1
40	<i>Anacamptis pyramidalis</i> (L.) Rich.	Orchidaceae	Cr	subMed	1
41	<i>Convolvulus cantabrica</i> L.	Convolvulaceae	Cr	Pont	1
42	<i>Alyssum alyssoides</i> (L.) L.	Brassicaceae	Th-H	Eur-Med	1
43	<i>Falcaria vulgaris</i> Bernh.	Apiaceae	Th-H	Eur-As	1
44	<i>Cerastium semidecandrum</i> L.	Caryophyllaceae	Th	Eur-Med	1
45	<i>Thlaspi perfoliatum</i> L.	Brassicaceae	Th	Eur-Med	1
46	<i>Crataegus monogyna</i> Jacq.	Rosaceae	Ph	subBoreal	+
47	<i>Pyrus pyraster</i> (L.) Burgsd.	Rosaceae	Ph	subMed	+
48	<i>Rosa canina</i> L.	Rosaceae	Ph	subMed	+
49	<i>Teucrium polium</i> L.	Lamiaceae	Ch	Pont-Med	+
50	<i>Thymus sibthorpii</i> Benth.	Lamiaceae	Ch	Bal-Dac	+
51	<i>Agrimonia eupatoria</i> L.	Rosaceae	H	Eur-Med	+
52	<i>Althaea cannabina</i> L.	Malvaceae	H	Med-As	+
53	<i>Eryngium campestre</i> L.	Apiaceae	H	Pont-Med	+
54	<i>Koeleria simonkaii</i> Adamovič	Poaceae	H	Bal	+
55	<i>Linaria dalmatica</i> (L.) Mill.	Scrophulariaceae	H	Med	+
56	<i>Peucedanum alsaticum</i> L.	Apiaceae	H	subMed	+
57	<i>Verbascum phoeniceum</i> L.	Scrophulariaceae	H	Eur-Sib	+
58	<i>Orchis simia</i> Lam.	Orchidaceae	Cr	subMed	+
59	<i>Myosotis ramosissima</i> Rochel	Boraginaceae	Th	Eur	+
60	<i>Linum nervosum</i> Waldst. & Kit.	Linaceae	H	subMed	r



Most of the associated species are hemicryptophytes (H): 36 species. This is understandable given the type of habitat. The phanerophytes (Ph), represented by trees and shrubs, are 8 species. The bushes (6 species) predominate, represented mainly by *Paliurus spina-christi* and *Ligustrum vulgare*. The tree species are represented by single individuals of *Pyrus pyraister* and *Ulmus minor* with a height of up to 2 m. The hamephytes (Ch) are 6 species. The cryptophytes (Cr) are 5 species. Of these, two species belong to the same family (Orchidaceae): *Anacamptis pyramidalis* and *Orchis simia*. The therophytes (Th) are 3 species. The intermediate group of therophytes-hemicryptophytes (Th-H) is represented by only 2 species. The low presence of therophytes and therophytes-hemicryptophytes is due to the dense cover of perennial herbaceous plants.

The distribution of the floristic elements confirms the second hypothesis about the origin of the disjunctive areal of this subspecies by reducing the initially wider areal. Among the associated species, 33 species (55.00%) refer to floristic elements more or less related to the Mediterranean region.

The habitat conditions are favorable for the development of *O. sphegodes* subsp. *helenae*. The shrubs and trees overgrowth offers wind protection, which is necessary for species of the genus *Ophrys* and they prefer such habitats, as indicated by Delforge [1]. The composition and height of the grass cover are also favorable for the development of individuals of the newly established subspecies. During flowering the individuals of *O. sphegodes* subsp. *helenae* have enough space and are easily spotted by insects. *Eucera longicornis*, the potential pollinator identified by Delforge [1], is widespread in Bulgaria [31].

The anthropogenic impact on the territories in the immediate vicinity of the population is limited. A trail passes through the territory of the population, which is poorly used by humans and wild animals. Proof of this is the complete overgrowth of this part of the trail, which is located in the northern part of the site. Due to this reason and due to the large slope, there is no grazing in the locality and its surroundings. At 18 m from the locality there is a forest culture from *Pinus nigra* J.F.Arnold.

Existing roads are far enough away and pose no threat: the nearest road to a durable surface is about 1250 m, and the nearest dirt road at a distance 780 m. The nearest arable land is 285 m away, at the foot of the mound and behind the forest.

In the vicinity are important archaeological sites. About 1 km to the northeast is the Dinea fortress. This is an ancient, late antique and medieval Roman fortress (3-9 century AD). 13.5 km to the south is the first Bulgarian capital Pliska (681-893 AD). It was one of the largest and richest cities in Europe at the time.

At the top of the mound, 140 meters west of the site, there was probably a watchtower.

In the future, the following abiotic, biotic and anthropogenic factors may have a negative impact on the state of the population: limited distribution, low number of individuals in the population, low reproductive potential, change in the dynamics of local species (e.g. dense overgrowing of open lawns with shrubs and trees), damage to the fruit boxes by insect larvae, afforestation with coniferous species, unregulated grazing during the period of flowering and fruiting, fires.

The locality of the newly established subspecies is not located within the boundaries of a protected area (under the Protected Areas Act of the Republic of Bulgaria) and is not located within the boundaries of protected zones of the European ecological network "NATURA 2000".

## Conclusions

The locality of *O. sphegodes* subsp. *helenae* is new for Bulgaria. The habitat fully corresponds to the habitats of the species described so far in Greece and Albania. It is characterized by rich floristic diversity. Among the associated species, two other species of the family Orchidaceae have been identified: *Anacamptis pyramidalis* and *Orchis simia*. The established abiotic and biotic conditions in the habitat determine good prospects for the existence of the species in the newly established locality. We propose that the entire territory of the mound near the Voyvoda village (its total area is approximately 10.3 ha) be declared a protected area in order to preserve the habitat from negative anthropogenic impacts. This area does not include the already established forest culture of *Pinus nigra*, a species that is not naturally distributed in this part of Bulgaria.

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