

# First record of a natural hybrid *Neotinea* × *dietrichiana* (*Orchidaceae*) in Serbia

Vladan Djordjević<sup>1</sup>, Spyros Tsiftsis<sup>2</sup>, Ksenija Jakovljević<sup>1</sup>,  
Jasmina Šinžar-Sekulić<sup>1</sup> & Snežana Vukojičić<sup>1</sup>

<sup>1</sup> Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade, 43 Takovska St., 11000 Belgrade, Serbia, e-mail: vlakiorhi@yahoo.co.uk (corresponding author); ksenija\_jakovljevic@yahoo.com; jsekulic@bio.bg.ac.rs; sneza@bio.bg.ac.rs

<sup>2</sup> Department of Botany, School of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece, e-mail: stsiftsi@bio.auth.gr

Received: April 24, 2012 ▷ Accepted: June 25, 2012

**Abstract.** *Neotinea* × *dietrichiana* (*Orchidaceae*), a natural hybrid between *Neotinea tridentata* and *N. ustulata*, has been found on the southwestern slopes of Mt Maljen (Western Serbia) for the first time in Serbia, representing its most continental record in the Balkans. Morphological and distribution data, as well as the ecological preferences of *N. × dietrichiana* are provided. The hybrid grows between 490 m and 510 m a.s.l., on serpentine substrate, as a member of xero-mesophilous steppe-like meadows. Hybrid specimens have been recorded at the sites where the two parental species grow in sympatry, and mostly where the population size of *N. tridentata* is larger than the population size of *N. ustulata*.

**Key words:** distribution, hybrid, *Neotinea*, *Orchidaceae*, Serbia, serpentine, steppe-like meadows

## Introduction

Natural hybridization is a relatively common phenomenon, which has played a significant role in plant evolution (Grant 1981). In food-deceptive orchids, i.e. those orchids that attract pollinators by mimicking rewarding signals, hybridization is a natural consequence of their unspecific pollination system and it may represent a potential stimulus for evolutionary change (Cozzolino & al. 2006). In fact, when closely related species of food-deceptive orchids grow in sympatry and have overlapping flowering periods and unspecific pollinators, they are exposed to opportunities for inter-specific hybridization (Moccia & al. 2007). However, this hybridization is not a consequence of habitat disturbance as it is commonly accepted for other hybridizing plants (Cozzolino & al. 2006). Con-

sidering that the relative proportion of the two parental species can be a significant parameter that determines likelihood of hybridization (Schatz 2006), determining the population size of parental species is important in knowledge of hybrids.

The genus *Neotinea* Rchb. (*Orchidaceae*) comprises six taxa (Bateman & al. 2003), whose distributions are limited to Europe, Asia Minor, Caucasus, and northwestern coastal regions of North Africa (Kretzschmar & al. 2007). The taxonomic status of *Neotinea tridentata* (Scop.) R.M. Bateman, Pridgeon & M.W.Chase and *N. ustulata* (L.) R.M. Bateman, Pridgeon & M.W. Chase was recently reconsidered using a molecular phylogeny tools (Bateman & al. 2003). Previously, these species were included within the genus *Orchis* (Soó 1980). *Neotinea* × *dietrichiana* (Bogenh.) H. Kretzschmar, Eccarius & H. Dietr.

is a natural hybrid between *N. tridentata* and *N. ustulata*. It was described for the first time by Bogenhard (1850) under the name *Orchis* × *dietrichiana*. A specimen of this hybrid from Italy has been characterized by using molecular markers, i.e. applying the Randomly Amplified Polymorphic DNA (RAPD) and Restriction Fragment Length Polymorphism (RFLP) analysis of plastid DNA (cpDNA) (Cozzolino & al. 1998). The RAPD has confirmed the hybridization, while the cpDNA pattern of the hybrid has demonstrated that *N. tridentata* contributes to the maternal lineage in the hybrid, and *N. ustulata* provides the pollen line (Cozzolino & al. 1998). However, the morphological differences between *N.* × *dietrichiana* and its parental species, as well as the ecological preferences of the hybrid have not been sufficiently studied. Furthermore, little is known about the relative proportions of the two parental species, i.e. about their population size within the hybrid zones.

The present paper reports the occurrence of *N.* × *dietrichiana* in Serbia. The aims of this study were: (i) to compare its morphological characters to those of its parental species; (ii) to present currently known distribution data of the hybrid; (iii) to determine the ecological preferences, population size and flowering period both of the hybrid and its two parental species.

## Material and methods

During floristic investigations carried out on the territory of Mt Maljen (Western Serbia) in the period between 2002 and 2005, data concerning the distribution, population size and ecological preferences of taxa of the genus *Neotinea* were recorded. The collected specimens of *N.* × *dietrichiana* and its two parental species were herbarized and deposited in the Herbarium of the Institute of Botany and Jevremovac Botanical Garden, University of Belgrade (BEOU). Identification of the hybrid specimens was done according to Kerner (1865), Schulze (1894), Camus & Camus (1928), Kretzschmar & al. (2007), and Cozzolino & al. (1998). Their morphological description was based on morphometric measurements of three collected specimens. Identification of the two parental species was done according to Soó (1980) and Delforge (2006), while the nomenclature followed Bateman & al. (2003). Their morphological descriptions were based on herbarium specimens (six spec-

imens of *N. tridentata*, and five specimens of *N. ustulata*), with some characters added according to Delforge (2006), *Flora Europaea* (Soó 1980) and Tali & al. (2004).

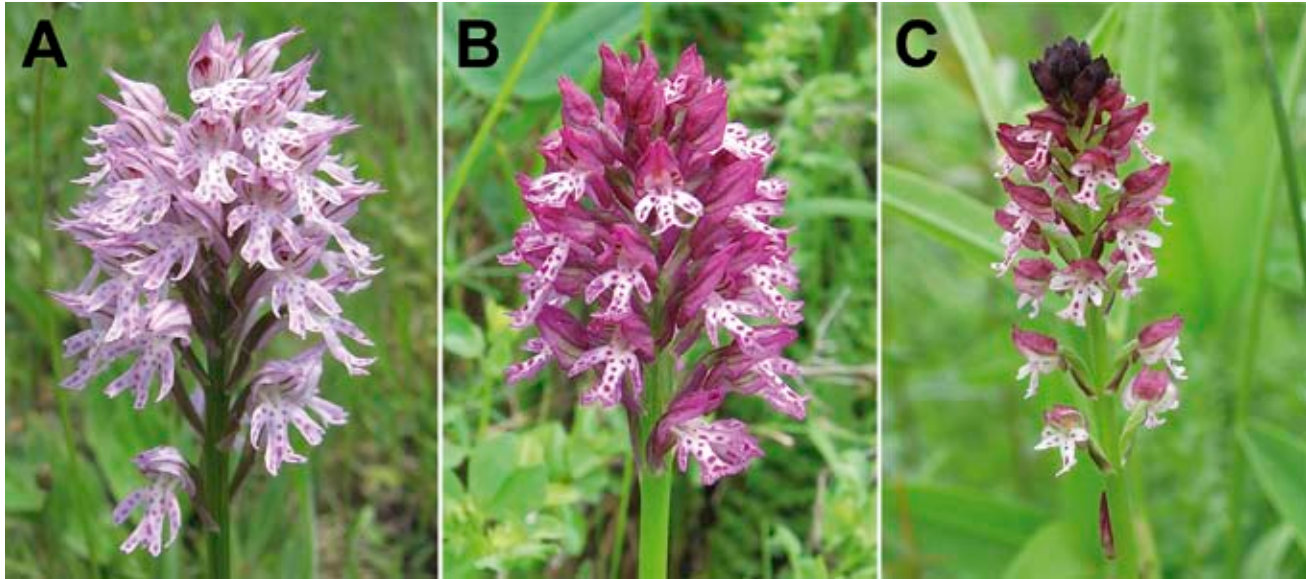
Distribution of the hybrid in Serbia is presented on a 10 km × 10 km grid, using the Universal Transverse Mercator (UTM) projection (Lampinen 2001). Plant community types were determined by phytosociological sampling, according to Braun-Blanquet (1964) methodology. The names of plant taxa, with a few exceptions, followed the *Flora Europaea* (*Flora Europaea* Database). The names of syntaxa followed *A Syntaxonomic Review of Vegetation in Serbia* (Kojić & al. 1998). The population size of *N.* × *dietrichiana* and its two parental species was determined by counting the total number of plant specimens.

## Results and discussion

***Neotinea* × *dietrichiana*** (Bogenh.) H. Kretzschmar, Eccarius & H. Dietr., *Orchid Gen. Anacamptis Orchis Neotinea*: 464 (2007) (Fig. 1B). Synonyms: *Orchis* × *dietrichiana* Bogenh., *Taschenb. Fl. Jena*: 351 (1850); *Orchis* × *austriaca* A.Kern. Hybrid formula: *N. tridentata* subsp. *tridentata* × *N. ustulata* var. *ustulata* (Figs. 1A and 1C).

### Morphology

A comparison of the observed morphological characters of *N.* × *dietrichiana* and its two parental species is given in Table 1, while the measurements of specimens from Serbia are typed in bold. The morphology of the hybrid (Fig. 1B) is intermediate between that of the two parental species (Figs 1A and 1C) in nearly all characters. However, some traits are dominantly inherited from one of the two parental species. The most important distinctive characteristics of the hybrid are the colour and disposition of the outer perianth segments, as well as the structure of the labellum and its lobes. The shape of the ripe inflorescence is not clearly spherical, but is longer than wide, resembling that of *N. ustulata*. The size of flowers is intermediate between that of the two parental species. The influence of *N. ustulata* is clearly recognized in the perianth segments, which make a dark-coloured galea (hood). However, the galea is acuminate as in *N. tridentata*. The influence of *N. tridentata* is to be recognized in the extended lobes of the labellum. The size of the spur is intermediate between that of the two parental species (Table 1).



**Fig. 1.** *Neotinea* × *dietrichiana* and its two parental species (all from the village of Stojići, southwestern slopes of Mt Maljen, Western Serbia, 26.05.2005, photos V. Djordjević). **A** – *N. tridentata* subsp. *tridentata*; **B** – *N. × dietrichiana*; **C** – *N. ustulata* var. *ustulata*.

**Table 1.** Comparison of morphological characters of *Neotinea* × *dietrichiana* and its two parental species.

	<i>N. tridentata</i>	<i>N. × dietrichiana</i>	<i>N. ustulata</i>
Tubers	2 ellipsoid or ovoid	2 ellipsoid or ovoid	1-2 (-3) subglobose or ellipsoid
Stem height	15-24.8-35-40 (-45) cm	26.1-36 cm	12-28-31.5-35 (-60) cm
Leaves	4-7-8-11 leaves, with sheaths above, glaucous green, oblong to lanceolate, unspotted, 2-9.5-10 cm long and 0.5-1.2-2 cm wide	8-10 leaves, with sheaths above, oblong-acuminate to broadly lanceolate, unspotted, 2-10.5 cm long and 0.5-1.5 cm wide	5-6-9-10 leaves, oblong-acuminate to broadly lanceolate, unspotted, 2-7.7-10 (-15) cm long and 0.5-1.4-2 (-3) cm wide
Bracts	lanceolate, acuminate, 1-veined, pale lilac, (3-) 4-11 mm long, shorter than to about as long as ovary	lanceolate, reddish, membranous, 4-7 mm long, shorter than ovary	ovate-lanceolate, 1-veined, reddish, membranous, 2-3 mm long, shorter than to about as long as ovary
Inflorescence	initially conical, becoming (near) ovoid, short, dense, 26-49 mm long and up to 20-35 mm wide	initially ovoid, becoming lax at the base, cylindrical, 28-39 mm long and up to 21-26 mm wide	initially dense, short and ovoid, becoming lax at the base, cylindrical, 23-57 (-100) mm long and up to 13-20 mm wide
Flowers	entirely lilac or crimson-pink, seldom whitish, veined purple; all perianth segments convergent into an acuminate galea	reddish-purple; all perianth segments form an acuminate galea	pink to greenish washed purple inside, purple to dark-blackish-brown outside; all perianth segments convergent into a tight, ovoid or shortly acuminate galea
Sepals	lanceolate, 8-10-13 mm long	lanceolate, 4-6 mm long	oval-lanceolate, 3.5-4.5 mm long
Petals	lanceolate, 4-7 (-10) mm long	lanceolate, 3-5 mm long	linear, near spatulate, keeled, 3-3.5 mm long
Labellum	3-lobed, (6-) 7-9 (-12) mm long, the same colour as the hood, but slightly paler, well spotted over the entire surface; lateral lobes oblong, near spatulate, obliquely truncated, 2-6 mm long; middle lobe 3-7 mm long, most often 2-lobed; secondary lobes broad, often near rhomboidal, seldom toothed or separated by a small tooth	3-lobed, 5-8 mm long, white or pale pink with purple spots; lateral lobes oblong to rhomboid, occasionally denticulate at apex, 3-5 mm long; middle lobe 4-6 mm long, divided into 2 secondary lobes with a small tooth between them	3-lobed, 3-5-8 mm long, white or pale-pink, with a few papillose purple spots; lateral lobes oblong, occasionally falcate, near spatulate, rounded, occasionally obliquely truncated, 2-4 mm long; middle lobe 3-5 mm long, divided into 2 secondary lobes, seldom entire, slightly divergent, occasionally separated by a small tooth
Spur	3-7-10 mm long, cylindrical, about as long as ovary	2.5-4 mm long, cylindrical, about half as long as ovary	1-2 mm long, cylindrical, shorter than ovary
Ovary	5-12 mm long	5-8 mm long	2-4 mm long



### General distribution

*N.* × *dietrichiana* has been recorded in: Russia, France, Austria, Czech Republic, Slovakia (Peitz 1972); Germany (Kretzschmar & al. 2007); Switzerland (Reinhard & al. 1991); Hungary (Molnár & al. 1995); Italy (Cozzolino & al. 1998), and Slovenia (Bovec-Vršič and Tolmin; Hertel, S. pers. comm.). In the Balkans, the hybrid has been recorded in Greece (Mt Menikion and Mt Falakron; Tsiftsis & al. 2007), Croatia (Kozina; Gözl & Reinhard 1986; Plitvička Jezera Lakes and near Jasenak; Kranjčev 2005), and Bosnia-Herzegovina (the plateau Borje and Gradina, near Sarajevo; Maly 1928).

The parental species *N. tridentata* is a Balkan-Pontic species, with a main range from the Pyrenees to the Caucasus, Iraq and the Caspian Sea. It is widespread and rather rare in the western part of its range, but common in Anatolia (Delforge 2006; Kretzschmar & al. 2007). *N. ustulata* is an Euro-Siberian species, which is distributed north to the Baltic; it is widespread and rare in the Mediterranean zone (Delforge 2006). Tali & al. (2004) noted that *N. ustulata* occurs almost across the entire Balkans.

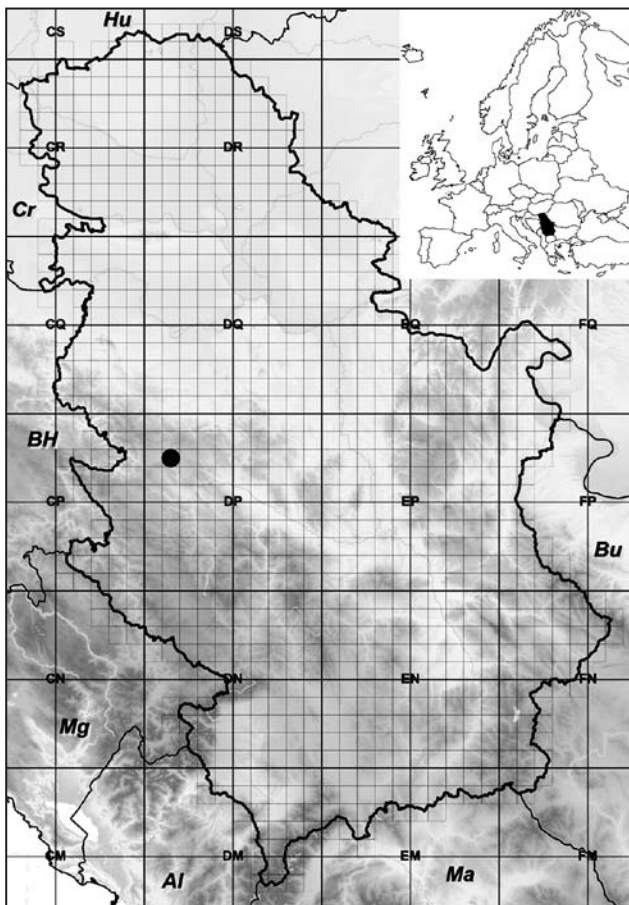


Fig. 2. Distribution of *Neotinea* × *dietrichiana* in Serbia.

### Distribution in Serbia

*N.* × *dietrichiana* has been recorded in Western Serbia: Mt Maljen, Stojići (Ražana Railway Station), UTM DP17, ass. *Danthonietum calycinae*, serpentine, alt. 490 m, exp. NW, 26.05.2005, coll./det. V. Djordjević (BEOU 16478); Mt Maljen, Stojići (Ražana Railway Station), UTM DP17, ass. *Danthonietum calycinae*, serpentine, alt. 490 m, exp. SW, 26.05.2005, coll./det. V. Djordjević (BEOU 16479); Mt Maljen, Stojići (Ražana Railway Station), UTM DP17, ass. *Chrysopogonetum grylli*, serpentine, alt. 510 m, exp. W, 26.05.2005, coll./det. V. Djordjević (BEOU 16480) (Fig. 2).

The finding of *N.* × *dietrichiana* in the village of Stojići (on the southwestern slopes of Mt Maljen, Western Serbia) is the first record of this hybrid on the territory of Serbia. This is the most continental known record of this hybrid in the Balkans, and the second record in the Central Balkans after almost 80 years. At the same time, the locality of the hybrid in Serbia is the northernmost limit of its distribution in the Central Balkans. It was found earlier near Sarajevo, in Bosnia-Herzegovina (Maly 1928). In a biogeographical aspect, Mt Maljen belongs to an intermediate zone connecting the Illyric and Moesic phytogeographical provinces. In addition, the mountain is exposed to the influence of the Pannonian climate. This position has enabled different floristic influences, so it is not surprising that the two parental species overlap here in their range and, therefore, have made sympatric zones.

### Habitat, ecology and population data

Like its two parental species, *N.* × *dietrichiana* has been found to grow on serpentine substrate on the southwestern slopes of Mt Maljen (Western Serbia). All specimens have been recorded on moderately moist soil, under a full-light regime. The habitats are west-, southwest- and northwest-exposed slopes, with 5–30° inclination. *N.* × *dietrichiana* is a member of the xero-mesophilous steppe-like meadow communities *Danthonietum calycinae* Cincović et Kojić 1958 and *Chrysopogonetum grylli* Gajić 1954, both of the alliance *Chrysopogoni-Danthonion calycinae* Kojić 1957, order *Festucetalia valesiaca* Br.-Bl. et Tx. 1943, and class *Festuco-Brometea* Br.-Bl. et Tx. 1943. The floristic compositions of these two communities are presented in the phytocoenological relevés (Table 2).

**Table 2.** The phytocoenological relevés in Serbian habitats of *Neotinea × dietrichiana*. 1 & 2 – *Danthonietum calycinae*; 3 – *Chrysopogonetum grylli*.

No. Relevé	1	2	3
Altitude (m)	490	510	510
Exposition	NW	W	W
Inclination (°)	20–30	20	20
Relevé area (m <sup>2</sup> )	25	25	25
Date	01.07.2005	01.07.2005	01.07.2005
<b>Species</b>			
<i>Danthonia calycina</i> (Vill.) Rchb.	4.4	4.4	
<i>Rhinanthus rumelicus</i> Vel.	1.1	2.2	2.2
<i>Trifolium montanum</i> L.	2.2	1.2	
<i>Dorycnium pentaphyllum</i> Scop. subsp. <i>herbaceum</i> (Vill.) Rouy	1.2	1.2	+2
<i>Filipendula vulgaris</i> Moench	1.2	1.2	1.2
<i>Cynosurus cristatus</i> L.	1.1	+1	+
<i>Inula britannica</i> L.	1.2	+	
<i>Ononis arvensis</i> L.	+1	1.1	+1
<i>Chrysopogon gryllus</i> (L.) Trin.			3.3
<i>Agrostis stolonifera</i> L.			1.2
<i>Melampyrum arvense</i> L.		+1	2.2
<i>Agrostis capillaris</i> L.	+1	+1	+1
<i>Lotus corniculatus</i> L.	+2	+1	+2
<i>Ranunculus montanus</i> Willd.	+1	+1	
<i>Potentilla erecta</i> (L.) Raeusch.	+1	+1	+1
<i>Achillea millefolium</i> L.	+1	+2	+1
<i>Briza media</i> L.	+1	1.1	1.2
<i>Anthoxanthum odoratum</i> L.	+2	+2	+1
<i>Trifolium pratense</i> L.	+1	+1	+1
<i>Hieracium pilosella</i> L.	+1	+2	
<i>Veronica chamaedrys</i> L.	+1	+1	
<i>Thymus</i> sp.	+2	+2	+2
<i>Dianthus carthusianorum</i> L.	+	+1	
<i>Plantago lanceolata</i> L.	+	+	+
<i>Leucanthemum vulgare</i> Lam.	+	+	+
<i>Sanguisorba minor</i> Scop.	+	+	+
<i>Anacamptis morio</i> (L.) R.M. Bateman, Pridgeon & M.W. Chase	+1	+	+1
<i>Galium mollugo</i> L.	+	+	+
<i>Campanula patula</i> L.	+	+	+
<i>Euphrasia stricta</i> Wolff	+	+	
<i>Prunella vulgaris</i> L.	+	+	
<i>Leontodon crispus</i> Vill.	+	+	
<i>Luzula campestris</i> (L.) DC.	+	+	

No. Relevé	1	2	3
Altitude (m)	490	510	510
Exposition	NW	W	W
Inclination (°)	20–30	20	20
Relevé area (m <sup>2</sup> )	25	25	25
Date	01.07.2005	01.07.2005	01.07.2005
<i>Cruciata laevipes</i> Opiz	+	+	+
<i>Ajuga reptans</i> L.	+		
<i>Trifolium repens</i> L.	+	+	
<i>Prunella laciniata</i> L.	+	+	
<i>Bellis perennis</i> L.	+1	+1	+1
<i>Neotinea ustulata</i> (L.) R.M. Bateman, Pridgeon & M.W. Chase	+1	+	+1
<i>Neotinea tridentata</i> (Scop.) R.M. Bateman, Pridgeon & M.W. Chase	+1	+1	+1
<i>Neotinea × dietrichiana</i> (Bogenh.) H. Kretzschmar, Eccarius & H. Dietr.	+	+	+
<i>Galium verum</i> L.	+	+	+
<i>Dactylis glomerata</i> L.		+	
<i>Centaurea phrygia</i> L.	+1	+1	+1
<i>Tragopogon pratensis</i> L.	+1	+1	+
<i>Polygala comosa</i> Schk.	+	+1	+
<i>Anacamptis pyramidalis</i> (L.) Rich.	r	+1	+1
<i>Medicago sativa</i> L.		+	+
<i>Rumex acetosa</i> L.		+	
<i>Ornithogalum pyramidale</i> L.		+1	+1
<i>Salvia pratensis</i> L.		+1	+1
<i>Ranunculus acris</i> L.	+1	+1	+1
<i>Coronilla varia</i> L.		+1	1.2
<i>Centaurea scabiosa</i> L.	+	+	+1
<i>Potentilla recta</i> L.	+	+	
<i>Fragaria vesca</i> L.	+	+	
<i>Stachys officinalis</i> (L.) Trevisan	+	+	+1
<i>Knautia arvensis</i> (L.) Coult.	+	+	
<i>Linum catharticum</i> L.	+	+	
<i>Trifolium patens</i> Schreb.		+	+1
<i>Euphorbia esula</i> subsp. <i>tommasiniana</i> (Bertol.) Nyman	+	+	
<i>Plantago media</i> L.	+	+	
<i>Crepis biennis</i> L.		+	+1
<i>Salvia verticillata</i> L.			+
<i>Linaria vulgaris</i> Mill.			+

Phytosociological sampling has been done in the beginning of July, while the abundance of *N.* × *dietrichiana* and its two parental species has been estimated at the end of May. The presence of Pontic species *Rhinanthus rumelicus*, Pontic-Submediterranean species *Inula britannica*, *Knautia arvensis*, *Trifolium montanum*, *Salvia pratensis*, *Chrysopogon gryllus*, *Filipendula vulgaris*, *Melampyrum arvense*, and *Veronica chamaedrys*, as well as of Mediterranean-Submediterranean-Pontic species *Leontodon crispus*, *Dorycnium pentaphyllum* subsp. *herbaceum* and *Sanguisorba minor* indicates the xerothermic conditions and steppe-like character of the habitats. The edificator species *Chrysopogon gryllus* is a thermophilous plant, while *Danthonia calycina* belongs to the intermediate group between mesothermic and thermophilous plants (Kojić & al. 1997), which also testifies to the thermophilous nature of the meadows. The high abundance of *Chrysopogon gryllus*, *Danthonia calycina*, *Rhinanthus rumelicus*, *Filipendula vulgaris*, *Briza media*, *Trifolium montanum*, and *Dorycnium pentaphyllum* subsp. *herbaceum* indicates the oligotrophic character of the habitats, considering that these species belong to the intermediate group between oligotrophic and mesotrophic plants (Kojić & al. 1997). Other orchid species that have been found in these two communities were *Anacamptis morio* and *A. pyramidalis*. In contrast to its two parental species, the hybrid has not been found to grow in the hygro-mesophilous meadow community *Molinietum coeruleae* W. Koch 1926 (*Molinion coeruleae* Horv. 1949, *Molinetalia coeruleae*

Koch 1926, *Molinio-Arrhenatheretea* Tx. 1937). Such absence of hybrid specimens suggests that increased moisture is probably the limiting factor for appearance of the hybrid.

Population size of *N.* × *dietrichiana* and its two parental species in relation to ecological factors and plant community types is presented in Table 3. *N.* × *dietrichiana* has been recorded at an altitude between 490 m and 510 m. Eighteen specimens of *N.* × *dietrichiana* have been found there, and its total population size is estimated under 50 specimens. Furthermore, its area of distribution is estimated to be below 1 km<sup>2</sup>. Hybrid plants have been found mostly at sites where the population size of *N. tridentata* was larger than the population size of *N. ustulata* (Table 3). Between 490 m and 540 m a.s.l., a total of 1569 specimens of *N. tridentata* and a total of 131 specimens of *N. ustulata* have been recorded. *N.* × *dietrichiana* has not been found on the higher belts of Mt Maljen, while allopatric populations of the two parental species have been recorded at higher altitudes (950–960 m) (Table 3). Population data suggest that the abundance of the two parental species on Mt Maljen was decreasing with altitude. According to Pellissier & al. (2010), the relative frequency of food-deceptive orchids decreases with increasing altitude, indicating that deception may be less profitable at high altitude, as compared to low altitude. The low abundance of the two parental species and the absence of hybrid plants at higher altitudes on Mt Maljen may have been caused by environmental factors correlated with altitude, such as temperature

**Table 3.** Population size of *Neotinea* × *dietrichiana* and its two parental species on Mt Maljen (Serbia) in relation to ecological factors (altitude, exposition and inclination) and plant community types

Locality	Altitude (m)	Exposition	Inclination (°)	Plant community	Date	Population size		
						<i>Neotinea tridentata</i>	<i>Neotinea</i> × <i>dietrichiana</i>	<i>Neotinea ustulata</i>
Stojići	490	NW	20–30	<i>Danthonietum calycinae</i>	26.05.2005	12	2	27
Stojići	490	SW	10	<i>Danthonietum calycinae</i>	26.05.2005	309	4	5
Stojići	500	SW	5	<i>Danthonietum calycinae</i>	26.05.2005	204	3	10
Stojići	500	SW	15	<i>Danthonietum calycinae</i>	26.05.2005	363	–	–
Stojići	510	Z/W	0–20	<i>Danthonietum calycinae</i>	26.05.2005	146	5	24
Stojići	510	W	20	<i>Chrysopogonetum grylli</i>	26.05.2005	151	4	20
Stojići	520	SW	5	<i>Danthonietum calycinae</i>	26.05.2005	198	–	3
Mrčići	535	Z	0	<i>Molinietum coeruleae</i>	26.05.2005	–	–	3
Mrčići	535	W	5	<i>Molinietum coeruleae</i>	26.05.2005	186	–	27
Mrčići	540	SW	5	<i>Molinietum coeruleae</i>	26.05.2005	–	–	12
Divčibare	950	Z	0	<i>Danthonietum calycinae</i>	20.05.2004	2	–	–
Divčibare	960	Z	0	<i>Danthonietum calycinae</i>	17.06.2002	–	–	6
Divčibare	960	Z	0	<i>Danthonietum calycinae</i>	17.06.2002	–	–	2
Divčibare	960	Z	0	<i>Danthonietum calycinae</i>	17.06.2002	–	–	3

and humidity, by the low presence of appropriate pollinators, and by the fact that orchids require the presence of a suitable mycorrhizal symbiont for seed germination.

However, according to literature data, *N. × dietrichiana* has a great altitudinal range, between 170 m in Germany (Kretzschmar & al. 2007) and 1668 m in Switzerland (Renz 1944). In Bosnia-Herzegovina, the hybrid has been recorded at 1200 m a.s.l. (Maly 1928). In Greece (Mt Menikion and Mt Falakron), *N. × dietrichiana* inhabits the openings of *Carpinus-Ostrya* shrubs, subalpine grasslands and the openings of *Pinus nigra* Arn. forests (Tsiftsis & al. 2007), and it has been recorded at an altitude between 400 m and 1600 m (Tsiftsis, S. field obs.). It has been assumed that in Greece the higher altitude range of the hybrid is related to the climatic conditions correlating with latitude. The presence of thermophilous Submediterranean species *Ostrya carpinifolia* Scop., *Carpinus orientalis* Mill. and *Pinus nigra* Arn. indicates xerothermic habitat conditions. However, the higher belts of Mt Maljen (Serbia), in which the hybrid is absent, are represented by forests of *Pinus sylvestris* L., beech forests and mixed beech-fir forests, indicating colder and more humid conditions. In Croatia (Kozina), it grows in grasslands and grasslands with *Juniperus* sp., between 470 m and 490 m a.s.l., with S-SSW and W-WNW exposition (Gözl & Reinhard 1986). The altitudinal range of the hybrid there is similar to that in Serbia, while the presence of *N. × dietrichiana* in the predominantly western exposition conforms with the findings of the hybrid in Serbia.

Data on the occurrence of *N. × dietrichiana* on Mt Maljen (Serbia) and literature data suggest the heliophilous and thermophilous character of the hybrid. Its presence on serpentine habitats in Serbia confirms this fact, considering that the open serpentine habitats, especially in the lowlands and at middle altitudes, are thermophilous and xeric (Jakovljević & al. 2011). The specific chemical properties of serpentine soils (high concentrations of iron, magnesium, nickel, cobalt and chromium, and reduced nutrients) contribute to the xerothermicity of the habitat. The pH values of serpentine substrate vary from basic to ultrabasic (pH 5.5–8.0) (Stevanović & al. 2003). However, although it is known that the two parental species of *N. × dietrichiana* prefer alkaline substrates, they are primarily characteristic of calcareous substrates, and not of serpentine (Delforge 2006; Tali & al. 2004).

The occurrence of *N. × dietrichiana* on Mt Maljen (Serbia) in sunny steppe-like meadows, on moderately moist soil is not surprising, considering the ecological preferences of the two parental species. *N. tridentata* grows in full light, seldom in slight shade, on soils that are moderately dry to moist, well aerated and rich in humus, with a pH of 5.5 to 8.0 (Vakhrameeva & al. 2008). It inhabits short and poor grasslands, poor meadows, open garrigues, mountain pastures, banks, open woodlands, and woodland edges, up to 1600 m (Delforge 2006). The majority of populations occur in the orders *Festuco-Sedetalia* and *Molinietalia*, and in the alliance *Mesobromion* (Oberdorfer 1994). *N. ustulata* is a light-requiring plant growing on moderately moist, often clayish soils, with a pH of 4.9 to 8.5, and hardly tolerates excessive moisture, as well as drought (Vakhrameeva & al. 2008; Tali & al. 2004). It inhabits short grasslands, mountain pastures, transitional zones of marshland, garrigues, and open woodlands, up to 2400 m (Delforge 2006; Tali & al. 2004; Haraštová-Sobotková & al. 2005). *N. ustulata* occurs in the alliances *Mesobromion*, *Cirsio-Brachypodium pinnati*, *Arrhenatherion*, *Festucion valesiacae*, *Violion caninae*, *Geranion sanguine*, and *Quercion pubescenti-petraeae* (Tali & al. 2004; Oberdorfer 1994; Haraštová-Sobotková & al. 2005). In Serbia, *N. ustulata* was recorded in the communities *Carpinetum orientalis serbicum* Rudski 1940. Rudski em. Jovanović 1953, *Danthonietum calycinae* Cincović et Kojić 1958, and *Potentilleteo-Caricetum humilis* R. Jovanović 1955 (Diklić 1976). Both parental species are members of the community *Inulo-Chrysopogonetum grylli* Stevanović 1984 *stipetosum pulcherimae* Stevanović 1984 on Mt Fruška Gora (Vojvodina, Northern Serbia) (Savić 1998). The hybrid *N. × dietrichiana* has not been recorded there, although there are similarities between this community and the community *Chrysopogonetum grylli* Gajić 1954 described on Mt Maljen.

### Flowering period

Similarly to its two parental species, the flowering of *N. × dietrichiana* lasts from mid-May to mid-June on the southwestern slopes of Mt Maljen (alt. 490–540 m). The optimal (full) flowering period is in the fourth week of May. On the higher belt of Mt Maljen (alt. 950–960 m), the flowering period of *N. tridentata* is the same as at lower altitudes, while the flowering of *N. ustulata* lasts from about the first week of June until the end of June, with an optimal (full) flowering peri-



od in the second and the third weeks of June. The different flowering periods of the two parental species are the possible reasons why the hybrid does not occur at higher altitudes on Mt Maljen. Although the existence of early- and late-flowering populations of *N. ustulata* has led many authors to distinguish the two separate varieties/ subspecies (*ustulata* and *aestivalis*) (Tali & al. 2004; Haraštová-Sobotková & al. 2005; Tali & al. 2006), all populations of *N. ustulata* on Mt Maljen are identified as early-flowering (var. *ustulata*).

### Pollination

Both parental species of *N. × dietrichiana* are cross-pollinated and food-deceptive orchids. The pollinators of *N. tridentata* are *Apis mellifera* L., *Halictus patellatus* Morawitz, *Osmia bicornis* L., and *O. niveata* Fabricius (Cozzolino & al. 2005). The pollinators of *N. ustulata* are *Echinomyia magnicornis* Zett. (Diptera, Tachinidae) (Delforge 2006) and *Leptura livida* Fabricius (Coleoptera, Cerambycidae) (Tali & al. 2004). In this study, the pollinators of the hybrid and its parental species have not been recorded. The hybrid might have originated by mistake made by one of the pollinators or through accidental cross pollination by some other pollinators.

### Conservation

*N. × dietrichiana* is protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) like all members of the family Orchidaceae, including hybrids (Hágsater & Dumont 1996). In addition, its two parental species are protected by law in most of Europe. It should be noted that hybridization is of limited importance as a basis for speciation in food-deceptive orchids and it does not pose a threat to their survival, but sympatric zones are important because they provide the stage for evolutionary processes in orchids (Cozzolino & al. 2006; Moccia & al. 2007). Consequently, conservation priorities should focus mostly on the hybrid zones and the sympatric zones, instead of the hybrid individuals (Cozzolino & al. 2006). The threat factors to *N. × dietrichiana* and its two parental species on Mt Maljen are plowing of meadows and their conversion into agricultural land, change in the grazing regime, increasing of moisture through irrigation, and habitat destruction by urbanization.

**Acknowledgements.** The authors thank Dr. Attila Molnár (University of Debrecen), Dr. Jeffrey J. Wood (Royal Botanical Garden – Kew, UK), Dr. Horst Kretschmar, and Dr. Karel Kreutz for

confirming the identification of the hybrid. We are grateful to Stefan Hertel and Djordjije Milanović for providing the distribution data of the hybrid. The authors also thank Dr. Salvatore Cozzolino (University of Naples) and Dr. Dmitar Lakušić (University of Belgrade) for providing literature and suggestions. This research was supported by the Ministry of Education and Science of the Republic of Serbia (Grant 173030).

### References

- Bateman, R.M., Hollingsworth, P.M., Preston, J., Yi-Bo, L., Pridgeon, A.M. & Chase, M.W. 2003. Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). – *Biol. J. Linn. Soc.*, **142**: 1-40.
- Bogenhard, C. 1850. Taschenbuch der Flora von Jena. Wilhelm Engelmann, Leipzig.
- Braun-Blanquet, J. 1964. Pflanzensoziologie. Grundzüge der Vegetationskunde. Springer, Vienna & New York.
- Camus, E.G. & Camus, A. 1928. Iconographie des Orchidées d'Europe et du Bassin Méditerranéen, pp. 262-263. P. Lechevalier, Paris.
- Cozzolino, S., Aceto, S., Caputo, P. & Menale, B. 1998. Characterization of *Orchis* × *dietrichiana* Bogenh., a natural orchid hybrid. – *Pl. Biosyst.*, **132**(1): 71-76.
- Cozzolino, S., Nardella, A.M., Impagliazzo, S., Widmer, A. & Lexer, C. 2006. Hybridization and conservation of Mediterranean orchids: Should we protect the orchid hybrids or the orchid hybrid zones? – *Biol. Conservation*, **129**(1): 14-23.
- Cozzolino, S., Schiestl, F.P., Müller, A., Castro, O.D., Nardella, A.M. & Widmer, A. 2005. Evidence for pollinator sharing in Mediterranean nectar-mimic: absence of pre-mating barriers? – *Proc. Roy. Soc. London, Ser. B, Biol. Sci.*, **272**: 1271-1278.
- Delforge, P. 2006. Orchids of Europe, North Africa and the Middle East. A. & C. Black Publishers, London
- Diklić, N. 1976. *Orchis* L. – In: Josifović M. (ed.), Flora of SR Serbia. Vol. 8, pp. 48-68. Serbian Acad. Sci. & Art, Belgrade (in Serbian).
- Flora Europaea Database – <http://rbg-web2.rbge.org.uk/FE/fe.html>, Royal Botanic Garden Edinburgh.
- Gözl, P. & Reinhard, H.R. 1986. Orchideen in Jugoslawien. – *Mitt. Bl. Arbeitskr. Heim. Orch. Baden-Württemberg*, **18**(4): 689-827.
- Grant, V. 1981. Plant Speciation. Columbia Univ. Press, New York.
- Hágsater, E. & Dumont, V. (eds) 1996. Orchids: Status, Survey and Conservation Action Plan. IUCN, Gland, Switzerland and Cambridge, UK.
- Haraštová-Sobotková, M., Jarsáková, J., Kindlmann, P. & Čurn, L. 2005. Morphometric and genetic divergence among populations of *Neotinea ustulata* (Orchidaceae) with different flowering phenologies. – *Folia Geobot.*, **40**: 385-405.
- Jakovljević, K., Lakušić, D., Vukojičić, S., Tomović, G., Šinžar-Sekulić, J. & Stevanović, V. 2011. Richness and diversity of Pontic flora on serpentine of Serbia. – *Cent. Eur. J. Biol.*, **6**(2): 260-274.
- Kerner, A. 1865. Die hybriden Orchideen der oesterreichischen Flora. – *Oesterr. Bot. Z.*, Wien, **15**: 203-236.



- Kojić, M., Popović, R. & Karadžić, B.** 1998. Vascular plants of Serbia as indicators of habitat. Inst. Biol. Istraž. "S. Stanković" Press., Belgrade (in Serbian).
- Kojić, M., Popović, R. & Karadžić, B.** 1998. Syntaxonomic Overview of the Vegetation of Serbia. Inst. Biol. Istraž. "S. Stanković" Press., Belgrade (in Serbian).
- Kranjčev, R.** 2005. Croatian Orchids. AKD, Zagreb (in Croatian).
- Kretschmar, H., Eccarius, W. & Dietrich, H.** 2007. The Orchid Genera *Anacamptis*, *Orchis* and *Neotinea*. Phylogeny, Taxonomy, Morphology, Biology, Distribution, Ecology and Hybridization. 2<sup>nd</sup> ed. EchinoMedia Verlag, Bürgel.
- Lampinen, R.** 2001. Universal Transverse Mercator (UTM) and Military Grid Reference System (MGRS). <http://www.fmnh.helsinki.fi/english/botany/afe/map/utm.htm>.
- Maly, K.** 1928. Contribution to the flora of Bosnia and Herzegovina. – Glasn. Zemaljsk. Muz. Bosne Hercegovine, **40**(1): 107-166 (in Serbo-Croatian).
- Moccia, M.D., Widmer, A. & Cozzolino, S.** 2007. The strength of reproductive isolation in two hybridizing food-deceptive orchid species. – Molec. Ecol., **16**: 2855-2866.
- Molnár, A., Sulyok, J. & Vidéki, R.** 1995. Wild orchids. Kossuth, Budapest (in Hungarian).
- Oberdorfer, E.** 1994. Pflanzensoziologische Exkursionsflora. Ulmer, Stuttgart, Germany.
- Peitz, E.** 1972. Zusammenstellung aller bisher bekannten Bastarde der in Deutschland verbreiteten Orchideen. – Jahresb. naturwiss. Ver. Wuppertal, **25**: 167-200.
- Pellissier, L., Vittoz, P., Internicola, A.I. & Gigord, L.D.B.** 2010. Generalized food-deceptive orchid species flower earlier and occur at lower altitudes than rewarding ones. – J. Plant Ecol., **3**(4): 243-250.
- Reinhard, H., Gözl, P., Peter R. & Wildermurth, H.** 1991. Die Orchideen der Schweiz und angrenzender Gebiete. Fototar, Egg.
- Renz, J.** 1944. *Orchis* × *dietrichiana* Bogenh. Herbarium Jany Renz 2672. Swiss Orchid Foundation at the Herbarium Jany Renz. – <http://orchid.unibas.ch>. (accessed 27.09.2011).
- Savić, D.** 1998. Ecology, distribution and conservation of species of the family *Orchidaceae* on Mt Fruška gora. *Master's Thesis*. Fac. Biol., Univ. Belgrade (in Serbian).
- Schatz, B.** 2006. Fine scale distribution of pollinator explains the occurrence of the natural orchid hybrid ×*Orchis bergonii*. – *Écoscience*, **13**(1): 111-118.
- Schulze, M.** 1894. Die Orchidaceen Deutschlands, Deutsch-Oesterreichs und der Schweiz. 7(3) t.7(b). Gera-Untermhaus, E. Kölers's Verlag, Berlin.
- Soó, R.** 1980. *Orchis* L. – In: **Tutin, T.G & al.** (eds), *Flora Europaea*. Vol. 5, pp. 337-342. Cambridge Univ. Press, Cambridge.
- Stevanović, V., Tan, K. & Iatrou, G.** 2003. Distribution of the endemic Balkan flora on serpentine I. – obligate serpentine endemics. – *Pl. Syst. Evol.*, **242**: 149-170.
- Tali, K., Fay, M.F. & Bateman, R.M.** 2006. Little genetic differentiation across Europe between early-flowering and late-flowering populations of the rapidly declined orchid *Neotinea ustulata*. – *Biol. J. Linn. Soc.*, **87**: 12-25.
- Tali, K., Foley, M.J.Z. & Kull, T.** 2004. Biological flora of the British Isles, 232. *Orchis ustulata* L. – *J. Ecol.*, **92**: 174-184.
- Tsiftsis, S., Karagiannakidou, V. & Tsiripidis, I.** 2007. The orchid flora of East Macedonia (NE Greece). – *J. Eur. Orch.*, **39**(3/4): 489-526.
- Vakhrameeva, M.G., Tatarenko, I.V., Varlygina, T.I., Torosyan, G.K. & Zagulski, M.N.** 2008. Orchids of Russia and Adjacent Countries (within the borders of the former USSR). A.R.G Gantner Verlag, Ruggell, Liechtenstein.
-

