

× *GYMNIGRITELLA SUAVEOLENS* (ORCHIDACEAE),
A NEW NATURAL HYBRID TO THE FLORA OF SERBIA

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Received on March 14, 2024

Presented by D. Ivanov, Corresponding Member of BAS, on September 17, 2024

Abstract

× *Gymnigritella suaveolens* (Orchidaceae), a natural hybrid between *Gymnadenia conopsea* and *Nigritella rhellicani*, was found at Mt Kopaonik for the first time in Serbia, representing its single known locality in the Central Balkans. Morphological characteristics, distribution data, habitat and ecological preferences are indicated. The hybrid was found at 1552 m a.s.l., on sericite schists, as a member of the *Festuco-Deschampsietum flexuosae* grassland community. Two hybrid specimens were recorded at the site where the two parental species grow in sympatry, and where the population size of *G. conopsea* was larger than the population size of *N. rhellicani*.

Key words: *Gymnadenia*, *Nigritella*, Orchidaceae, hybrid, Serbia, Balkan Peninsula

Introduction. Natural hybridization is a relatively common phenomenon in vascular plants and is considered one of the most important mechanisms in plant evolution. Due to the important role of hybridization in the speciation and evolution of plants, it is of great importance to identify hybrid individuals, their spatial distribution and habitat preferences. Natural hybridization between orchid species occurs when the parental species grow in sympatry, their flowering periods overlap and they usually have non-specific pollination systems and at

The study was supported by the Science Fund of the Republic of Serbia, Grant No 7750112 – Balkan biodiversity across spatial and temporal scales – patterns and mechanisms driving vascular plant diversity (BalkBioDrivers).

<https://doi.org/10.7546/CRABS.2024.10.03>

least one common pollinator [1]. The potential distribution of natural hybrids can usually be determined on the basis of the habitat in which the parental species are abundant and sympatric, defined as potential “hybridogenic” populations [2]. Determining the population size of the parental species is of great importance for the knowledge of hybrids, as the relative proportion of the two parental species can be an important parameter that determines the rates of hybrid production, seed production and seedling performance [3].

The genus *Gymnadenia* R.Br. comprises 26 species and subspecies [4], which are distributed in Europe and Asia, in the east to western and central Asia, China and Japan and in the south to the Himalayas [5]. There are 34 hybrids within the genus *Gymnadenia* [4]. Some studies suggest that the genus *Gymnadenia* also includes taxa of the genus *Nigritella* Rich. [6, 7], whose centre of diversification is in the Alps [8, 9]. However, more recent studies have recognised the genus *Nigritella* as distinct and confirmed its taxonomic validity [8, 10]. The two genera (*Gymnadenia* and *Nigritella*) are closely related [11], and hybrids are sometimes found between them.

× *Gymnigritella suaveolens* (Vill.) E.G.Camus (syn. *Gymnadenia* × *suaveolens* (Vill.) Rchb.f.) is a natural hybrid between *Gymnadenia conopsea* (L.) R.Br. and *Nigritella rhellicani* Teppner & E.Klein. In most cases, *G. conopsea* contributes to the maternal lineage in the hybrid, and *N. rhellicani* provides the pollen line [12]. This is consistent with the prediction that pollen from *Nigritella* should be much more easily transferred to the stigma of a *Gymnadenia* plant than vice versa [13]. However, the reverse combination of hybrids has also been observed [12]. The morphological differences between × *Gymnigritella suaveolens* and its parental species, as well as the ecological preferences of the hybrid have not been sufficiently studied. Moreover, little is known about their population size of two parental species within the hybrid zones.

This paper reports the first finding of × *Gymnigritella suaveolens* in Serbia. The aims of this study were: (a) to compare morphological characters of the hybrid to those of its parental species; (b) to present currently known distribution data of the hybrid; (c) to determine the ecological and habitat preferences, population size and flowering period of both the hybrid and its two parental species.

Materials and methods. During the floristic and chorological investigation of the orchids of Mt Kopaonik (Serbia) in July 2023, data were collected on the morphology, distribution and ecological preferences of × *Gymnigritella suaveolens* and its parental species. The hybrid was identified according to VÖTH [14], VLADIMIROV et al. [15] and ECCARIUS [16], whereas the nomenclature follows GRIEBL [17]. For documentation purposes, one specimen of × *Gymnigritella suaveolens* (without tubers and roots) was collected and deposited in the Herbarium of the Institute of Botany and Botanical Garden “Jevremovac”, University of Belgrade (BEOU). The morphological descriptions of the hybrid and its parental species are provided here on the basis of our own observations and measurements

and according to DELFORGE [5] and Eccarius [16]. For taking measurement, three flowers of the hybrid were collected and stored in 50% ethanol and later measured with a vernier calliper. The values of the measured characteristics of the hybrid specimen from Serbia are given in bold.

The distribution of $\times$ *Gymnigritella suaveolens* in Serbia was mapped on a grid map with squares of 10×10 km, based on the Universal Transverse Mercator (UTM) projection, grid zone 34T. In addition, the geographic coordinates (longitude and latitude) and altitude were recorded using a Garmin eTrex 32 handheld GPS device in the World Geodetic System 84 (WGS 84) format. The methodology of BRAUN-BLANQUET [18] was used to determine the plant community of the site with $\times$ *Gymnigritella suaveolens*, whereas the habitat type was determined according to the EUNIS habitat classification (<http://eunis.eea.europa.eu/>). The geological substrate was determined using the geological map of the study area on a scale of 1:100 000. During the field surveys, the abundance of $\times$ *Gymnigritella suaveolens* and its parental species was determined by counting the total number of individuals.

Results and discussion. $\times$ *Gymnigritella suaveolens* (Vill.) E.G.Camus in J. Bot. (Morot) 6: 484 (1892) (Fig. 1B). Synonyms: *Gymnadenia* $\times$ *suaveolens* (Vill.) Rchb.f.; *Gymnadenia odoratissima* subsp. *suaveolens* (Vill.) Gand.; *Nigritella* $\times$ *suaveolens* (Vill.) W.D.J.Koch; *Orchis* $\times$ *suaveolens* Vill., *Gymnadenia* $\times$ *chanousiana* G.Foelsche & W.Foelsche, *Gymnadenia* $\times$ *moritziana* (Brügger ex Nyman) Oddone. Hybrid formula: *Gymnadenia conopsea* (L.) R.Br. (Fig. 1A) $\times$ *Nigritella rhellicani* Teppner & E.Klein (Fig. 1C).

Morphology. A comparison of the observed morphological characteristics of $\times$ *Gymnigritella suaveolens* and its two parental species is presented in Table 1,

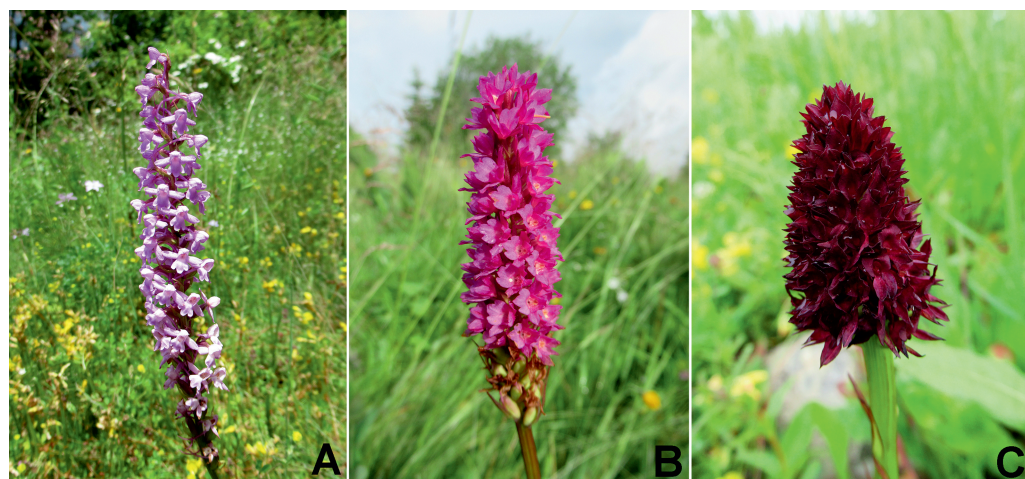


Fig. 1. $\times$ *Gymnigritella suaveolens* and its parental species: (A) *Gymnadenia conopsea*; (B) $\times$ *Gymnigritella suaveolens*; (C) *Nigritella rhellicani* (all from Mt Kopaonik, Treska, 2 July 2023, photos by V. Djordjević)

with the measurements of the hybrid specimen from Serbia in bold. The morphology of the hybrid (Fig. 1B) is intermediate between those of the two parental species (Fig. 1A and C) in almost all characteristics. The most important distinguishing features of the hybrids are the colour and position and the structure of the labellum and its lobes. The habit of $\times$ *Gymnigritella suaveolens* is similar to that of the two parental species, but much taller, and with longer and elongated cylindrical inflorescences compared to *N. rhellicani*, where the inflorescence is mostly capitate, ovoid or conical. The bracts are more or less reddish as in *N. rhellicani*. An important characteristic of the hybrid is that the resupination of the flowers usually occurs halfway, so that the labellum is turned to one side. In the upper part of the inflorescence the flowers are usually not resupinate, as in *N. rhellicani*, so that the labellum is directed upwards. The flowers of the hybrids vary greatly in the colour of the purplish-red (magenta), usually in a shade between light and dark magenta and more rarely in electric pink, cyclamen pink and chimney red. In $\times$ *Gymnigritella suaveolens* the labellum is clearly three-lobed, unlike in *N. rhellicani*, where the labellum is entire or only very slightly three-lobed. Furthermore, the labellum is as long as wide or slightly wider than long, in contrast to *N. rhellicani*, where the labellum is longer than wide (Table 1).

Flowering period. On July 2, 2023, both specimens of $\times$ *Gymnigritella suaveolens* at Mt Kopaonik were at the peak of flowering. This coincides with the flowering time of the parental species in the Central Balkans, where *G. conopsea* flowers from mid-May to the end of July, while *N. rhellicani* flowers from mid-June to mid-July.

Pollination. Both parental species of $\times$ *Gymnigritella suaveolens* are cross-pollinated and rewarding orchids that produce nectar. The pollinators of *G. conopsea* are insects from the order Lepidoptera (Hesperiidae, Pieridae, Lycaenidae, Nymphalidae, Zygaenidae, Sphingidae, Geometridae and Noctuidae), but also bumblebees *Bombus terrestris* (Linnaeus, 1758) and *B. pascuorum* (Scopoli, 1763) (Hymenoptera), as well as representatives of the orders: Coleoptera – *Paracorymbia fulva* De Geer, 1775; Diptera – *Empis livida* Linnaeus, 1758, *E. tessellata* Fabricius, 1794; Hemiptera – *Spilostethus saxatilis* (Scopoli, 1763) [12, 13, 19]. The pollinators of *N. rhellicani* are insects from the order Lepidoptera (Pieridae, Nymphalidae, Satyridae, Zygaenidae, Noctuidae, Geometridae, and Pyralidae) and some species of the orders Coleoptera, Hymenoptera and Diptera [12, 13]. In this study, the pollinators of the hybrid and its parental species were not recorded. The occurrence of intergeneric hybrids proves that the same insects occasionally transfer pollen between plants of different genera. It can be assumed that the pollinaria attach to different parts of the proboscis depending on the depth of the spur. Insect sucking nectar from the long spur of *G. conopsea* is likely to attach the pollinaria to the proximal part of the proboscis, near the head, whereas the pollinaria would attach to the distal part of the proboscis when visiting a flower of *N. rhellicani* with a short spur [12].

T a b l e 1

Comparison of morphological characters of $\times$ *Gymnigritella suaveolens*
and its two parental species

Character	<i>Gymnadenia conopsea</i>	$\times$ <i>Gymnigritella suaveolens</i>	<i>Nigritella rhellicani</i>
Plant height	(10–) 25–60 (–100) cm	46.8 cm	5–30 (–35) cm
Leaves	5–12 basal leaves, linear-lanceolate, 5–25 cm long and 0.5–4 cm wide	6 basal leaves, linear-lanceolate, 8.9–18.6 cm long and 0.4–1 cm wide	7–11 (–14) basal leaves in a rosette, linear, 1.5–7 cm long and 0.3–0.9 cm wide
Bracts	9–20 mm long and 2–4 mm wide, green to washed violet	8–16 mm long and 1.5–2.5 mm wide, green washed purple to reddish	8–14 mm long and 1.2–2.5 mm wide, green washed purple to reddish
Inflorescence	dense, elongated, cylindrical, 5–25 (–30) cm long and 1.3–3 (–4.5) cm wide	dense, initially conical or ovoid, later almost cylindrical, elongated, 6.4 cm long and up to 1.7 cm wide	dense, initially slightly conical, but then round or ovoid, 1.5–3.2 cm long and 1.5–2.3 cm wide
Flowers	resupinate flowers (the lip is directed downwards); purplish-pink, liliac, pale pink, rarely whitish	mostly half-resupinate flowers (the lip is turned to the side); not resupinate flowers (the lip is turned upwards) in the upper part of inflorescence; bright to dark magenta	not resupinate flowers (the lip is directed upwards); dark brown-red, grey-brown-red, brown-red or red, rarely pink or cyclamen pink
Sepals	elongate ovate to linear, 4–7 (–10) mm long and 2.7–4.5 mm wide	lanceolate to linear-lanceolate, 4–6.5 mm long and 1.5–2.5 mm wide	lanceolate, 4.5–7.5 (–9) mm long and (1.1–) 1.5–2.5 mm wide
Petals	ovate to ovate-lanceolate, 4–7 mm long and 3–5 mm wide	ovate-lanceolate, 4–5 mm long and 1.5–2 mm wide	lanceolate, 4.5–7 mm long and 1.2–1.8 mm wide
Labellum	3.5–6 (–7.5) mm long and 5–9 mm wide; deeply 3-lobed; wedge-ovate. The width of lateral lobes $\pm$ equal to width of middle lobe. The middle lobe of the labellum is longer or the same length as the lateral lobes	4–5.5 mm long and 4.5–5 mm wide, shallowly to deeply 3-lobed or slightly 3-lobed; ovate to almost triangular. The width of lateral lobes $\pm$ equal to width of middle lobe. The middle lobe of the labellum is longer than the lateral lobes	4.5–7 (–8.2) mm long and 3–5 (–5.5) mm wide; entire or very slightly 3-lobed; almost triangular, ovate-acuminate to near rhomboidal
Spur	filiform, curving downwards, (10–) 14.5–20 mm long and 0.8–1.1 mm wide	cylindrical, 4–6 (–9) mm long and 0.9–1.1 mm wide	bulbous, 1–1.7 mm long and 0.9–1.1 mm wide

General distribution. $\times$ *Gymnigritella suaveolens* has been recorded in Austria, Bulgaria, France, Germany, Greece, Italy, Switzerland, Croatia, and Bosnia and Herzegovina [4, 8, 14, 15, 20]. *Gymnadenia conopsea* is an Eurasian species distributed in the temperate and boreal region of Eurasia, the Caucasus, Asia Minor, Siberia, east to China and Japan [5, 19]. *Nigritella rhellicani* is distributed in the Alps, Dinarides, and Carpathians [5, 8]. It has been recorded in Austria, Switzerland, Germany, France, Italy, Slovenia, Croatia, Bosnia and Herzegovina, Serbia, Montenegro, North Macedonia, Albania, Greece, Bulgaria, and Romania [4, 5, 8, 15].

Distribution in Serbia. $\times$ *Gymnigritella suaveolens* has been recorded in Central Serbia: Mt Kopaonik, Treska, N 43.262209°, E 20.785772°, MGRS 34T DN88, 1552 m a.s.l., ass. *Festuco-Deschampsietum flexuosae*, sericite schists, exp. N, incl. 20°, July 2, 2023, coll. V. Djordjević, S. Krdžić, det. V. Djordjević (BEOU 71501) (Fig. 2).

The finding of $\times$ *Gymnigritella suaveolens* at the locality of Treska (Mt Kopaonik, Central Serbia) is the first record of this hybrid on the territory of Serbia and at the same time the only known record of this hybrid in the Central Balkans.

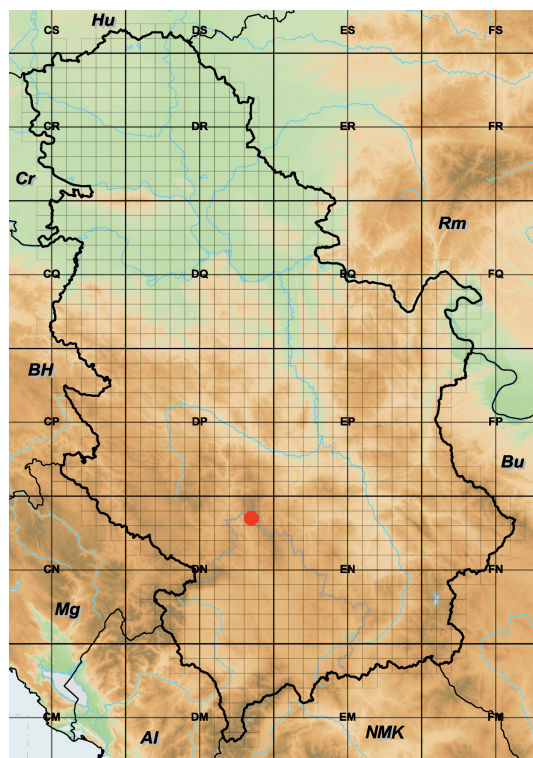


Fig. 2. Distribution of $\times$ *Gymnigritella suaveolens* in Serbia

Previously, it had been found in Bosnia and Herzegovina without the exact locality being specified [20].

Habitat, ecology and population data. × *Gymnigritella suaveolens* and its two parental species were found at the locality of Treska (Mt Kopaonik) in the grassland community *Festuco-Deschampsietum flexuosae*. The following accompanying taxa were recorded at the site with × *Gymnigritella suaveolens*: *Deschampsia flexuosa* (L.) Trin., *Festuca nigrescens* Lam., *Agrostis capillaris* L., *Koeleria macrantha* (Ledeb.) Schult., *Trifolium alpestre* L., *Genista sagittalis* L., *Hypericum barbatum* Jacq., *Leucanthemum vulgare* Lam., *Rosa pendulina* L., *Valeriana officinalis* L., *Veratrum album* L., *Trollius europaeus* L., *Polygonum bistorta* L., *Bromopsis erecta* (Huds.) Fourr., and *Astrantia major* L. According to the EUNIS classification, this habitat type belongs to the *Agrostis-Festuca* grassland (E1.72 code). The hybrid was found on sericite schists, on moderately moist soil, under full light conditions, at an altitude of 1552 m, on a north-facing slope with an inclination of 20°.

The occurrence of × *Gymnigritella suaveolens* at Mt Kopaonik (Serbia) in sunny grassland on moderately moist soil is not surprising, considering the ecological preferences of the two parental species. *Gymnadenia conopsea* occurs from lowland areas to high mountain regions, usually in full light, rarely in slight shade, on moderately dry to moist soils with acidic to alkaline reactions [5, 19]. It mainly inhabits herbaceous vegetation types (meadows, pastures and fens) and is less common in forest communities [5, 19]. *Nigritella rhellicani* inhabits exclusively high-altitude pastures and meadows under full light conditions, on moderately moist and extremely moist, acidic to alkaline soils [5]. In Bulgaria, the hybrid was found on Mt Slavyanka, in a subalpine grassland, at an altitude of 2100–2150 m, whereas in Greece (Mt Falakro), the hybrid specimens were found in a subalpine grassland, at an altitude of about 2050–2100 m, on limestone [15].

Two individuals of × *Gymnigritella suaveolens* were found at the locality of Treska (Mt Kopaonik), at the site where the population size of *G. conopsea* (23 individuals) was larger than the population size of *N. rhellicani* (4 individuals). Previous studies in Bulgaria (Mount Slavyanka) and Greece (Mount Falakro) have also shown that hybrid individuals occur at sites where the population size of *G. conopsea* (several thousand individuals) is larger than the population size of *N. rhellicani* (one to several hundred individuals) [15]. In contrast to these localities in Bulgaria and Greece, the hybrid zone in Serbia (Mt Kopaonik) has a much smaller population size of both parental species, which indicates a greater endangerment of the orchids and an increased risk of extinction.

Conservation. × *Gymnigritella suaveolens* and its two parental species are protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). According to Serbian legislation, *G. conopsea* is classified as protected, while *N. rhellicani* is a strictly protected species in Serbia. Conservation priorities should focus mainly on the hybrid zones and sympatric

zones, instead of the hybrid individuals. The threat factors for $\times$ *Gymnigritella suaveolens* and its two parental species on Kopaonik Mountain are habitat destruction due to urbanisation, plowing of grasslands and their conversion to agricultural land, changes in the grazing regime and tourism.

Acknowledgements. The authors thank the support of NP Kopaonik for orchid research.

REFERENCES

- [1] PELLEGRINO G., F. BELLUSCI, A. MUSACCHIO (2009) Genetic integrity of sympatric hybridising plant species: the case of *Orchis italica* and *O. anthropophora*, *Plant Biol.*, **11**(3), 434–441.
- [2] SCHATZ B. (2006) Fine scale distribution of pollinator explains the occurrence of the natural orchid hybrid $\times$ *Orchis bergonii*, *Écoscience*, **13**(1), 111–118.
- [3] FIELD D. L., D. J. AYRE, R. J. WHELAN, A. G. YOUNG (2008) Relative frequency of sympatric species influences rates of interspecific hybridization, seed production and seedling performance in the uncommon *Eucalyptus aggregata*, *J. Ecol.*, **96**(6), 1198–1210.
- [4] Plants of the World Online (POWO) (2024) (continuously updated). <http://www.plantsoftheworldonline.org/>. Facilitated by the Royal Botanic Gardens, Kew. [accessed 05 Feb 2024].
- [5] DELFORGE P. (2006) *Orchids of Europe, North Africa and Middle East*, A&C Black Ltd. Publishers, London.
- [6] TEPPNER H., E. KLEIN (1998) Etiam atque etiam – *Nigritella* versus *Gymnadenia*: Neukombinationen und *Gymnadenia dolomitensis* spec. nova (Orchidaceae: Orchideae), *Phyton (Horn)*, **38**, 220–224.
- [7] BATEMAN R. M., P. M. HOLLINGSWORTH, J. PRESTON et al. (2003) Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae), *Bot. J. Linn. Soc.*, **142**, 1–40.
- [8] HEDRÉN M., R. LORENZ, H. TEPPNER et al. (2018) Evolution and systematics of polyploid *Nigritella* (Orchidaceae), *Nord. J. Bot.*, **36**, njb-01539.
- [9] GRIEBL N. (2009) Die Gattung *Nigritella* in Österreich, *Ber. Arbeitskreis. Heimische Orchid*, **26**(1), 76–105.
- [10] BRANDRUD M. K., O. PAUN, R. LORENZ et al. (2019) Restriction-site associated DNA sequencing supports a sister group relationship of *Nigritella* and *Gymnadenia* (Orchidaceae), *Mol. Phylogenet. Evol.*, **136**, 21–28.
- [11] PRIDGEON A. M., R. M. BATEMAN, A. COX et al. (1997) Phylogenetics of subtribe Orchidinae (Orchidoideae, Orchidaceae) based on nuclear ITS sequences. 1. Intergeneric relationships and polyphyly of *Orchis sensu lato*, *Lindleyana*, **12**, 89–109.
- [12] HEDRÉN M., R. LORENZ, D. STÅHLBERG (2018) Evidence for bidirectional hybridization between *Gymnadenia* and *Nigritella*, *J. Eur. Orch.*, **50**(1), 43–60.
- [13] VÖTH W. (2000) *Gymnadenia*, *Nigritella* und ihre Bestäuber, *J. Eur. Orch.*, **32**, 547–573.

- [14] VÖTH W. (2004) Verbreitungskarten von in Österreich anzutreffenden *Gymnadenia*-, *Nigritella*-, $\times$ *Gymnigritella*-, $\times$ *Pseudadenia* und $\times$ *Pseuditella*-Arten (Orchidaceae), Linz. Biol. Beitr., **36**(1), 493–519.
- [15] VLADIMIROV V., M. AYBEKE, K. TAN (2018) New floristic records in the Balkans: 37, Phytol. Balc., **24**(3), 397–461.
- [16] ECCARIUS W. (2022) Die Orchideengattung *Gymnadenia* mit einem Exkurs zu Gattung *Pseudorchis*: Phylogenie, Taxonomie, Morphologie, Biologie, Verbreitung, Ökologie und Hybridisation, Selbstverlag der Verfasser, 336 pp.
- [17] GRIEBL N. (2011) Die Orchideenhybriden Österreichs, Ber. Arbeitskreis. Heimische Orchid, **28**(2), 132–179.
- [18] BRAUN-BLANQUET J. (1964) Pflanzensoziologie [Phytosociology]. Grundzüge der Vegetationskunde, Dritte Auflage, Springer-Verlag, Wien, New York.
- [19] MEEKERS T., M. J. HUTCHINGS, O. HONNAY, H. JACQUEMYN (2012) Biological flora of the British Isles: *Gymnadenia conopsea* s.l., J. Ecol., **100**, 1269–1288.
- [20] FRITSCH K. (1909) Neue Beiträge zur Flora der Balkanhalbinsel, insbesondere Serbiens, Bosniens und der Herzegowina, Erster Teil. Mitt. Naturwiss. Ver. Steiermark, **45**, 131–183.

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