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Morphological and karyological differentiation of *Gymnadenia densiflora* and *G. conopsea* in the Czech Republic and Slovakia

Morfologická a karyologická diferenciace *Gymnadenia densiflora* a *G. conopsea* v České republice a na Slovensku

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Gymnadenia densiflora was recently either misinterpreted or not accepted as a distinct taxon by several authors. To resolve its taxonomic position and differentiation from the related *G. conopsea*, a detailed study of the morphology, chromosome numbers and distribution of these two taxa in the Czech Republic, Slovakia and neighbouring areas was carried out. Chromosome counts showed an invariable diploid chromosome number (2n = 40) for *G. densiflora*, while *G. conopsea* is diploid, tetraploid and rarely also pentaploid (2n = 40, 80, 100). Results of morphometric analyses (principal component analysis, cluster analysis, classificatory and canonical discriminant analysis) confirmed a good morphological separation between *G. densiflora* and *G. conopsea*. Characters such as the width of the second lowermost leaf, height of the plant, number of flowers in the inflorescence, number of leaves, and the ratio of height of the plant and distance from the stem base to the base of the uppermost sheathed leaf contributed most to this separation. Our study supports the recognition of *G. densiflora* as a distinct species.

Keywords: chromosome numbers, morphometrics, *Gymnadenia*, *Orchidaceae*

Introduction

Gymnadenia densiflora (Wahlenb.) A. Dietr. was described in 1806 from Sweden by Wahlenberg (1806) under the name *Orchis conopsea* β [var.] *densiflora*. This name was later transferred to the genus *Gymnadenia* and referred to as either *G. densiflora* (Wahlenb.) A. Dietr., *G. conopsea* subsp. *densiflora* (Wahlenb.) K. Richt. or *G. conopsea* var. *densiflora* (Wahlenb.) Hartm. Wahlenberg's name was recently lectotypified by Moberg & Nilsson (1991: 291) by a specimen from Thunberg's herbarium in Uppsala (UPS-THUNB no. 21193), while for Linnaeus' basionym of the name *G. conopsea* (L.) R. Br., *Orchis conopsea* L., a lectotype was chosen by Baumann et al. (1989: 482), namely an illustration in Fuchs (1542: 712) entitled *Satyrium basilicum.* Both lectotypifications provided the basis for the unequivocal interpretation of these names. Although in the past the taxon corresponding to *G. densiflora* was accepted in many floras (e.g. Ascherson &

Graebner 1905–1907, Soó 1973), it is not mentioned in the Flora Europaea (Moore 1980) and some recent Central European floras or identification keys (e.g. Adler et al. 1994). Referring to our preliminary results, it was accepted at the level of subspecies by Wisskirchen & Haeupler (1998). As a separate taxon it was accepted also by Bisse (1963 a,b), Möseler (1987), Möseler & Patzke (1987), Mrkvicka (1993) and some other authors, but our preliminary results indicated that at least some authors misinterpreted this taxon.

More recently, molecular techniques were used to investigate patterns of genetic variation within and among populations, and marginally addressed taxonomic questions in *Gymnadenia conopsea* s.l. Scacchi & de Angelis (1989) analyzed allozyme variation in Italian populations and detected strong differentiation between two ecotypes (from dry and humid habitats), which, however, were not morphologically distinct. Effects of variation in flowering time in *G. conopsea* s.l. on the genetic structure, differentiation and extent of gene flow have been studied in populations in Sweden (Gustafsson & Lönn 2003) and Switzerland (Soliva & Widmer 1999). Studies on allozyme and morphological variation in Swiss early- and late-flowering variants, assigned to the subspecies *G. conopsea* subsp. *conopsea* and subsp. *densiflora*, respectively, revealed pronounced genetic divergence, separating the populations into two distinct groups. However, no corresponding differentiation in floral characters, and only a weak one in vegetative characters was observed (Soliva & Widmer 1999). In Sweden, both early and late phenological variants morphologically corresponding to *G. conopsea* subsp. *conopsea*, as well as late-flowering subsp. *densiflora* were recognized and studied using microsatellites and nrDNA ITS sequences (Gustafsson & Lönn 2003). The early- and late-flowering types of subsp. *conopsea*, which show also different habitat preferences, display unexpectedly strong divergence in ITS sequences. The ITS sequence of the latter, however, is identical to that found in plants identified as subsp. *densiflora*, which suggests that the phenological differentiation in subsp. *conopsea* probably occurred before that between subsp. *conopsea* and subsp. *densiflora.*

Although the taxonomic recognition of the taxon corresponding to *G. densiflora* was not disputed in the above two studies, extensive variability in morphological traits in *G. conopsea* sensu lato, phenological variation and repeated taxonomic misinterpretations complicate the unequivocal circumscription of these two taxa. In this study emphasis is placed on detailed morphological analyses, reviewing and presenting new data on chromosome numbers, with the aim of re-evaluating the taxonomic recognition and position of *G. densiflora*, and its relationship to *G. conopsea* (s. str.). This study was largely based on material from the Czech Republic, Slovakia and neighbouring areas (Poland, Austria, Germany, Italy and Slovenia).

Material and methods

Morphometric analysis

For the morphometric analysis, 22 population samples (in total 157 specimens) of *Gymnadenia conopsea* s.l. from the Czech Republic and Slovakia were collected (for localities see Table 1). The sampling was designed to cover as much morphological variation as possible, and to include morphological types previously classified as both *G. conopsea* and *G. densiflora* (or *G. conopsea* subsp. *conopsea* and *G. conopsea* subsp. *densiflora*, respectively). The number of specimens studied was strongly limited by the number of localities and the population sizes.

Table 1. – List of populations and number of plants of *Gymnadenia densiflora* and *G. conopsea* used for karyological and morphometric analyses. Localities are arranged according to countries; for samples included in morphometric analyses, population codes (in parentheses) and exact collection dates (reflecting the flowering time) are given. The three numerical columns include: (i) chromosome number, (ii) number of individuals used for karyological analysis, (iii) number of individuals used for morphometric analysis. The chromosome numbers typed in normal font refer to counts from root-tips, those in italics are counts from flower buds (*n* or *2n* values); chromosome numbers without a symbol in superscript represent new data. Symbols and indices: * DNA ploidy level assessed by flow cytometry, ^{1} published by Jongepierová & Jongepier (1989), ^{2} unpublished chromosome counts/ploidy level assessments by other authors. Unless otherwise stated voucher specimens are deposited in herbarium BRNM.

Gymnadenia densiflora

Czech Republic:

SE Moravia, Bílé Karpaty stepní, Nature reserve Zahrady pod Hájem, near the village of Velká nad Veličkou, I. Jongepierová 1994, 1995, 1996, count. A. Krahulcová	2n = 40 *2n = 40* *n = 20*	2 7	–
SE Moravia, Bílé Karpaty stepní, Nature reserve Čertoryje, near the village of Kněždub, I. Jongepierová 1995, 1996, count. A. Krahulcová	*2n = 40*	3	–
SE Moravia, Bílé Karpaty stepní, Nature reserve Nad Vápenkou, near the village of Javorník, I. Jongepierová 1994, count. A. Krahulcová	*2n = 40* *n = 20*	3	–
(D-LA) SE Moravia, Bílé Karpaty lesní, Nature reserve Lazy, near the village of Bylnice, I. Jongepierová, 24. 7. 1987, 1995, count. A. Krahulcová	*2n = 40*	3	5
(D-HR) SE Moravia, Bílé Karpaty lesní, Nature reserve Hrnčárky, near the village of Strání, I. Jongepierová 26. 7. 1988, 22. 7. 1993, 1994, count. Č. Čihalík, A. Krahulcová	*2n = 40* *2n = 40*^{1}	2	1
SE Moravia, Bílé Karpaty lesní, Nature reserve Machová, near the village of Javorník, I. Jongepierová 1988,1994, count. Č. Čihalík, A. Krahulcová	*2n = 40* *n = 20* *2n = 40*^{1}	3	–
(D-KA) SE Moravia, Bílé Karpaty lesní, Nature reserve Kalábová, near the village of Březová, I. Jongepierová 8. 7. 1987, 27. 6. 1988, 1994, count. Č. Čihalík, A. Krahulcová	*2n = 40* *2n = 40*^{1}	3	4
(D-ST) SE Moravia, Bílé Karpaty lesní, Střelnice, near the village of Březová, I. Jongepierová 8. 7. 1987, count. A. Krahulcová	*2n = 40*	1	16
(D-CE) SE Moravia, Bílé Karpaty lesní, Nature reserve Cestiska, near the village of Březová, I. Jongepierová 15. 7. 1993	–	–	1
(D-SU) SE Moravia, Bílé Karpaty lesní, Suchovské mlýny, near the village of Suchov, I. Jongepierová 2. 7. 2002	–	–	4
(D-RU) SE Moravia, Vsetínská kotlina, Růžďka, near the town of Vsetín, J. Pavelka 30. 6. 1993, 29.6. 1995, count. A. Krahulcová	*2n = 40,* *n = 20*	2	5
E Bohemia, Jaroměřské Polabí, in the present area of Rozkoš reservoir (prior to flooding) near the town of Náchod, F. Krahulec 1972 PR, count. J. Měsíček	*2n = 40*^{2}	–	–
C Bohemia, Džbán, Líský, NEE of the town of Slaný, J. Hadinec et al. 2004, flow cytometric analysis J. Suda	diploid (2x)*	1	–
C Bohemia, Milešovské stredohoří, Chrášťany, between Solanská hora hill and ruin of Oltářík castle, K. Kubát 1987 LIT, F. Procházka, I. Jongepierová & R. Dundr 2001, count. K. Kubát	*2n = 40*^{2}	–	–
C Bohemia, Všetatské Polabí, Mělnická Vrutice, R. Dundr 1988, count. K. Kubát	*2n = 40*^{2}	–	–
C Bohemia, Všetatské Polabí, near the village of Mělnická Vrutice, F. Procházka & I. Jongepierová 2001, flow cytometric analysis J. Vrána	diploid (2x)*	1	–
N Bohemia, Milešovské stredohoří, near the village of Chrášťany, under Solanská hora hill, F. Procházka, I. Jongepierová & R. Dundr 2001, flow cytometric analysis J. Vrána	diploid (2x)*	1	–
W Bohemia, Tachovská brázda, Chotěnov, near the town of Mariánské Lázně, V. Melichar, F. Procházka & T. Urfus 2002, flow cytometric analysis J. Suda & T. Urfus	diploid (2x)*	2	–

Slovakia:

(D-NL) W Slovakia, Považský Inovec, gamekeeper's house Jániš, near the spring of Bojnianka, near the village of Nová Lehota, J. Kučera, 15. 7. 2002	–	–	4
(D-PR) C Slovakia, Lúčanská Malá Fatra, near the village of Predvrícko, J. Kučera, 9. 7. 2002	–	–	12

C Slovakia, Veľká Fatra, Gaderská dolina valley, D. Bernátová & J. Kochjarová, 1995, count. A. Krahulcová	$2n = 40$	2	–
C Slovakia, Veľká Fatra, Žarnovická dolina valley, J. Kochjarová, 1995, count. A. Krahulcová	$2n = 40$	1	–
C Slovakia, Veľká Fatra, Blatnická dolina valley, D. Bernátová & J. Kochjarová, 1995, count. A. Krahulcová	$2n = 40$ $n = 20$	3	–
C Slovakia, Turčianska kotlina, near the town of Vrútky, K. Kubát 1977 LIT, D. Bernátová 1980 LIT, I. Jongepierová 1988, count. K. Kubát, Č. Čihalík	$2n = 40$[1] $2n = 40$[2]	–	–
Germany:			
Sachsen-Anhalt, Grosswilsdorf, Tote Taler, F. Procházka & I. Jongepierová 2001, flow cytometric analysis J. Vrána	diploid (2x)*	1	–
Italy:			
Dolomiti, Falzarego, V. Čurn 1997, count. A. Krahulcová	$2n=40$	1	–
Gymnadenia conopsea			
Czech Republic:			
SE Moravia, Bílé Karpaty stepní, Nature reserve Čertoryje, near the village of Radějov, I. Jongepierová 1996, count. A. Krahulcová	$2n = 40$ $n = 20$	7	–
SE Moravia, Bílé Karpaty stepní, Nature reserve Zahrady pod Hájem, near the village of Velká nad Veličkou, I. Jongepierová 1996, I. Jongepierová & V. Čurn 1997, count. A. Krahulcová	$2n = 40$ $n = 20$ $2n = 80$	1 1 3	–
SE Moravia, Bílé Karpaty stepní, Nature reserve Drahy, near the village of Horní Němčí, I. Jongepierová & V. Čurn 1998, count. A. Krahulcová	$2n = 80$	1	–
SE Moravia, Bílé Karpaty lesní, Nature reserve Hutě, near the village of Žitková, I. Jongepierová 1996, count. A. Krahulcová	$2n = 40$ $n = 20$	5	–
SE Moravia, Bílé Karpaty lesní, Nature reserve Machová, near the village of Javorník, I. Jongepierová 1996, count. A. Krahulcová	$2n = 40$ $n = 20$	3	–
(C-JA) SE Moravia, Bílé Karpaty lesní, Javorník, nearby the railway, I. Jongepierová 18. 6. 1993	–	–	3
(C-KJ) SE Moravia, Bílé Karpaty lesní, Nature reserve Jazevčí, near the village of Javorník, I. Jongepierová 8. 6. 1987	–	–	28
SE Moravia, Bílé Karpaty lesní, Nature reserve Lazy, near the village of Bylnice, I. Jongepierová 1995, count. A. Krahulcová	$2n = 40$ $n = 20$	2	–
(C-DU) SE Moravia, Bílé Karpaty lesní, Nature reserve Dubiny, near the village of Březová, I. Jongepierová 25. 6. 1987	–	–	13
SE Moravia, Bílé Karpaty lesní, Babiratka, near the village of Javorník, I. Jongepierová 1996, count. A. Krahulcová	$2n = 40$	4	–
(C-DO) SE Moravia, Bílé Karpaty lesní, Nature reserve Dolnoněmčanské louky, near the village of Horní Němčí, I. Jongepierová 20. 6. 1989, I. Jongepierová & V. Čurn 1997, count. A. Krahulcová	$2n = 40$ $n = 20$	3	6
SE Moravia, Bílé Karpaty lesní, Nature reserve Porážky, near the village of Slavkov, I. Jongepierová & V. Čurn 1998, count. A. Krahulcová	$2n = 40$	2	–
(C-KV) SE Moravia, Hustopečská pahorkatina, Nature reserve Kamenný vrch, near the village of Kurdějov, I. Jongepierová 10. 6. 1993, 1995, count. A. Krahulcová	$2n = 80$ $n = 40$	3	7
(C-ZA) SE Moravia, Hustopečská pahorkatina, Nature reserve Zázmoníky, near the village of Bořetice, I. Jongepierová 25. 6. 1989, V. Čurn 1998, count. A. Krahulcová	$2n = 80$	1	8
(C-HM) S Moravia, Chřiby, Nature reserve Moravanské louky, I. Jongepierová 10. 6. 1993	–	–	2
(C-VS) SE Moravia, Javorníky, Zádělský, 20.6. 1993; Nový Hrozenkov, 25. 6. 1993; Halenkov, 20. 6. 1993; Vsetín, Jasenice, 25. 6. 1993, J. Pavelka	–	–	17
(C-KR) NE Moravia, Javorníky, near the village of Karolínka, J. Pavelka 3. 7. 1993	–	–	3
(C-VK) NE Moravia, Javorníky, near the village of Velké Karlovice, J. Pavelka 3. 7. 1993	–	–	2
N Bohemia, České stredohoří, Nature reserve Bílé stráně, near the town of Litoměřice, I. Jongepierová & V. Čurn 1997, count. A. Krahulcová	$2n = 40$ $2n = 40$	1 2	–

N Bohemia, Jizerské hory, near the village of Malá Strana, I. Jongepierová 1995, count. A. Krahulcová	*n = 20*	1	–
N Bohemia, Český ráj, Podloučky, near the town of Turnov, I. Jongepierová 1995, 1996, count. A. Krahulcová	*2n = 80* n = 40	4	–
(C-VU) N Bohemia, Krkonoše, near the village of Velká Úpa, F. Krahulec 15. 6. 1993	–	–	4
S Bohemia, Českomoravská vrchovina, near the village of Horní Čepí, I. Jongepierová & V. Čurn 1998, count. A. Krahulcová	2n = 80	1	–
S Bohemia, Šumavsko-novohradské podhůří, village of Opolenec u Sudslavic, near the town of Vimperk, V. Čurn 1997, 1998, count. A. Krahulcová	2n = 80 2n =100	3 1	–
S Bohemia, Šumavsko-novohradské podhůří, village of Milčice, near the town of Sušice, V. Čurn 1998, count. A. Krahulcová	2n = 80	1	–
C Bohemia, Český kras, Nature reserve Karlštejn, near the village of Karlštejn, I. Jongepierová & V. Čurn 1998, count. A. Krahulcová	2n = 80	1	–
Slovakia:			
W Slovakia, Záhorská nížina, Nature reserve Veterník, near the town of Skalica, I. Jongepierová & V. Čurn 1998, count. A. Krahulcová	2n = 80	2	–
(C-VV) W Slovakia, Strážovské vrchy, Vápeč hill, near the village of Horná Poruba, P. Batoušek 18. 6. 1989, J. Kochjarová 1997, count. A. Krahulcová	*2n = 80* *n = 40*	1	10
(C-DK) W Slovakia, Devínska Kobyla, Devínska Kobyla hill, I. Jongepierová 15. 6. 1993, J. Kochjarová 1996, count. A. Krahulcová	*2n = 80*	6	2
C Slovakia, Krivánska Malá Fatra, Jánošíkove diery, Terchová, near the settlement of Štefanová, L. Hrouda 1997, count. A. Krahulcová	2n = 40	1	–
C Slovakia, Krivánska Malá Fatra, Varín, dolina Kúr valley, near the village of Krasňany, I. Jongepierová, V. Čurn & A. Dobošová 1999, count. A. Krahulcová	2n = 40	1	–
C Slovakia, Krivánska Malá Fatra, Belá, Pod Sokolím, near the village of Nižné Kamence, I. Jongepierová, V. Čurn & A. Dobošová 1999, count. A. Krahulcová	2n = 40	3	–
C Slovakia, Veľká Fatra, Čremošianske lazy, near the village of Čremošné, J. Kochjarová 1995, count. A. Krahulcová	*n = 20*	1	–
C Slovakia, Veľká Fatra, Konský dol, near the village of Blatnica, J. Kochjarová 1995, count. A. Krahulcová	*n = 40*	1	–
C Slovakia, Lúčanská Malá Fatra, between the villages of Porúbka and Turie, I. Jongepierová, V. Čurn & A. Dobošová 1999, count. A. Krahulcová	2n = 80	2	–
C Slovakia, Nízke Tatry, above the caves of Demänovská jaskyňa Slobody, near the village of Demänová, I. Jongepierová & V. Čurn 1999, count. A. Krahulcová	2n = 40	1	–
C Slovakia, Slovenský raj, near the village of Vernár, I. Jongepierová & V. Čurn 1999, count. A. Krahulcová	2n = 40	2	–
C Slovakia, Slovenský raj, between the village of Vernár and ice-cave Dobšinská ľadová jaskyňa, I. Jongepierová & V. Čurn 1999, count. A. Krahulcová	2n = 40	1	–
C Slovakia, Slovenský raj, near the village of Hrabušice, I. Jongepierová & V. Čurn 1999, count. A. Krahulcová	2n = 40	1	–
C Slovakia, Slovenský raj, Nature reserve Kopanecké lúky, near the village of Stratená, J. Kochjarová 1997, count. A. Krahulcová	*2n = 40* n = 20	1	–
C Slovakia, Poľana, Jaseňový vrch hill, near the village of Povrazník, J. Kochjarová 1995, count. A. Krahulcová	*n = 20*	3	–
N Slovakia, Západné Beskydy, Rovná hora, between the villages of Terchová and Zázrivá, I. Jongepierová, V. Čurn & A. Dobošová 1999, count. A. Krahulcová	2n = 40 2n = 80	2 1	–
N Slovakia, Chočské vrchy, near the village of Osádka, I. Jongepierová & V. Čurn 1999, count. A. Krahulcová	2n = 40	3	–
S Slovakia, Slovenský kras, under Vysoká hill, near the village of Silická Jablonica, I. Jongepierová & V. Čurn 1999, count. A. Krahulcová	2n = 80	1	–
Austria:			
Lower Austria, Lunz am See, V. Čurn 1998, count. A. Krahulcová	2n = 40 2n = 80	1 1	–

Morphological characters measured or scored for each specimen included traits traditionally used for identifying *G. densiflora*, as well as those that were useful in our preliminary studies: HP – height of the plant (cm); ratio of height of the plant (HP) to the distance from the base of the stem to the base of the uppermost sheathed leaf (USL); WL – maximum width of the second lowermost leaf (cm); NL – number of sheathless leaves; NF – number of flowers in the inflorescence; WLB – width of labellum (mm); SF – height of flower multiplied by its width, a character used to estimate flower size; FD – flower density, estimated as the ratio of the length of inflorescence (in cm) to number of flowers in the inflorescence. Variation in the scent of flowers was also recorded and used as a character (flower scent, FS) in multivariate analyses. Weak, vanilla-like, but slightly unpleasant (musty) scent vs. a strong and pleasant spicy to carnation-like odour could be distinguished. In addition to the morphological characters, phenological variation was studied, i.e. flowering time (represented by the collection date), before or after 10 June, was recorded for each specimen. This character was not included in the multivariate analyses, but is discussed in the text.

Principal component analysis (PCA, Krzanowski 1990), UPGMA (unweighted pair-group method using arithmetic averages) and complete linkage cluster analysis (Everitt 1986) were performed, using population samples characterized by the mean values of characters as operational taxonomic units. They were used to generate hypotheses, in order to find if any groupings of populations exist in the data corresponding to the traditionally recognized taxa, *G. conopsea* and *G. densiflora*. In both PCA and cluster analyses, the above-mentioned morphological characters and scent of the flowers were included. Based on two groups of populations resulting from both PCA and cluster analyses, classificatory and canonical discriminant analyses were carried out, using individual plants as operational taxonomic units and morphological characters. Scent of the flowers was not used in these analyses because of the lack of within-group variation. In the classificatory discriminant analysis, a non-parametric k-nearest neighbours method was used together with cross-validation (Klecka 1980, Krzanowski 1990, SAS Institute 2000). In addition, a descriptive data analysis, including computation of mean, median, standard deviation and 1, 10, 90 and 99 percentiles, was carried out on the data for both groups of populations.

All computations were performed at Charles University, Prague, using the UNIVARIATE, CANDISC and DISCRIM procedures of the SAS statistical package (SAS Institute 2000) and the program SYN-TAX 2000 (Podani 2001).

Chromosome numbers

Chromosome numbers of some of the plants from the morphometrically evaluated populations and numerous other plants of *Gymnadenia conopsea* s.l., from altogether 49 localities in the Czech Republic, Slovakia and neighbouring areas, were counted (Fig. 1, for localities see Table 1). In addition, previously published data by Jongepierová & Jongepier (1989), and unpublished data of several other authors are included in the analysis (Table 1). Either young inflorescences or root tips were used for temporary squashes. Young inflorescences were fixed in a mixture of glacial acetic acid and 96% ethanol (1 : 3) overnight and stored in 70% ethanol. Flower buds about 3 mm in size were macerated in 1 N HCl at 60 °C for 2–3 min, then washed in distilled water and stained in lactopropionic orceine. Chromosomes were counted either in cells undergoing somatic mitoses in young anthers at the premeiotic stage or meiotic metaphase in pollen mother cells, or both. To assess the chromosome num-

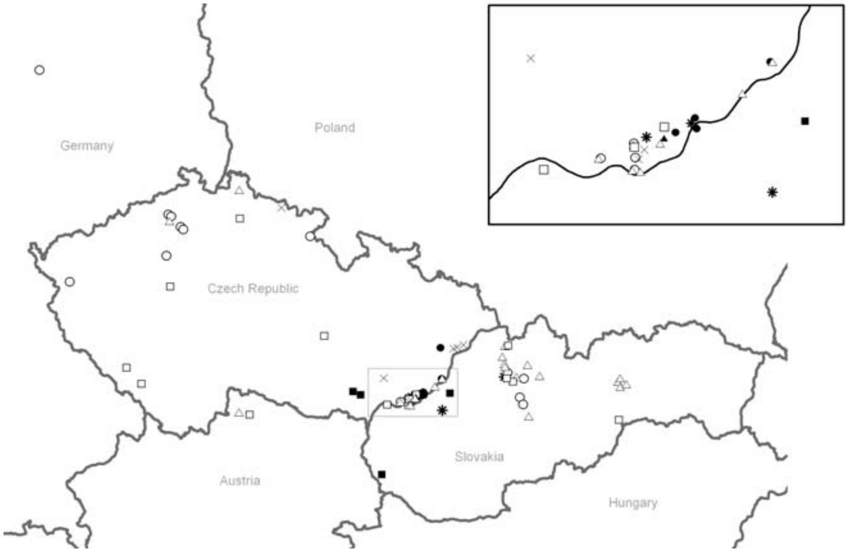

Fig. 1. – Distribution map of populations of *Gymnadenia densiflora* and *G. conopsea* used in the morphometric and karyological analyses. ▲ *G. conopsea*, 2x, morphologically analysed; △ *G. conopsea*, 2x; ✕ *G. conopsea*, unknown ploidy level; ■ *G. conopsea*, 4x, morphologically analysed; □ *G. conopsea*, 4x; ● *G. densiflora*, 2x, morphologically analysed; ○ *G. densiflora*, 2x; ✳ *G. densiflora*, unknown ploidy level. The locality in Italy is not shown.

ber at the flowering stage (providing the most important morphological characters for identifying *G. densiflora* and *G. conopsea*) root-tips were examined. Before fixation, the root tips were pretreated in para-dichlorbenzene for 3 hours and then macerated in 1 N HCl at 60 ºC for 7 min.; otherwise the procedure was the same as for the flower buds.

Chromosome counts were completed by flow cytometric analyses of *G. densiflora* from five additional localities (Table 1). In this case, the relative DNA content, corresponding to a particular ploidy level, was assessed and expressed as “DNA ploidy level”. The procedure of nuclei isolation and DAPI staining followed the two-step protocol originally described by Otto (1990) (for details see also Rosenbaumová et al. 2004: 221), using *Pisum sativum* cv. Ctirad (2C = 9.09 pg, Lysák & Doležel 1998) as an internal standard.

Distribution data

The distribution of *G. densiflora* in Central Europe was based entirely on the study of herbarium specimens and authors’ observations in the field. Field observations by František Krahulec, Průhonice, and Michal Hájek, Brno, both in the Czech Republic, were also taken into consideration. The following herbaria were consulted: BRA, BRNU, BRNM, KRAM, LIT, MP, OLM, PR, PRC, SAV, SLO, W and WU. Phytogeographic arrangement of the distribution data follows Skalický (1988) for the territory of the Czech Republic, and Futák (1984) for Slovakia. Polish localities are arranged following Kondracki (1981), and the distribution in Austria follows the political division of the country. Distribution of *G. conopsea* s. str. is not included here, but will be published elsewhere as part of a detailed taxonomical treatment of this species.

Results

Morphometric analysis

Both UPGMA and complete linkage cluster analysis divided the population samples into the same two groupings, except for one population (D-HR) in the UPGMA (only a complete linkage cluster diagram is shown, Fig. 2). Principal component analysis (Fig. 3) also clearly indicated two groups of populations separated along the first component axis, corresponding to the two clusters resolved in the dendrograms. Neither the second nor the third axis indicated a further division of the populations. Among the characters that contributed most significantly to the first axis, and thus to the between-group differentiation, were flower scent (FS), width of the second lowermost leaf (WL), number of flowers in the inflorescence (NF), height of the plant (HP) and number of leaves (NL) (Table 2). These two groups of populations correspond to the two morphological types usually considered as *G. conopsea* and *G. densiflora.* The histogram resulting from the canonical discriminant analysis (Fig. 4), based on individual plants and groups resulting from the principal component analysis and cluster analyses, shows good separation of both groups with only a slight overlap. Among the characters strongly correlated with the canonical axis were the height of the plant (HP), number of leaves (NL), the ratio of height of the plant to the distance from the stem base to the base of the uppermost sheathed leaf (HP/USL) and the number of flowers in the inflorescence (NF) (Table 2). This was reflected also in the results of the k-nearest neighbours non-parametric classificatory discriminant analysis (k = 2), where 94.2% and 94.3% of the plants of *G. densiflora* and *G. conopsea*, respectively, were classified correctly (Table 3).

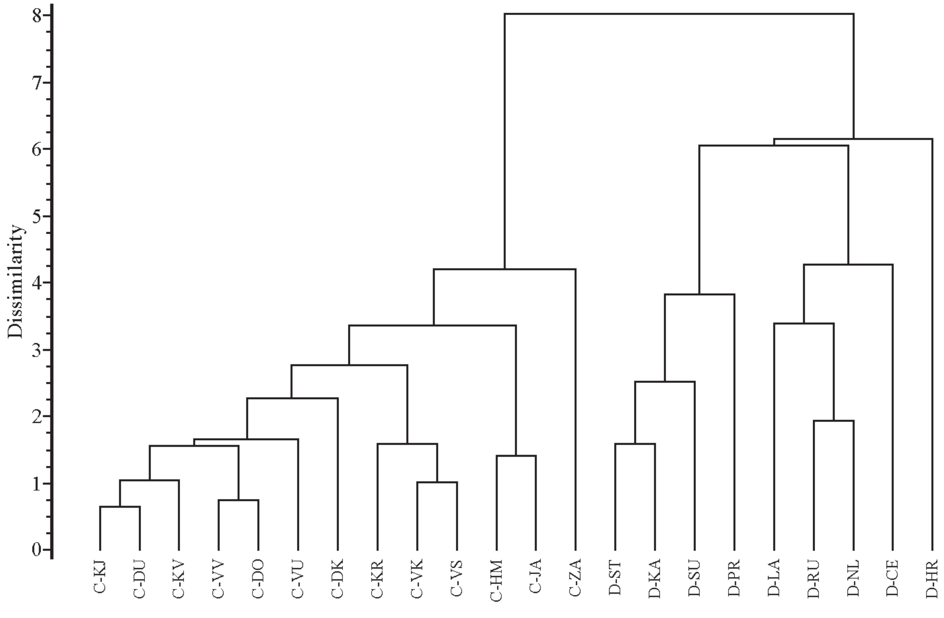

Fig. 2. – Dendrogram of the complete linkage cluster analysis of population samples of *Gymnadenia conopsea* (C) and *G. densiflora* (D). For population codes see Table 1.

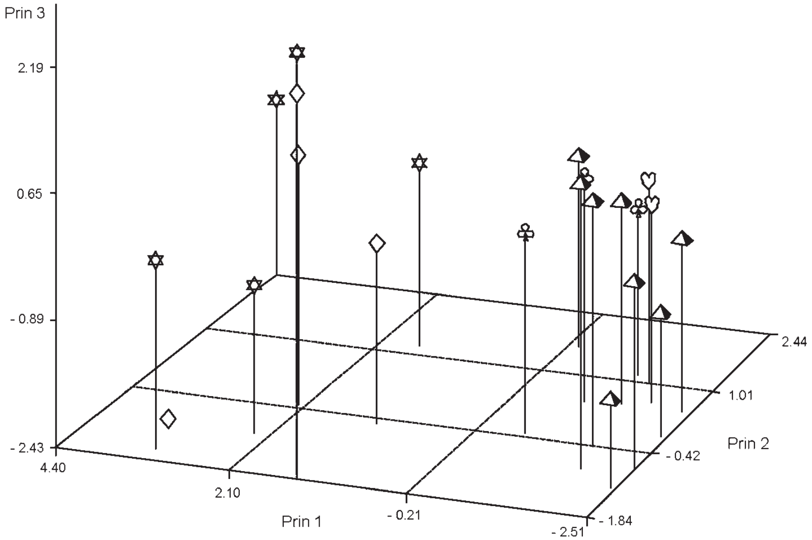

Fig. 3. – Ordination diagram of the principal component analysis of population samples of *Gymnadenia conopsea* and *G. densiflora*. Diamond – *G. densiflora* with 2n = 40, star – *G. densiflora* with unknown chromosome number, heart – *G. conopsea* with 2n = 40, club – *G. conopsea* with 2n = 80, pyramid – *G. conopsea* with unknown chromosome number. The first three components express 64.7, 15.5 and 10.8% of variation.

Results of the descriptive data analysis, which show considerable shift in several characters between these two taxa, are presented in Table 4.

There appears to be considerable phenological differentiation between *G. conopsea* and *G. densiflora*. The former has a much longer flowering period, with nearly half of the specimens flowering before 10 June (40 out of 105 examined plants), while *G. densiflora* (52 examined plants) flowered after this date (data are based on the flowering time of the morphometrically analysed plants).

Although flower scent might be considered a subjective character, in this case it seems to be reliable and allows for the identification of *G. conopsea* by its weak vanilla-like, but slightly unpleasant (musty) scent, and *G. densiflora* by its strong and pleasant, spicy to carnation-like scent.

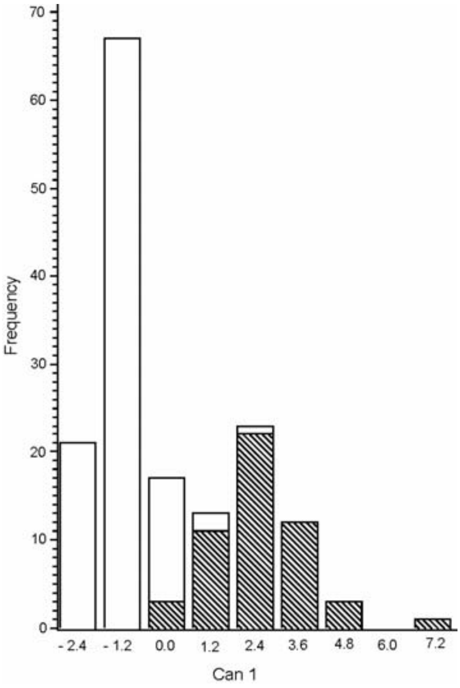

Fig. 4. – Histogram of the canonical discriminant analysis of *Gymnadenia conopsea* (white) and *G. densiflora* (hatched) based on individual specimens.

Table 2. – Eigenvectors of the principal component analysis (Prin1, Prin2 and Prin2) expressing correlation of the characters with the axes, and total canonical structure (Can1, correlation of morphological characters and the canonical axis). WL – width of the second lowermost leaf; HP – height of the plant; NF – number of flowers in the inflorescence; NL – number of sheathless leaves; WLB – width of labellum; FS – flower scent; HP/USL – the ratio of height of the plant to the distance from the stem base to the base of the uppermost sheathed leaf; SF – height of flower multiplied by its width, a character used to estimate flower size.

Character	Prin1	Prin2	Prin3	Can1
WL	0.406	–0.032	–0.319	0.489
HP	0.392	–0.003	–0.284	0.888
NF	0.399	–0.046	–0.262	0.766
NL	0.367	–0.376	–0.028	0.883
WLB	0.311	0.566	0.183	0.573
FS	0.407	–0.164	0.075	–
HP/USL	0.211	–0.453	0.747	0.864
SF	0.285	0.550	0.389	0.495

Morphological description of Gymnadenia densiflora

Perennial (40–) 54–82 (–100) cm high. Stem straight, roundish in cross-section or with more or less conspicuous lines in the upper part, and with 1–2 rudimentary scaly, sheathy leaves at the base. Leaves ± arranged into two rows, mostly concentrated in the lower third of the stem, basal leaves narrowly lanceolate with a sheath, those close to the inflorescence becoming sheathless, slightly keeled, green to dark-green. Lower leaves 3–6 (–8) in number, the second leaf (15.3–) 16.3–25.5 (–29) cm long, (1.3–) 1.6–3.3 (–4.7) cm wide. Sheathless leaves (3–) 4–9 (–12) in number. Inflorescence cylindrical, but tapering towards the top before flowering, (5–) 6–15 (–24) cm long, consisting of many flowers [(29–) 42–111 (–172)]. Flowers pinkish to purple, with an intensive pleasant smell reminiscent of carnation, lilac or cloves, (5–) 8–12 (–13.9) mm high, (8–) 10.3–14.2 (–16.1) mm wide. Lateral sepals more or less patent. Upper perianth segments connivent, shorter. Labellum shallowly three-lobed, (3–) 4–6 (–6.4) mm high and (4.6–) 5.5–8.7 (–10.5) mm wide. Spur slender, arcuate, (9.6–) 11.3–16 (–17.1) mm long. Ovary cylindrical, slightly twisted, about 8 mm long. Bracts of the inflorescence mostly lanceolate, often exceeding the ovary. Fruit broadly cylindrical.

Flowering time: end of June to the first half of August.

Chromosome numbers

The plants morphologically corresponding to *G. densiflora* showed the same diploid chromosome number 2n = 2x = 40 at all localities studied, whereas those morphologically identified as *G. conopsea* were more variable (Table 1, Fig. 5). In *G. conopsea*, populations with two ploidy levels, diploid (2n = 2x = 40) and tetraploid (2n = 4x = 80) were found, and even a single plant with the chromosome number 2n = 5x = 100, corresponding to the pentaploid level, was recorded (Table 1).

Distribution of Gymnadenia densiflora

In the area studied (covering Czech Republic, Slovakia and marginally also Austria, Germany, Poland, Slovenia and Italy), *G. densiflora* was found in the following countries, re-

Table 3. – Results of the classificatory discriminant analysis of *Gymnadenia conopsea* and *G. densiflora.*

Actual group	Predicted group membership (number of observations and percentage classified into groups)		
	G. conopsea	*G. densiflora*	Total
G. conopsea	99 (94.3%)	6 (5.7%)	105 (100%)
G. densiflora	3 (5.8%)	49 (94.2%)	52 (100%)

Table 4. – Results of the descriptive data analysis of *Gymnadenia conopsea* (n = 105) and *G. densiflora* (n = 52). WL – width of the second lowermost leaf; HP – height of the plant; NF – number of flowers in the inflorescence; NL – number of sheathless leaves; WLB – width of labellum; HP/USL – height of plant / distance from the stem base to the base of the uppermost sheathed leaf; SF – size of flowers (height of flower multiplied by its width); FD – flower density (length of inflorescence in cm / number of flowers).

Character	Species	Mean	Median	SD	Percentiles	
					10 (1)	90 (99)
WL	*conopsea*	1.13	1.1	0.28	0.8 (0.7)	1.45 (2.10)
	densiflora	2.34	2.1	0.75	1.6 (1.3)	3.3 (4.7)
HP	*conopsea*	40.74	40	8.44	31.0 (25.0)	53 (59.9)
	densiflora	67.29	66.5	13.21	54.1 (40.0)	82.0 (100.5)
NF	*conopsea*	39.7	40	11.2	24.4 (17.1)	54.0 (65.8)
	densiflora	77.5	74	30.9	42.1 (29.0)	110.8 (171.9)
NL	*conopsea*	2.4	2	1.2	1 (1)	4.0 (7.8)
	densiflora	6.4	6	2.1	4 (3)	9.0 (11.5)
WLB	*conopsea*	5.4	5.2	1.11	4.1 (3.0)	7.0 (8.2)
	densiflora	6.8	6.7	1.38	5.5 (4.6)	8.7 (10.5)
HP/USL	*conopsea*	2.25	2.13	0.57	1.75 (1.38)	2.98 (4.13)
	densiflora	2.98	2.79	0.96	2.1 (1.5)	4.12 (5.92)
SF	*conopsea*	103.96	108	27.1	70.0 (48.2)	136.1 (174.1)
	densiflora	144.77	143.37	43.97	88.8 (71.4)	193.8 (269.9)
FD	*conopsea*	0.41	0.38	0.13	0.28 (0.20)	0.58 (0.89)
	densiflora	0.51	0.46	0.19	0.32 (0.27)	0.71 (1.10)

gions and habitats (for details see Appendix): In the Czech Republic *G. densiflora* grows in sloping springs with tuff formation, fens, communities on wet and dried-out marl, and in grassland vegetation on shallow calcareous clay or marl. It is found in the Labe (= Elbe) basin in central and E Bohemia, rarely also in Lounsko-labské středohoří, Tachovská brázda and Milešovské středohoří, more frequently in Moravia with the largest populations in the Bílé Karpaty Mts; in Slovakia it occurs at sites with calcareous soils, mostly concentrated in Carpathian mountain areas. In Austria it can be found growing on calcareous soils in the Alps. In Poland this species is confined to the southern part of the country, mostly to the Carpathians (see also Zając & Zając 1997: 57).

Discussion

The diploid level, which we invariably recorded for *Gymnadenia densiflora*, confirms the previously published chromosome number 2n = 40 for three populations from the Bílé Karpaty Mts (Czech Republic, localities Machová, Březová and Strání) and one from Slovakia (Vrútky) by Čihalík (in Jongepierová & Jongepier 1989: 131), as well as that of

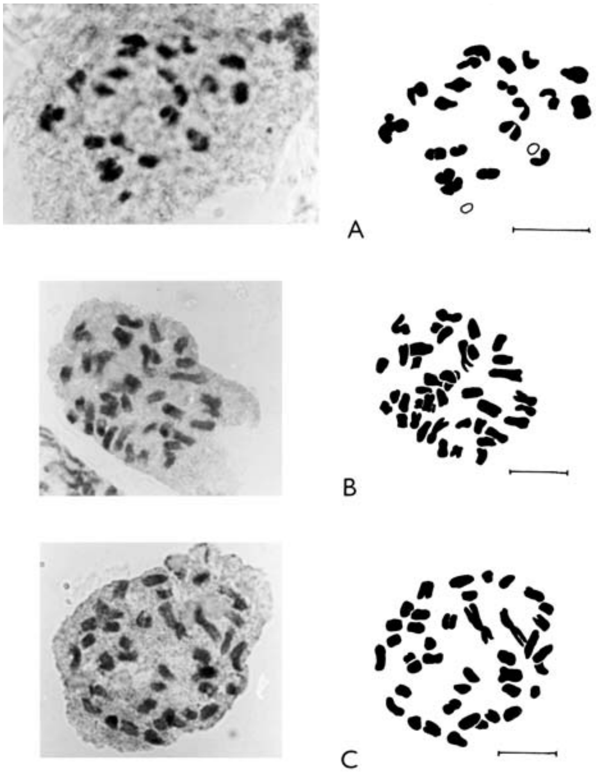

Fig. 5. – Microphotographs of chromosomes of *Gymnadenia densiflora* with explanatory drawings: A – meiosis in pollen mother cell, locality Slovakia, Veľká Fatra Mts, Blatnická dolina valley, $2n = 19_{II} + 2_{I}$; the bivalents are black, the two univalents empty; B & C – somatic metaphases, locality Czech Republic, Bílé Karpaty Mts, Nad Vápenkou, 2n = 40. [Scale bars = 10 µm].

Averyanov (1979: 868) and Safonova (in Agapova 1993: 25) for plants from Russia (St. Petersburg Region) and Estonia. However, it contradicts that given by several other authors who report tetraploid and hexaploid chromosome numbers of 2n = 80 and ca. 120 for *G. densiflora* – either as *G. densiflora* or *G. conopsea* subsp. *densiflora* (Löve & Löve 1961: 114; Hagerup in Bisse 1963a: 188; Groll 1965; Mrkvicka 1993).

The first to report a tetraploid chromosome number for *G. densiflora* were Löve & Löve (1961: 114). They assigned all previously published 2n = 40 records for *G. conopsea* s. l. to *G. conopsea* (s. str.), and 2n = 80 (the records by Heusser 1938 and Sokolovskaya & Strelkova 1940, both originally published for *G. conopsea*) to *G. densiflora*, most likely without a detailed study of the voucher specimens of the chromosome counts. Such an arbitrary assignment of different ploidy levels to different taxa, without providing any supporting evidence, is not rare in the works of Löve & Löve; another documented case is in the genus *Cardamine* (*C. amara* L. vs. *C. opicii* J. Presl et C. Presl, see Löve & Löve 1974: 344 and Marhold 1992: 122, 1999). It is apparent, however, that Löve & Löve (1961) influenced other authors, who widely followed their treatment.

Other tetraploid records for *G. densiflora* come from Central Europe, close to the area studied by the present authors. Hagerup's record of 2n = 80 was published by Bisse

(1963a) in his study of *G. conopsea*, in which he also described a new subspecies, *G. conopsea* subsp. *montana*. However, Bisse's concept of three subspecies is rather controversial; herbarium specimens that he cited for each of his subspecies are heterogeneous, as is mentioned by Jongepierová & Jongepier (1989: 130). Moreover, he did not mention the exact locality for Hagerup's chromosome number report or any voucher specimen, and thus this report cannot be considered reliable. Groll (1965) in his karyological study followed the taxonomic treatment of Bisse (1963b) and identified the plants with 2n = 40 as *G. conopsea* subsp. *conopsea* and those with 2n = 80 as *G. conopsea* subsp. *densiflora*. He noted, however, that some of the plants with 2n = 40 had a different morphology, namely more than 150 flowers per inflorescence, leaves more than 3 cm wide and a very intense scent, and classified these plants as *G. conopsea* subsp. *montana*. The morphological characters he provided for these plants perfectly fit to our concept of *G. densiflora*. Groll left no voucher specimens (Ch. Dobeš, Heidelberg, pers. comm.), but from his notes on the morphology of the taxa he studied we conclude that both 2n = 40 and 2n = 80 reports in his study correspond to *G. conopsea* s. str. and that only the diploid number 2n = 40 he recorded for "subsp. *montana* Bisse" corresponds to *G. densiflora* in the sense of the present paper. Mrkvicka's (1993) data of 2n = 80 and ca. 120 are also not documented by voucher specimens (A. C. Mrkvicka, Wien, pers. comm.), but from his detailed morphological description of the taxa he studied it might be concluded that he confused some of the *G. conopsea* s. str. populations with *G. conopsea* subsp. *densiflora.* Only individuals of the "riesenwüchsige und breitblütige Sippe" [robust taxon with wide flowers] mentioned in his paper and identified by him as "ssp. *montana* Bisse" correspond to *G. densiflora* in our sense. It should be mentioned that *G. conopsea* subsp. *montana* Bisse is a rather confusing name. Its holotype is most probably lost (F. Fukarek, Greifswald, pers. comm.) and other herbarium material mentioned in the protologue (Bisse 1963a: 188, deposited in GFW) is heterogeneous, consisting of both *G. conopsea* s. str. and *G. densiflora* (Jongepierová & Jongepier 1989). Our assumption that at least Central European tetraploids should be classified as *G. conopsea* is also supported by data published by Wegener (1966), who reported a tetraploid chromosome number (2n = 80) for *G. conopsea* subsp. *conopsea* from the eastern part of Germany.

Although chromosome numbers other than diploid for *G. densiflora* from other parts of its distribution range cannot be completely excluded, it may be concluded that at least in Central Europe this species is diploid, and all other previous and above-mentioned records of polyploidy in this species are based on misinterpretations. On the other hand, *G. conopsea* (s. str.) includes diploids, tetraploids, pentaploids and possibly also hexaploids, thus it is an heterogeneous assembly.

In addition to the partial karyological differentiation observed between these two taxa, our morphometric studies have confirmed good separation, based on a combination of both floral and vegetative characters. These data support the species level treatment for *G. densiflora*. Bateman et al. (1997, 2003) arrived at the same conclusion after a large-scale phylogenetic study of *Orchidinae.* On the basis of significant divergence in nrDNA ITS sequences between *G. conopsea* s. str. and *G. densiflora* they advocated species status for the latter. These results are, however, contradicted by the study of Gustafsson & Lönn (2003), who also used ITS sequences. They argued that late-flowering populations of *G. conopsea* s. str. are closely related to *G. densiflora* (both treated as subspecies), and that there is a strong divergence within *G. conopsea* s. str. with different phenology. This may point to the different concepts or circumscription adopted for these two taxa or to complex evolutionary processes resulting from restriction in gene flow due to phenological differentiation, or even both. In neither of these studies nor other recent investigations on the differentiation within *G. conopsea* s.l. at the molecular level (Scacchi & de Angelis 1989, Soliva & Widmer 1999), was the ploidy level of individuals investigated, and consequently a direct comparison with our findings is not possible. Only for the Swedish plants studied by Gustafsson & Lönn (2003) a diploid status is indicated by the fact that in the microsatellite analyses no more than two alleles per individual were found (S. Gustafsson, pers. comm.), but no detailed morphological analyses were done in that study. Saliva & Widmer (1999) explored allozyme and cpDNA sequence differentiation between two phenological variants assigned to *G. conopsea* and *G. densiflora* (both treated as subspecies), along with a morphometric analysis. The two taxa were resolved as genetically strongly differentiated, and showing a certain degree of morphological separation, but only in vegetative characters. Among them were also traits that in our study contributed best to the separation between the species (plant height, number of leaves and number of flowers), and in this respect the pattern found in Swiss populations is comparable and consistent with our results. Yet, in our material of *G. conopsea* s. str. there were both early- and late-flowering populations (Table 4), but no late-flowering *G. conopsea* s. str. was found in the Swiss populations; all Swiss late phenological variants were identified as *G. densiflora*. Another study on populations of *G. conopsea* s. str. from Lower Austria (Mrkvicka 1993) revealed a strong correlation between flowering time and ploidy level. The diploid number was reported exclusively in early-flowering populations (May, June), while tetraploids and hexaploids were found only in late-flowering populations (July). This observation, however, might be biased by the very restricted area of that study.

Future investigations are definitely needed to get more comprehensive data on *Gymnadenia* species, and especially on the evolutionary processes leading to the differentiation patterns. These should integrate detailed studies on variation in ploidy level, flowering time, morphology, habitat, patterns in genetic variation and correlations between them. To get consistent patterns, evaluation over a much greater geographical scale is also required, as most of the current studies, including the present one, cover only a small part of the distribution. According to the literature (e.g. Kreutz 1987, 2000, Lang 1989, Vakhrameeva et al. 1991) and herbarium material, *G. densiflora* occurs all over Europe, from Great Britain to the Urals and from Sweden to Italy and Croatia, while *G. conopsea* occurs all over Europe from Great Britain and Scandinavia to the Mediterranean, eastwards to China and Japan (Procházka 1980, Landwehr 1977).

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Souhrn

Taxon odpovídající *Gymnadenia densiflora* byl v poslední době buď špatně interpretován, nebo nebyl některými autory vůbec uznáván jako vyhraněný taxon. To byl důvod pro tuto studii, zahrnující morfologické zhodnocení a informace o počtu chromozomů a rozšíření tohoto taxonu v České republice a na Slovensku. U *G. densiflora* byl zjištěn neměnný diploidní počet chromozomů (2n = 40), zatímco příbuzný druh *G. conopsea* je diploidní, tetraploidní nebo vzácně pentaploidní (2n = 40, 80, 100). Výsledky morfometrické analýzy (analýza hlavních komponent, shluková analýza, klasifikační a kanonická diskriminační analýza) potvrdily dobré morfologické rozlišení *G. densiflora* a *G. conopsea*, ke kterému nejvíce přispěla šířka druhého nejnižšího listu, výška rostliny, počet květů v květenství, počet listů a poměr mezi celkovou výškou rostliny a vzdáleností od báze stonku k bázi nejhořejšího listu s pochvou.

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Appendix 1. – List of localities of *Gymnadenia densiflora* based on revised herbarium specimens and field data by M. Hájek, F. Krahulec and the present authors (marked *). Localities from Table 1 are not repeated here.

Czech Republic. Thermophyticum: Lounsko-labské středohoří: Litoměřice, Bílé stráně (Kubát 16. 8. 1987 LIT, Jongepierová et Procházka 9. 7. 2001 *). Všetatské Polabí: Neratovice (Velenovský 6. 1893 PRC, Velenovský 6. 1889 PR, Binder 20. 6. 1889 PR). – Between the towns of Neratovice and Všetaty (Liebaldt 18. 6. 1911 PR). – Všetaty (Krajina 4. 5. 1926 PRC). – Mělnická Vrutice (Dundr 2. 7. 1990 BRNM). Hradecké Polabí: Česká Skalice, Rozkoš (Krahulec 8. 7. 1969, 6. 7. 1969 MP). – Česká Skalice, Dubno (Krahulec 8. 1998 *). Pardubické Polabí: Dašice (Košťál 7. 1889 PR, MP). – Dašice, Zminný (J. Hadač 6. 1936 MP, Froněk 16. 7. 1956, 30. 7. 1956 MP, Procházka et M. Hájek 1971 *, M. Hájek. 8. 7. 1972 *). Znojemsko-brněnská pahorkatina: Kuřim, Třeštěnec (Saul 5. 8. 1981 BRNM, Kuřim 2. 7. 1989 J. Šmiták BRNM. Mikulovská pahorkatina, Pavlovské kopce: Mikulov (Frohlich s. a. BRNU). Jihomoravský úval, Dyjsko-svratecký úval: Břeclav (s. coll. 6. 7. 1902 BRNM). – Nový Přerov, in the direction of Novosedly (Staněk 22. 6. 1923 BRNU). Bílé Karpaty stepní: Kněždub, Nature reserve Čertoryje (Podpěra 6. 7. 1939 BRNU, Jongepierová & Jongepier 10. 7. 1987, 10. 7. 1988 BRNM, Jongepierová 7. 2002 *). – Kněždub, Horní hora (Dostál 22. 6. 1966 PR). – Velká nad Veličkou, Nature reserve Zahrady pod Hájem (Jongepierová & Jongepier 19. 6. 1987, 9. 7. 1988, 6. 1993 BRNM). – Velká nad Veličkou, Hliník (Jongepierová 7. 2001 *). – Velká nad Veličkou, Nad Vápenkou (Jongepierová 7. 2001 *). – Horní Němčí, above the village (Skřivánek 2. 7. 1942 PRC). – Horní Němčí, Nature reserve Drahy (Jongepierová 2. 7. 1996 *, Jongepierová 7. 2001 *). – Korytná, Horní louky (Staněk 6. 1924 BRNU).

Mesophyticum: Milešovské středohoří: Chrášťany, between Solanská hora hill and ruin of the castle Oltářík (Dundr 2. 7. 1990 BRNM). Orlické opuky: Černíkovice, Černíkovický rybník (Černohous 10. 7. 1975 MP, Procházka 20. 7. 1976 MP). Dolní Poorličí, Křivina: Týniště nad Orlicí, Rašovice (Šourek 6. 7. 1939 PR). Litomyšlská pánev: Stradouň (J. Dostál 6. 7. 1976 MP). Českomoravské mezihoří, Českotřebovský úval: Česká Třebová (Hadinec & Kovář 9. 7. 1976 MP, Kovář 28. 7. 1970 MP). Českomoravské mezihoří, Opatovské rozvodí: Semanín (Košťál 7. 1891 MP). – Between Semanín and Opatov (Kovář 12. 8. 1973 MP). – Česká Třebová, Nový rybník (Kovář 9. 7. 1977 PRC, Černohous 8. 7. 1975 MP). Železné hory, Sečská vrchovina: Čejkovice near Chrudim (sine coll. 6. 1887 PR). Středomoravské Karpaty, Chřiby, Zdounky (Jongepierová 7. 2000 *). Bílé Karpaty lesní: Hrubá Vrbka, Vojšické louky (Podpěra 24. 6. 1927 BRNU, Uhrová 6. 1927 BRNU). – Javorník, Hradisko (Béňa 28. 5. 1913 BRNU, BRNM, Podpěra 20. 6. 1930 BRNU). – Javorník, Nature reserve Machová (Podpěra 16. 7. 1933 BRNU, Podpěra 19. 7. 1933 BRNU, Jongepierová 7. 2002 *). – Javorník, Dlúhé lúky (Pospíšil 11. 7. 1957 BRNM). – Javorník, Zajíčkův mlýn (Podpěra 19. 7. 1930 BRNU). – Hrubá Vrbka, Březinky (Jongepierová 7. 2001 *). – Hrubá Vrbka, Léšť (Jongepierová 7. 2001 *). – Suchov, under Šumárník (Podpěra 6. 7. 1939 BRNU). – Suchov, above the valley of Trnovka (J. Dostál 17. 7. 1942 PRC). – Suchov, Kazivec valley (Bílý 27. 7. 1923 BRNM). – Between the villages of Suchov and Vápenky (Podpěra 12. 7. 1929 BRNU). – Between the villages of Suchov and Velká nad Veličkou (J. Dostál 6. 1966 PR). – Suchov, U Baladů (Hájek 7. 1995 *, Jongepierová 7. 1997 *). – Suchov, Trnovský mlýn (Jongepierová 7. 2001 *). – Strání, Nature reserve Hrnčárky (Jongepierová 7. 2001 *). – Březová, Studený vrch (Šourek 2. 8. 1944 PR, PRC,). – Březová, Nature reserve Kalábova (Jongepierová & Jongepier, 18. 6. 1993 BRNM, Jongepierová 7. 2002 *). – Březová, Nature reserve Cestiska (Jongepierová 7. 2002 *). – Lopenik, Nature reserve U Zvonice (Jongepierová 2. 7. 1996 *, Jongepierová 7. 2001 *). – Komňa, Nature reserve Lom Rasová (Jongepierová & Jongepier 25. 6. 1993 BRNM, Jongepierová 7. 2002 *). – Lopeník, slope of Mt. Hrubár (Hájek 1996 *). – Lopeník, Od Březovska (Hájek 1994 *). Javorníky: Valašské Klubouky, Nature reserve Javorůvky (Hájek 1997 *). Střední Pobečví, Vsetínská kotlina: Vsetín, Růžďka (Pavelka 12. 7. 1993 BRNM).

Oreophyticum: Orlické hory, Český hřeben: Deštné, Luisino údolí (Procházka 6. 1964 MP).

Slovakia. Pannonicum. Záhorská nížina: Kostolište (Vicherek 20. 9. 1962 BRNU). – Malacky, vineyard (Valenta 9. 7. 1936 BRA). – Lakšárská Nová Ves (Bertová 9. 5. 1968 BRA). – Between the village of Lozorno and the forest Búr (Ptačovský 15. 6. 1925 SAV). – Between the village of Jablonové and the forest Búr (Ptačovský 24. 6. 1924 SAV). – Between the villages of Pernek and Kuchyňa (Májovský 29. 6. 1964 SLO). – Veľké Leváre, Abrod (Májovský 5. 7. 1967 SLO, M. Štech 3. 7. 1995 BRNM). Podunajská nížina: Bratislava (Eschfaller s.a. BRNU). – Between the town of Palárikov and Nature reserve Šúr (near Svätý Jur) (Holuby 15. 6. 1915 BRA, PRC).

Carpaticum occidentale. Biele Karpaty (southern part): Bošácka dolina (Holuby 7. 1887 BRA). – Chocholná, Macejovská, 4 km NNW of the village (Hájek 1998 *). Malé Karpaty: Plavecké Podhradie (Dočolomanský 9. 7. 1956 BRA). – Plavecký Mikuláš, 2 km ENE of the village, Nature reserve Bukoviny (J. Hrbatý 1998 herb. Hrbatý). – Lozorno, Volhovisko (Dočolomanský 9. 7. 1956 BRA). – Pernek, Čmele (709 m) (Valenta 10. 7. 1935 BRA). Strážovská hornatina: Zliechov, Gabris (J. Dostál 9. 7. 1980 PR). – Lietavská Svinná, N of Skalky (778 m) (Velísek 9. 7. 1993 BRNM). – Hrabové, nearby the stream S of the elevation point 575 m (Velísek 10. 7. 1993 BRNM). – Horná Poruba (Jongepierová & Jongepier 6. 8. 1988 BRNM). Kremnické vrchy:

Turček, Kaltwasser (Tlusták 17. 7. 1996 BRNM). Slovenské rudohorie: Dobšiná (J. Šmarda 7. 1923 PR). – Stratená, Hnilec (J. Dostál 26. 7. 1970 PR). – Rožňava, Ramzsás (Geger 11. 6. 1868 BRA). Muránska planina: Tisovec, valley of the stream Kačkava (Soják 11. 8. 1958 PR). – Červená Skala (Hendrych 11. 7. 1948 PR). Malá Fatra (Lúčanská Fatra): Kamenná Poruba near Rajec (Unzeitig 1935 BRNU). – Vrútky (Kubát 30. 8. 1977 LIT, D. Bernátová 7. 1980 LIT). Veľká Fatra: Hrabov, Málinô Brdo (Kliment 21. 7. 1988 BRA). – Gaderská dolina (Májovský 21. 6. 1948 SLO, Kubát 17. 6. 1975 LIT). – Ľubochnianska dolina, Čierny kameň (Králik 10. 7. 1973 SLO). – Blatnická dolina (Jongepierová & Jongepier 2. 9. 1989 BRNM). – Kraľovany, Nature reserve Rojkovské rašelinisko (Hájek 1998 *). – Rojkov, Nature reserve Rojkovská travertínová kopa (Hájek 1998 *). Chočské vrchy: Komjatná (Májovský 14. 7. 1951 SLO). – Prosiek, Prosiecka dolina (Čvančara 22. 8. 1979 OLM). – Valaská Dubová (Deyl 7. 1959 PR). – Liptovská Anna (Hájek 7. 1997 herb. Hájek). Nízke Tatry: Liptovský Ján, Ohnište (Fott 22. 6. 1928 PRC). Belianske Tatry: Holubyho dolina (Futák 15. 7. 1943 SLO). Západné Tatry: Oravice (Hrabětová 20. 8. 1970 BRNU). Pieniny: Aksamitka (Domin 11. 7. 1932 PRC). Turčianska kotlina: Vrútky, "za Treťou lesnou" (J. Dvořák 19. 6. 1971 BRA). Biele Karpaty (severná časť): Červený Kameň, Nature reserve Brezovská dolina (Hájek 1997 herb. Hájek). Západné Beskydy: Nižný Kelčov, settlement Liškov, W margin of the village (Hájek 25. 7. 1995 herb. Hájek, Hájek 1998 *). – Vychylovka, NE of the settlement Chmúrovci (Hájek 1998 herb. Hájek). – Nová Bystrica, near the settlement Pišojovci (Hájek 1998 *). – Nová Bystrica, reservoir Nová Bystrica, nearby the Staňov potok stream (Hájek 1998 *). – Klin, above the village, near the path to Ťaskovka (Hájek 1998 *). – Skorušiná (Hrabětová 13. 8. 1970 BRNU). – Oravský Biely Potok, Studený potok (Pavlík 14. 7. 1929 OLM). Spišské vrchy: Jarabina (s. coll. 24. 7. 1962 PR). – Kremná (Chrtek & Deylová 13. 9. 1984 PR). – Litmanová (Chrtek & Deylová 10. 9. 1984 PR). – Kamienka, valley of the stream Riečka (Chrtek & Deylová 11. 9. 1984 PR). – Údol (Chrtek 14. 7. 1984 PR). – Spišské Hanušovce (Fatranská 22. 6. 1957 SLO). – Spišská Magura, Lendak (Hájek 3. 8. 1995 *).

Carpaticum orientale. Bukovské vrchy: Runina, Riaba skala (Soják 2. 7. 1957 PR).

Austria. Lower Austria. Dürrenstein, Lunz a. See, between the lakes of Mittersee and Obersee (Jongepierová & Jongepier 22. 7. 1995 BRNM). – Ötscher, 1880 m (Vetter 9. 8. 1906 W); near Gießhübl (Sabransky 5. 1880 W). – Near Rekawinkel [W of Pressbaum], 400 m, 550 m (Vetter 24. 6. 1906, 28. 7. 1906 W). – Schneeberg (Wettstein 7. 1890 WU). – Schneeberg, Saugraben (Khek 4. 7. 1880 W). – Mamauwiese, [WNW of] Puchberg (Schneider 30. 6. 1917 W). – Between Kernhof and Gschaid (Vetter 30. 6. 1916 W). – Moosbrunn (Fleischmann 24. 6. 1893 W, Handel-Mazetti 18. 6. 1902 WU, Onno 13. 6. 1937 W). – Near Moosbrunn (Korb 19. 7. 1918 W). – Near Hölles, [NW of] Wiener Neustadt (Sonklar 6. 1865 W). Wien. Neuwaldeck [Neuwaldegg], near Kirchdorf (s. coll. 6. 1891 WU). – Wienerwald, W of Wien (Mrkvicka 1996 photo seen).

Upper Austria: Warscheneck, In der Filzen, 1706 m (Jongepierová & Jongepier 24. 7. 1994 BRNM). – near Windischgarsten (Windischgarten?), opposite the river Teichl (Fleischmann 21. 8. 1919 W). – Windischgarsten, N of Hengstpass, Dörflmooralm, 1119 m (Jongepierová & Jongepier 25. 7. 1995 BRNU). – Near Molln, opposite Breitenau (Fleischmann 20. 7. 1919 W). – Near the river Traun (Böck 7. 1863 WU). Salzburg: Between Glanegg and Fürstenbrunn [SW of Salzburg] (Vierhapper 15. 6. 1872 WU).

Vorlarlberg: [Franstanzer] Ried, Frastanz [3 km SE of Feldkirch] (Schneider 24. 6. 1934 W). – Bregenzerwald, Laternser Tal, Innerlaterns, ca. 1050 m (Seipka 23. 7. 1971 W).

North Tyrol: Innsbruck, Vill, Viller Moor (A. Kerner 1873 WU). – Trins, in the Gschnitz Valley (Kerner s. a. W, Fl. Exs. Austro-Hung. no. 670). – Gschnitztal (Wettstein ca. 1889 WU).

Carinthia: Ovir [Hochobir, SE of Klagenfurt, SE of St. Margareten i. Rosental] (Welwitsch 1830 W). – Ferlach [S of Klagenfurt] (Schneider 14. 7. 1932 W).

Styria. Pötschen Pass [W of Bad Aussee] (Schneider 18. 7. 1936 W). – Wegscheid, near the path to Kastenriegel [S of Mariazell] (Klammerth 8. 1906 W). – E of Mariazell, Terz (Jongepierová & Jongepier 21. 7. 1990 BRNM). – Rohr i. Gebirge, Schwarzau i. Gebirge (Jongepierová & Jongepier 21. 7. 1990 BRNM).

Slovenia. Laybach [Ljubljana], s. a. Freier W; [Kamniške Alpe], Gründowitz [Grintavec] (Welwitsch 1830 W).

Poland. Kotlina Orawsko-Nowotarska: Pieninsky Pas Skalkowy, Falsztyn (Grodzińska 9. 7. 1968 KRAM). Pogórze Spisko-Gubałowskie: Chochołów, S of the village, near the river Czarny Dunajec (Hájek 7. 1998 *). – Chochołów, 1.5 km SW of the village, slopes of Mt. Góra Bieskid (Hájek 7. 1998 herb. Hájek). Rów Podtatrzański: Kościelisko, SW of the village, Polana Biały Potok (Hájek 7. 1998 herb. Hájek). Pieniny: near the road from Krościenko to Trzy Korony (Piękoś 15. 7. 1970 KRAM). Tatry Zachodnie: Zakopane, Kużice, Dolina Bystrej (Piękoś 12. 8. 1960 KRAM). – Dolina Bystrej, 990 m (Piękoś 12. 7. 1961 KRAM). – Dolina Małej Łąki (B. Zemanek 28. 7. 1968 KRAM). – Jaszczurówka, 870 m (Tacik 23. 8. 1978 KRAM). Bieszczady Zachodnie: Krzemień (M. Jagiełło 19. 7. 1980 KRAM). – Between the villages of Kalnica and Przystup (M. Jagiełło 18. 7. 1980 KRAM).