Geographic differentiation in Nigritella nigra s.l. from the Pyrenees, the Jura, the Eastern Alps and the Southern Carpathians.- J. Eur. Orch. 54 (3-4): 266-328. 2022

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Journal Europäischer Orchideen

Vol. 54 Heft 3-4 Dezember 2022

Mitteilungsblatt des AHO Baden-Württemberg

Arbeitskreis Heimische Orchideen Baden-Württemberg

Abbildung Titelseite: *Nigritella nigra* subsp. *bucegiana*, Rumänien, Südkarpathen, Dâmbovița, Moroeni, Bucegi, Plaiul lui Păcală, 6.7.2021, fot. Nora E. Anghelescu.

J. Eur. Orch.
54 (3-4): 266 – 328. 2022.

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Geographic differentiation in *Nigritella nigra* s.l. from the Pyrenees, the Jura, the Eastern Alps and the Southern Carpathians

Keywords
Orchidaceae, *Nigritella nigra*, *Nigritella nigra* subsp. *bucegiana*, geographic differentiation, nuclear SSR, morphometrics, Bucegi, Romania.

Summary
Hedrén, M., Anghelescu, N.E. & R. Lorenz (2022): Geographic differentiation in *Nigritella nigra* s.l. from the Pyrenees, the Jura, the Eastern Alps and the Southern Carpathians.- J. Eur. Orch. 54 (3-4): 266-328.
Dark-coloured, apomictic and polyploid members of the *Nigritella nigra* s.l. complex are distributed over major European mountain regions. Here, we analysed the differentiation between regional populations in molecular markers and morphology. Based on nuclear SSR marker data, populations from the Pyrenees were closely similar to those in the Western Alps and the Jura Mountains, but were differentiated from those in the Eastern Alps. In the Eastern Alps, populations from the Northern Calcareous Alps were minimally differentiated from populations in the Southern Calcareous Alps. Populations from Scandinavia are related to populations in the Alps, but carry unique genotypes and are triploid as opposed to the tetraploid Alp populations. Plants from the Southern Carpathian region did not share any genotypes with populations from elsewhere. The differentiation in morphology was generally small, but plants from the Southern Carpathians were still characterized by relatively short and wide floral parts and are here segregated as *Nigritella nigra* subsp. *bucegiana*.

Zusammenfassung
Hedrén, M., Anghelescu, N.E. & R. Lorenz (2022): Geographische Differenzierung innerhalb *Nigritella nigra* s.l. aus den Pyrenäen, Jura, Ostalpen und Südkarpathen.- J. Eur. Orch. 54 (3-4): 266-328.

Dunkel gefärbte, apomiktische und polyploide Angehörige des Komplexes *Nigritella nigra* s.l. besitzen eine weite Verbreitung in den europäischen Gebirgsregionen. In diesem Beitrag berichten wir über Untersuchungen zur Differenzierung der molekularen Marker und der Morphologie regionaler Populationen von *N. nigra*. Nach Daten nuklearer SSR Marker zeigen die Pyrenäen-Populationen eine große Ähnlichkeit zu den Populationen der Westalpen und des Jura, unterscheiden sich jedoch von denen der Ostalpen. Innerhalb der Ostalpen können die Vorkommen der nördlichen Kalkalpen geringfügig von denen der südlichen Kalkalpen unterschieden werden. Die Populationen aus Skandinavien stehen den Alpen-Populationen nahe, besitzen aber einzigartige Genotypen und unterscheiden sich weiter durch ihren triploiden Chromosomensatz von den tetraploiden Populationen der Alpen. Nach den hier vorgelegten Analysen besitzen die dunkelblütigen *Nigritella*-Pflanzen der Karpathen keine Genotypen der Populationen aus den anderen Gebietens. Ihre morphologischen Unterschiede sind insgesamt gering, die karpathischen Pflanzen besitzen jedoch relativ kürzere und leicht breitere Blütenblätter. Sie werden hier als eigene Unterart abgetrennt und als *Nigritella nigra* subsp. *bucegiana* beschrieben.

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1. Introduction

Dark-coloured members of the orchid genus *Nigritella* Rich. are distributed in the major European mountain regions. These plants were for a long period contained within *N. nigra* (L.) Rchb.f., but following embryological and cytological studies (Teppner & Klein 1989, 1990, 1993) it was shown that they separate into sexual and diploid members including *N. rhellicani* Teppner & E.Klein and *N. gabasiana* Teppner & E.Klein, and apomictic and polyploid members including *N. nigra* s.str. and related taxa in the *N. nigra* s.l. polyploid complex. Among these polyploids, it has been known for a long time that the Scandinavian population, *N. nigra* s.str. is a triploid apomict with $2n = 3x = 60$ (originally given as $2n = 64$ (Afzelius 1932: 367)), whereas populations from Continental Europe are tetraploid apomicts with $2n = 4x = 80$ (Teppner & Klein 1990, 1993). Based on morphology and distribution, the latter have variously been separated into *N. nigra* subsp. *iberica* Teppner & E.Klein from the Pyrenees, *N. nigra* subsp. *gallica* E.Breiner & R.Breiner from the Massif Central and Western Alps and *N. nigra* subsp. *austriaca* Teppner & E.Klein from the Eastern Alps. Additionally, populations of this complex are also known from the Jura Mountains (Boillat 1998), the Southern Alps (including the Dolomites),

Fig. 1: *Nigritella nigra* subsp. *bucegiana*, Romania, Dâmbovița, Bucegi, Plaiul lui Păcală, 30.6.2019, phot. NA.

Fig. 2: *Nigritella nigra* subsp. *bucegiana*, Romania, Prahova, Bucegi, Vârful Piatra Arsă, 3.7.2018, phot. RL.

Fig. 3-4: *Nigritella nigra* subsp. *bucegiana* with pyramidal spike at beginning bloom and ovate spike at end of bloom, Romania, Dâmbovița, Bucegi, Muntele Cocora, 4.7.2018, phot. RL.

Fig. 5-6 : *Nigritella nigra* subsp. *bucegiana*, Romania, Bucegi. Fig. 5 (left): Prahova, Vârful Piatra Arsă, 3.7.2018, phot. RL. Fig. 6 (right): Dâmbovița, Plaiul lui Păcală, 5.7.2019, phot. NA.

and the Southern Carpathians. The aim of this study was to clarify the relationships between the regional populations of the polyploid *Nigritella nigra* complex.

Here, we compiled morphological and biometrical data and extracted molecular data from two nuclear SSR (simple sequence repeat) loci. We included relevant results from a previous study (HEDRÉN et al. 2017) of nuclear SSR variation in *Nigritella*, but we considerably expanded the dataset by including material from the Pyrenees, which was only poorly investigated before, and samples from the Jura Mountains and the Southern Carpathians, from which regions no samples at all have been analysed before. Some supplementary material was also included from new locations in the Eastern Alps. For morphometric analyses, we included data that was previously used in LORENZ & PERAZZA (2012), but most of the measurements provided have been added here.

2. Material and Methods

2.1 List of investigated samples and their provenance

The sampled populations are listed separately for each taxon, together with their name and number of accessions used for biometrical statistics, genotype data obtained from the molecular analysis, if available (GA), followed by genotypes (GT) found in these samples and their frequency (within brackets). Subsequently follow details of used Excel-worksheets and number of samples, provenance i.e., country, province, locality, UTM-coordinates, altitude, habitat, substrate, date, number of observed plants at the site, phenology, site number (FO x) and observer/collector (M. Hedrén = MH, R. Lorenz. = RL and N. Anghelescu = NA). Mostly, the samples have been used for both molecular and morphological analyses (as indicated by “a”) or for molecular analyses only (“b”).

2.1.1 *Nigritella nigra* subsp. *bucegiana* (nbuc Buc 1, n = 13)
GA a: n=12; GT 58 (10), 62 (2).

Nnbu01 (n = 5): Romania, Prahova, Sinaia, Bucegi, Muntele Furnica, UTM_{WGS84} 35T 382270 5024392, 2020 m asl, alpine grassland, calcareous conglomerate, 28.6./3.7.2018, >10 ex., peak bloom, FO Lo 18.063c, leg. MH & RL; terra paratypi (Fig. 45, 46).

Nnbu02 (n = 8): Bucegi, Vârful Piatra Arsă, UTM_{WGS84} 35T 35T 382469 5025723, 1995 m asl, alpine grassland, calcareous conglomerate, 3.7.2018, >25 ex., peak bloom - dying, FO Lo 18.071d, leg. MH & RL; terra paratypi.

2.1.2 *Nigritella nigra* subsp. *bucegiana* (nbuc Buc 2, n = 19)
GA a: n=15; GT 55 (2), 58 (11), 60 (2); b: n=35; GT 54 (1), 55 (1), 58 (32),

60 (5), 61 (2).

Nnbu10 (n = 4): Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora, Plaiul lui Păcală, UTM_{WGS84} 35T 380537 5026893, 1979 m asl, alpine grassland, calcareous conglomerate, 4.7.2018, 15 ex., beginning to bloom, FO Lo 18.073b, leg. MH & RL; terra holotypi (Fig. 45, 46).

Nnbu11 (n = 6): Bucegi, Muntele Cocora, UTM_{WGS84} 35T 380577 5026944, 1976 m asl, alpine grassland, calcareous conglomerate, 4.7.2018, 20 ex., peak bloom - dying, FO Lo 18.073c, leg. MH & RL; terra paratypi.

Nnbu12 (n = 2): Bucegi, Muntele Cocora, UTM_{WGS84} 35T 380605 5026973, 1978 m asl, alpine grassland, calcareous conglomerate, 4.7.2018, 50 ex., peak bloom - dying, FO Lo 18.073d, leg. MH & RL; terra paratypi.

Nnbu13 (n = 2): Bucegi, Muntele Cocora, UTM_{WGS84} 35T 380591 5027587, 2026 m asl, alpine grassland, calcareous conglomerate, 4.7.2018, 30 ex., peak bloom - dying, FO Lo 18.073f, leg. MH & RL; terra paratypi.

Nnbu14 (n = 5): Bucegi, Muntele Cocora, UTM_{WGS84} 35T 380676 5027106, 1983 m asl, alpine grassland, calcareous conglomerate, 4.7.2018, 25 ex., peak bloom - dying, FO Lo 18.073g, leg. MH & RL; terra paratypi.

2.1.3 *Nigritella nigra* subsp. *bucegiana* (s. morph. dat.)

GA b: n=4; GT 58 (5).

Romania, Dâmbovița, Moroeni, Bucegi, Cabana Dichiu, UTM_{WGS84} 35T 379506 5020034, 1645 m asl, alpine grassland, calcareous conglomerate, 27.6.2018, 10 ex., end of flowering -dying, FO Lo 18.061b-e, leg. MH & RL;

GA b: n=2; GT 58 (2).

Romania, Dâmbovița, Moroeni, Bucegi, Vârful Dichiu, UTM_{WGS84} 35T 379006 5019334, 1700 m asl, alpine grassland, calcareous conglomerate, 27.6.2018, 020 ex., end of flowering -dying, FO Lo 18.062b-c, leg. MH & RL;

GA b: n=8; GT 56 (1), 57 (1), 58 (8), 59 (1).

Romania, Dâmbovița, Moroeni, Bucegi area, Plaiul lui Păcală, 35T 380567 5026860, 1960 m asl, alpine grassland, calcareous conglomerate, 2020-2021, beginning of flowering - peak bloom, leg. NA

2.1.4 *Nigritella nigra* subsp. *austriaca* (naus Tren 1, n = 24)

Naus01, Naus01a (n = 24): Austria, Steiermark, Hochschwabgruppe, Tragöß, Trenchtling, Edelweißboden, UTM_{WGS84} 33T 502827 5264172, 1822 m asl, alpine grassland, lime, 29.6.2012, > 1.000 ex., beginning to bloom - peak bloom - dying, FO Lo 12.165b, Terra typica, leg. RL (Fig. 47).

2.1.5 *Nigritella nigra* subsp. *austriaca* (naus Tren 2, n = 15)

GA a: n=8; GT 33 (7), 34 (1); b: n=1; GT 33 (1).

Naus02a, Naus02b (n = 15): Austria, Steiermark, Hochschwabgruppe, Tragöß, Trenchtling, Edelweißboden, UTM_{WGS84} 33T 502763 5264221, 1809 m asl, alpine grassland, lime, 29.6.2017, > 500 ex., peak bloom, FO Lo 17.103ab, Terra typica, leg. RL.

2.1.6 ***Nigritella nigra*** **subsp.** ***austriaca*** (naus Höch 1, n = 12)

Naus10, Naus11 (n = 12): Austria, Steiermark, Hochschwabgruppe, Aflenz, Bürgeralm, Höchstein, UTM_{WGS84} 33T 517338 5271415, 1755 m asl, alpine grassland, lime, 8.7.2004, ca. 100 ex., peak bloom, FO Lo 04.171a, leg. RL.

2.1.7 ***Nigritella nigra*** **subsp.** ***austriaca*** (naus Höch 2, n = 18)

Naus11a, Naus11b (n = 18): Austria, Steiermark, Hochschwabgruppe, Aflenz, Bürgeralm, Höchstein, UTM_{WGS84} 33T 517343 5271401, 1755 m asl, alpine grassland, lime, 1.7.2012, ca. 100 ex., peak bloom, FO Lo 12.168 -12.168c, leg. RL (Fig. 47).

2.1.8 ***Nigritella nigra*** **subsp.** ***austriaca*** (naus SeisA 2, n = 16)

Naus30a, Naus30a2, (n = 16): Italy, Südtirol, Kastelruth, Seiseralm, Grunser Bichl, UTM_{WGS84} 32T 0702270 5154956, 2120-2170 m asl, alpine grassland, lime, 14.7.1999, ca. 250 ex., beginning to bloom - peak bloom, FO Lo 99.349bc, leg. RL (Fig. 46).

2.1.9 ***Nigritella nigra*** **subsp.** ***austriaca*** (naus SeisA 3, n = 10)

GA a: n=11; GT 26 (8), 35 (2), 42 (1). (Lo 06.234c);

Naus30b, (n = 10): Italy, Südtirol, Kastelruth, Seiseralm, Grunser Bichl, UTM_{WGS84} 32T 702102 515510, 2155 m asl, alpine grassland, lime, 11.7.2013, ca. 50 ex., beginning to bloom - peak bloom, FO Lo 13.156c, leg. MH, RL.

2.1.10 ***Nigritella nigra*** **subsp.** ***austriaca*** (s. morph. dat.)

GA b: n = 1; GT 33 (1).

Austria, Salzburg, Salzkammergut, Schafberg, SO-Kamm, ca 47°47'N, 13°26'E, 30.6.1998, leg. K. Redl.

GA b: n = 3; GT 33 (3).,

Austria, Steiermark, Hochschwab-Gruppe, Aflenzer Bürgeralm, ca. 47°34'19''N, 15°13'33''E, 26.6.2009, leg. N. Griebl.

GA b: n = 2; GT 33 (2).

Austria, Steiermark, Hochschwab-Gruppe, Aflenzer Bürgeralm, ca. 47°34'19''N, 15°13'33''E, 5.7.1998, leg. D. Ståhlberg & E. Klein.

GA b: n = 1; GT 33 (1).

Austria, Steiermark, Hochschwab-Gruppe, Aflenzer Bürgeralm, ca. 47°34'19''N, 15°13'33''E, 20.6.1998, leg. E. Klein.

GA b: n = 8; GT 33 (7), 41 (1).

Austria, Steiermark, Hochschwab-Gruppe, Trenchtling, ca 47°31'45''N, 15°02'30''E, 26.6.2013, leg. W. Foelsche.

GA b: n = 2; GT 26 (2).

Germany, Bayern, Chiemgauer Berge, Feichtenalm, ca 47°31'46''N, 11°40'11''E, 28.6.1998, leg. K. Redl.

GA b: n = 4; GT 26 (4).

Italy, Südtirol, Dolomites area, Grödner Joch/Passo Gardena, 46°31'N,11°45'E, 8.7.2009, leg. U. Heidtke.

GA b: n = 1; GT 37 (1).

Italy, Alto Adige, South Tyrol, Dolomites area, Passo Pordoi, ca 46°29'N, 11°49'E, 4.7.2003, leg. M. Hedrén & S. Hansson.

GA b: n = 1; GT 26 (1).

Italy, Friuli-Venezia Giulia, Dolomites area, Monte Grappa, ca 45°51'29''N, 11°48'13''E, 14.6.2012, leg. B. Dolinar 11797.

GA b: n = 1; GT 35 (1).

Italy, Südtirol, Dolomites area, Dürrenstein, ca 46°39'N, 12°12'E, 17.7.2013, leg. M. Hedrén, R. Lorenz & R. Giotta Lo 13.181d2.

2.1.11 *Nigritella nigra* subsp. cf. *iberica* (njur Chas W, n = 20)

GA a: n=19; GT 4 (1), 13 (17), 16 (1); b: n=4; GT 4 (4).

Nnjur01, Nnjur01a (n = 20): Switzerland, Bern Jura, Col de Chasseral, UTM_{WGS84} 32T 350812 5220331 – 350900 5220450, 1500-1520 m asl, alpine grassland, lime, 28.6.2019, ≥ 60 ex., peak bloom - dying, FO Lo 19.081a – 19.081b, leg. RL (Fig. 48).

2.1.12 *Nigritella nigra* subsp. cf. *iberica* (njur Chas E, n = 24)

GA a: n=16; GT 4 (8), 13 (8); b: n=6; GT 4 (3), 9 (1), 13 (1), 15 (1).

Nnjur02, Nnjur03 (n = 24): Switzerland, Bern Jura, Col de Chasseral, UTM_{WGS84} 32T 352508 5221660 – 353290 5221794, 1540-1600 m asl, alpine grassland, lime, 29.6.2019, > 200 ex., peak bloom - dying, FO Lo 19.082c – 19.082de, leg. RL (Fig. 48).

2.1.13 *Nigritella nigra* subsp. *iberica* (nibe Creu 1, n = 16)

GA a: n=14; GT 4(4), 12 (3), 13 (5), 14 (1), 21 (1); b: n=5; GT 4 (2), 6 (2), 13 (1).

Nibe10, Nibe10a (n = 16): Spain, Catalunya, Pyrenees, Col de la Creueta, UTM_{WGS84} 31T 413987 4685828 – 413897 4685657, 1930-1990 m asl, alpine grassland, lime, 29.6.2019, > 400 ex., peak bloom, FO Lo 19.088d – 19.088e, leg. RL (Fig. 49)

2.1.14 *Nigritella nigra* subsp. *iberica* (nibe Creu 2, n = 8)

GA a: n=4; GT 2 (1), 4 (1), 11 (1), 49 (1).

Nibe10b (n = 8): Spain, Catalunya, Pyrenees, Col de la Creueta, UTM_{WGS84} 31T 413902 4685410, 2065 m asl, alpine grassland, lime, 29.6.2019, > 200 ex., peak bloom, FO Lo 19.088g, leg. RL.

2.1.15 ***Nigritella nigra*** **subsp.** ***iberica*** (nibe Ando, n = 19)

GA a: n=7; GT 4 (4), 17 (3).

Nibe15 (n = 7): Andorra, Pyrenees, Port d'Envaliria, UTM_{WGS84} 31T 370579 4712003, 2285 m asl, alpine grassland, lime slate, 4.7.2019, 10 ex., peak bloom, FO Lo 19.093a, leg. RL (Fig. 49).

GA a: n=6; GT 4 (3), 17 (1), 18 (1), 20 (1); b: n=7; GT 4 (4), 13 (2), 19 (1).

Nibe17 (n = 12): Andorra, Pyrenees, Col Botella - Port de Cabus, UTM_{WGS84} 31T 394127 4710791 – 370260 4711733, 2295 – 2360 m asl, alpine grassland, lime slate, 5.7.2019, 60 ex., peak bloom, FO Lo 19.094c-e, leg. RL (Fig. 49).

2.1.16 ***Nigritella nigra*** **subsp.** ***iberica*** (nibe Ampr, n = 12)

GA a: n=10; GT 1 (1), 3 (1), 4 (7), 8 (1); b: n=7; GT 4 (5), 7 (1), 17 (1).

Nibe25 (n = 12): Spain, Aragon, Pyrenees, Cerler Ampriu, UTM_{WGS84} 31T 301255 4715285, 2130 m asl, alpine grassland, lime slate, 8.7.2019, > 20 ex., peak bloom - dying, FO Lo 19.099f, Terra typica, leg. RL (Fig. 49).

2.1.17 ***Nigritella nigra*** **subsp.** ***iberica*** (s. morph. dat.)

GA b: n=3; GT 4 (1), 17 (2).

Spain, Aragon, Pyrenees, Bujaruelo, Sandaruelo, UTM_{WGS84} 30T 737422 4733385, 2115 m asl, alpine grassland, lime slate, 10.7.2019, 15 ex., end bloom - dying, FO Lo 19.101fg, leg. RL.

GA b: n=2; GT 4 (2).

Bujaruelo, Puerto Viejo, Lo 19.105b, 12.7.2019.

GA b: n = 1; GT 5 (1).

France, Pyrenees, Fontrabiouse, Val de Galbe, ca 42°37'25''N, 2°04'09''E, 13.6.1998, leg. M. Lewin

GA b: n = 1; GT 4 (1).

France, Pyrénées-Orientales, Porté-Puymorens, ca 42°33'N, 1°50'E, 11.7.1998, leg. M. Lewin

GA b: n = 1; GT 10 (1).

France, Isère, Mt de Lans, ca 45°02'09''N, 06°07'44''E, 14.6.1998, O. Gerbaud

GA b: n = 1; GT 13 (1).

France, Savoie, Croix de Nivolet, ca 45°36'49''N, 5°57'56''E, 14.6.1998, leg. O. Gerbaud.

2.1.18 ***Nigritella nigra*** **subsp.** ***nigra*** (s. morph. dat.)

GA b: n = 5; GT 47 (5).

Sweden, Härjedalen, Ljusnedal, Klinken, ca 62°43'22''N, 12°17'07''E, 9.7.1998, leg. MH

GA b: n = 1; GT 48 (1).

Sweden, Jämtland, Oviken, Oviken, ca 62°59'39''N, 14°23'42''E, 8.7.1998, leg. MH.

2.1.19 ***Nigritella rhellicani*** (rhe Rein, n = 24, dark coloured)

Nrhel10, Nrhel10a (n = 24): Italy, Südtirol, Rein in Taufers, Knuttenalm-Ochsenlenke, UTM_{WGS84} 33T 279960 5208434 - 279577 5208688, 2350 - 2520 m asl., alpine grassland, slate, 19.7.2012, > 1.500 ex., peak bloom, FO Lo 12.211, leg. RL (Fig. 48)

2.1.20 ***Nigritella rhellicani*** (rhe Puf s, n = 27, dark coloured)

Nrhel20 (n = 7): Italien, Südtirol, Seiseralm, Puflatsch, UTM_{WGS84} 32T 700570 5159256, 2100 - 2150 m asl, 16.7.1997, ca. 1.500 ex., peak bloom, FO Lo 97.290, leg. RL.

Nrhel21 (n = 8): Puflatsch, UTM_{WGS84} 32T 700260 5159140, 2125 m asl, 19.7.1999, ca. 300 Ex., peak-end bloom, FO Lo 99.364, leg. RL.

GA b: n=12; other genotypes than those reported here 18.7.2013, ca. 300 Ex., beginning - peak bloom, FO Lo 13.185, leg. MH, RL.

Nrhel25 (n = 12): Puflatsch, UTM_{WGS84} 32T 699235 5159040, 2050 m asl, 11.7.2012, >1.000 Ex., peak bloom, FO Lo 12.193e, leg. RL.

2.2 Material for molecular analyses

Molecular data from altogether 340 samples of tetraploid *Nigritella nigra* s.l. were compiled and analysed in this study, as follows: 67 from the Pyrenees, two from the Western Alps, 45 from the Jura Mountains, 103 from the Southern Alps including the Dolomites, 29 from the Northern Calcareous Alps, 88 from the Southern Carpathians and six from Scandinavia (List 2.1.1 - 2.1.18). Data for 249 samples analysed added here, whereas 91 of the samples were extracted from HEDRÉN et al. (2017). All examined samples are listed in Table 7.

No data from diploid sexual *Nigritella* were included in this study as these are easily distinguished and well separated from asexual polyploids.

2.3 Molecular analyses

Portions of plants, either vegetative leaves or inflorescences were sampled for DNA extraction. In most cases, the rest of the plant was left intact for continued growth and development. The material was dried in silica gel (CHASE & HILLS 1991) and stored at ambient temperature until DNA extraction. DNA was extracted from about half an inflorescence or a ca 2 cm long portion of a leaf according to the 2× CTAB procedure (DOYLE & DOYLE 1990). Vouchers in the form of dried flowers will be deposited in the Lund University botanical museum (LD).

Fig. 7:. *Nigritella nigra* subsp. *austriaca*, Austria, Steiermark, Trenchtling, 29.6.2012, phot. RL.

Fig. 8: *Nigritella nigra* subsp. *austriaca*, Italy, Südtirol, Seiseralm, 17.7.2006, phot. RL.

Fig. 9: *Nigritella nigra* subsp. *iberica*, Switzerland, Jura, Chasseral, 29.6.2019, phot. RL.

Fig. 10: *Nigritella nigra* subsp. *iberica*, Spain, Pyrenees, Huesca, Ampriu, 8.7.2019, phot. RL.

Fig. 11-12: *Nigritella nigra* subsp. *bucegiana*, Romania, Dâmbovița, Bucegi, Plaiul lui Păcală, left: 9.7.2020; right: 3.7.2021, phot. NA.

Fig. 13-14: *Nigritella nigra* subsp. *bucegiana*, Romania, Dâmbovița, Bucegi, Plaiul lui Păcală, 28.6.2020, phot. NA.

Two nuclear SSR loci originally described for *Gymnadenia* by GUSTAFSSON & THORÉN (2001), Gc51 and Gc77, were used to characterise the genotype differentiation patterns in *Nigritella nigra*. Previously, the same two loci have been adopted to study overall differentiation in the genus (HEDRÉN et al. 2017).

Individual PCR reactions were performed in a reaction volume of 6.4 µL containing 4.4 µL ddH2O, 0.66 µL 10 x reaction buffer (100 mM Tris-HCl pH 8.3, 500 mM KCl, 15 mM MgCl2), 0.56 µL dNTPs (2.5 mM of each nucleotide), 0.25 µL Cy5-labelled primer (10 pg/µL), 0.1 µL unlabelled complementary primer (25 pmol/µL), 0.03 µL AmpliTaq polymerase (5 u/µL; Applied Biosystems), and 0.6 µL template DNA (14 ng/µL).

The SSR loci were amplified by an initial denaturing at 94 °C for 3 minutes, followed by 36 cycles of 94 °C for 30 seconds, annealing at 52 °C (Gc51) or 57 °C (Gc77) for 30 seconds, extension at 72 °C for 45 seconds, and concluded with a final extension period at 72 °C for 10 minutes.

The PCR products from each reaction were mixed with 8.5 µl formamide containing appropriate size markers to enable exact size determination of the amplified fragments. The PCR products were then heated at 94°C for ca 15-20 minutes before loading onto acrylamide gels and separated by size on an ALFexpress II automated sequencer (Amersham Pharmacia Biotech). Fragment sizes were determined by using ALFwin™ fragment analyser 1.03.01 software (Amersham Pharmacia Biotech).

2.4 Material for morphological analyses

For each investigated *Nigritella* population, flowers from about ± 12 separate plants were collected. For morphology, the floral leaves of one or two flowers from the lower part of the inflorescence were dried between adsorbent hygienic paper, under high pressure to avoid shrinkage, and fixed on A4 cardboards covered by transparent foil; an example is shown in Fig. 45. Measures of the single flower parts were done with a 7× magnifying glass with a 0,1 mm scale (for details, see LORENZ et al. 2015: 127). Large selections of prepared flowers are presented in Figs. 46-49.

3. Data analyses

3.1 Analysis of molecular data

The samples were analysed for the presence of fragments at each of the two studied loci and the combined fragment patterns were recognized as genotypes. All the different genotypes were then compared with each other for the proportion of shared fragments using the Jaccard similarity coefficient, J (JACCARD 1908). The resulting matrix of pair-wise comparisons was subjected to a principal coordinates analysis, PCO (GOWER 1966) and the overall differentiation of genotypes was illustrated by plotting the scores of individual genotypes along the first two principal coordinates. Jaccard coefficients and the PCO were calculated in the computer program NTSYS-pc 2.2 (ROHLF 1994).

3.2 Analysis of morphometric data

Lengths and widths of 17 traits of the investigated populations of *Nigritella nigra* s.l. are presented in Tables 3-7 with means and standard deviation in comparison to diploid *N. rhellicani* from the Central Alps and the Dolomites. Single data for the most differentiating characters are presented by 2-factor diagrams in Figs. 19-22.
Since a large number of continuous biometric data was available, further multivariate statistical analyses, discriminant analyses, were performed by XLSTAT (Addinsoft). The results are presented for the strongest two factors as scatter plots with 95% confidence ellipsoids in Fig. 17. Further evaluation by agglomerative hierarchical clustering (AHC) using Mahalanobis distances is shown in Fig. 18.

4. Results

4.1 Nuclear SSR differentiation patterns

Altogether 26 fragments of different sizes were amplified at the two nuclear SSR loci, 16 at Gc51 and 10 at Gc77 (Table 1). The primers used for locus Gc51 often gave rise to more fragments than expected at a single locus for the given ploidy level of the sample (3x or 4x). This finding was in accordance with a previous study (HEDRÉN et al. 2017) in which it was observed that the number of fragments in the dark-coloured *N. rhellicani* and *N. nigra* was often higher than their corresponding ploidy levels, suggesting either that the locus was duplicated in these taxa, or that two separate loci were amplified by the same primer pair.

In contrast, at locus Gc77, the number of bands visualized on the electropherograms was not higher than the known ploidy levels.

Whereas most of the plants expressed the two fragments of 80 bp and 92 bp at locus Gc51, no plants from the Carpathians had any of these fragments. Among plants from other regions than the Carpathians, most of the variation at locus Gc51 was due to shifting positions of fragments in the range of 110 – 130 bp. At locus Gc77, most of the plants gave rise to a strong fragment of 105 bp plus one or two weaker fragments at 107 – 121 bp. However, in all plants from the Carpathians, the 105 bp fragment was replaced by a fragment of 103 bp.

A total of 62 genotypes were distinguished on basis of the combined variation patterns at the two SSR loci (Table 1). A few genotypes were strongly dominant, whereas most of the genotypes were confined to a single individual each; only 18 genotypes were found in more than one individual. The genotypes found in more than ten plants were: 26 (74 plants), 58 (70 plants), 4 (50 plants), 13 (35 plants), and 33 (22 plants).

Table 1: Genotypes identified in *N. nigra*. The genotypes are defined by the combined fragment patterns expressed at the two SSR loci Gc51 and Gc77. For each locus, the fragments found in any accession are given in base pairs (vertically). Both SSR loci have a repeat length of 2 bp; Gc51 gives rise to fragments of even lengths (80 – 138 bp) and Gc77 of odd lengths (103 – 133 bp). The presence of a given fragment in a given genotype is indicated by "1", and its absence by "0". The numbers of plants with a given genotype is reported per region.

Genotype	Pyrenees	W Alps	Jura	S Alps	N Alps	Carpathians	Scandinavia	Gc51 000011111111111 899911112222233 024602680246802 8	Gc77 1111111111 0001111123 3571357913
1	1							1100100100001000	0100101000
2	1							1100100010001000	0100101000
3	1							1100100001100000	0100101000
4	34		16					1100100001001000	0100101000
5	1							1100100001001000	0100011000
6	2							1100100001000100	0100101000
7	1							1100100001000010	0100101000
8	1							1100100001000000	0100101000
9			1					1100100000101000	0110001000

10		1						1100100000011000	0100101000
11	1							1100100000000000	0100101000
12	3							1100011000000100	0110001000
13	8	1	26					1100010010000100	0110001000
14	1							1100010010000100	0110000010
15			1					1100010010000100	0100101000
16			1					1100010010000100	0100001000
17	7							1100010001001000	0100101000
18	1							1100010001000100	0110001000
19	1							1100010000100100	0100101000
20	1							1100010000100000	0100101000
21	1							1100010000000100	0110001000
22				1				1100000100101000	0100101000
23					1			1100000010101100	0100101000
24				1				1100000010101001	0100101000
25				1				1100000010101000	0100110000
26				70	4			1100000010101000	0100101000
27				1				1100000010101000	0100100010
28				1				1100000010101000	0100011000
29				1				1100000010101000	0100010100
30				1				1100000010101000	0100010000
31				5				1100000010101000	0100001000
32				1				1100000010101000	0100000010
33					22			1100000010100100	0100101000
34					1			1100000010100000	0100101000
35				3				1100000010010000	0100101000
36				1				1100000010001000	0100110000
37				1				1100000010001000	0100101000
38				1				1100000010001000	0100001000
39				1				1100000010001000	0100000010
40				1				1100000010001000	0100000000
41					1			1100000010000100	0100101000
42				2				1100000010000100	0100001000
43				2				1100000001101000	0100101000
44				1				1100000001001000	0100001000
45				2				1100000000101000	0100101000
46				1				1100000000100000	0100010100
47							5	1100000000011000	0110001000
48							1	1100000000010100	0100001000
49	1							1100000000001000	0100101000

50				1				1010000010001000 0100001000
51				1				1010000010001000 0100000100
52				1				1001000010001000 0100001000
53				1				0100000010101000 0100100100
54						1		0000001101010100 1000100000
55						3		0000000111010100 1000100000
56						1		0000000101010100 1010001000
57						1		0000000101010100 1000101000
58						70		0000000101010100 1000100000
59						1		0000000101010010 1000001000
60						7		0000000101010000 1000100000
61						2		0000000101001100 1000100000
62						2		0000000100000100 1000100000

Three of the genotypes were shared between regions, whereas the rest were confined to a single region each. Genotype 4 was shared between the Pyrenees and the Jura, and genotype 13 was shared between the Pyrenees, the Western Alps and the Jura. Genotype 26 was shared between the Southern Calcareous Alps and the Northern Calcareous Alps in the Eastern Alps. Genotype 33 was only found in the Northern Calcareous Alps and genotype 58 only in the Carpathians.

The lowest Jaccard coefficients between any of the five most common genotypes were those between genotype 58 and the other ones, J = 0.077 – 0.167. The two genotypes shared between the Pyrenees and the Jura, 4 and 13, had a similarity of J = 0.333, and the two common genotypes in the Eastern Alps, 26 and 33, had a similarity of J = 0.778. The similarity between any of 4 or 13 and any of 26 or 33 was J = 0.455 – 0.600.

The overall differentiation between genotypes was visualized by a PCO (Fig. 15-16). Here, genotypes from the Carpathians were positioned close to each other, but well differentiated from those of the other regions. Plants from the Pyrenees and the Jura shared two common genotypes, but these were clearly differentiated from each other on Axis 3. Rare genotypes from these regions were mostly located close to any of the two common genotypes. Genotypes from the Eastern Alps attained low values for Axis 1 and 2, but showed still substantial overlap with genotypes from the Pyrenees and the Jura. Except for genotype 26, which was shared between the Northern and Southern Calcareous Alps, all plants had different genotypes, but these were still largely overlapping in the PCO plots. Plants from Scandinavia had unique genotypes, but still located close to genotypes from the Alps.

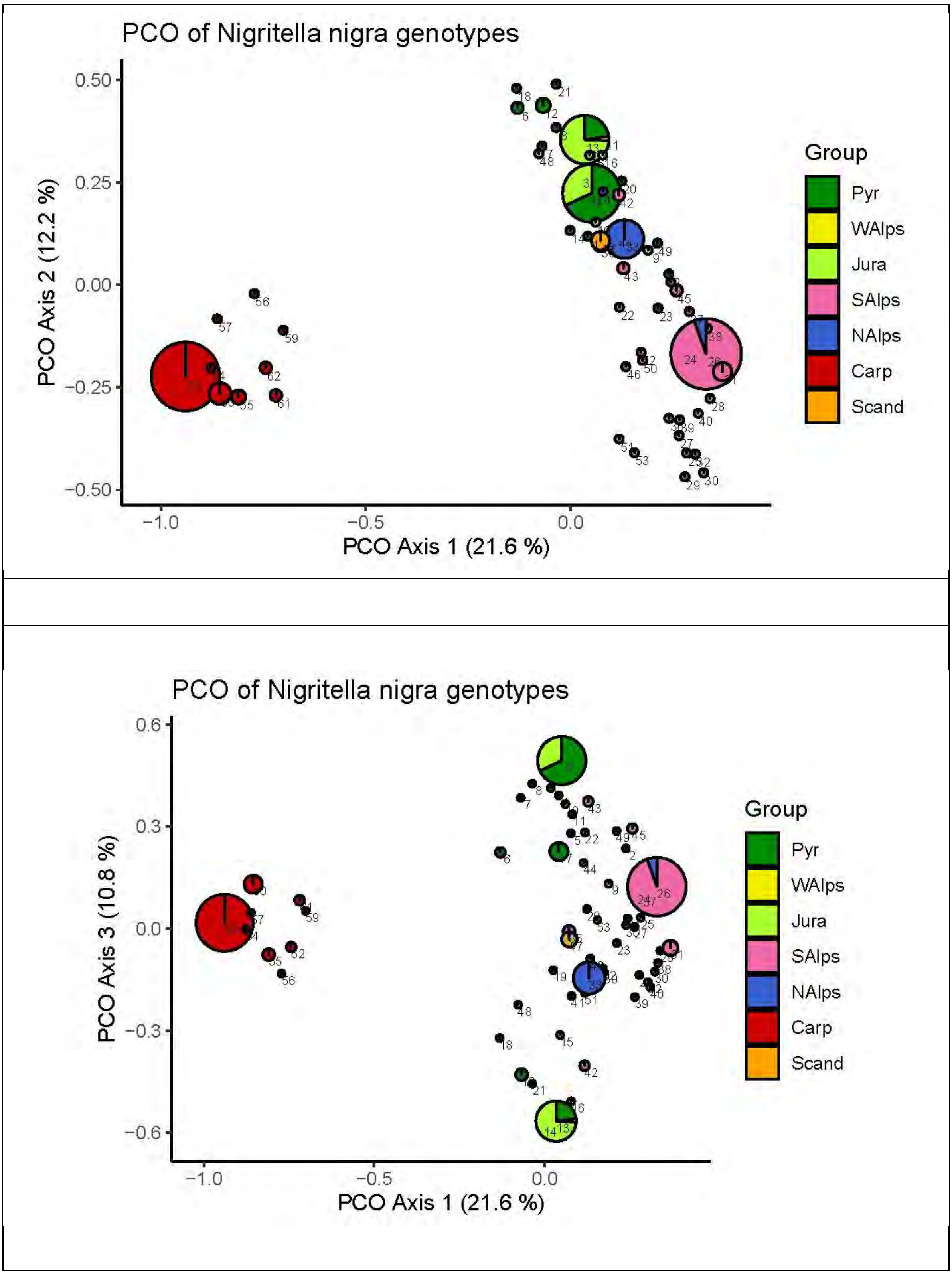


Fig. 15-16: Principal coordinates analysis (PCO) showing differentiation between nuclear genotypes in polyploid *Nigritella nigra* s.l. as given by Jaccard coefficients (above Fig. 15, Axis 1 and 2; below Fig. 16, Axis 1 and 3).

4.2 Morphological differentiation patterns

The means and standard deviation of the measurements of the single floral traits of the investigated taxa are presented in Tables 4-7. First, one can find a good correspondence of the ranges between our new data and the min-max-data communicated by TEPPNER & KLEIN (1990, 1993) for *Nigritella nigra* subsp. *austriaca* and *N. nigra* subsp. *iberica* as shown in Table 2.

Table 2: Comparison of Min-Max-biometric data of *Nigritella nigra* s.l. communicated by TEPPNER & KLEIN (1993: 205; TK) with means ± 2s of the here presented data (HAL) for populations from the Eastern Alps (subsp. *austriaca*), Pyrenees (subsp. *iberica*) and, additionally, Scandinavia (subsp. *nigra*), the latter based on data collected last summer in Jämtland, Hedmark, Sør-Trøndelag and Troms. Smaller values of current data in comparison to Teppner & Klein are marked in blue, greater values in red. Overall, the ranges of the values of the compared characters agree well, only for the Scandinavian samples there are small differences, which could be caused by our larger collection area.

Species		*Nigritella*					
subspecies		*austriaca*		*iberica*		*nigra*	
provenance		Aust. Dolom	Austria	Pyrenees	Pyrenees	Skandin.	Skandin.
source		HAL	TK	HAL	TK	HAL	TK
data value		M ± 2s	min-max	M ± 2s	min-max	M ± 2s	min-max
characters	n	107		55		64	
61	petals length	5,2 – 7,4	5,2 – 7,4	5,4 – 7,6	6,5 – 8,2	5,7 – 7,8	6,4 – 9,5
62	petals width	0,9 – 1,5	0,85 – 1,3	0,9 – 1,6	0,9 – 1,5	1,0 – 1,9	1,2 – 1,7
63	lat. sepals L	5,8 – 8,0	6,1 – 8,2	6,1 – 8,6	6,8 – 9,5	6,7 – 8,5	7,2 – 10,5
64	lat. sepals W	1,6 – 2,2	1,3 – 2,1	1,5 – 2,6	1,5 – 2,3	1,5 – 2,5	1,4 – 2,2
23	med. sepal L	5,6 – 7,9	5,8 – 7,5	5,5 – 8,1	6,7 – 8,4	6,1 – 8,4	7,0 – 10,2
24	med. sepal W	1,3 – 2,2	1,5 – 1,8	1,3 – 2,3	1,5 – 2,1	1,4 – 2,4	1,4 – 2,1
27	lip L	6,1 – 9,3	6,8 – 10	6,1 – 9,2	7,8 – 10	7,1 – 9,9	7,9 – 12
28	lip W	3,8 – 5,6	s.d.	3,5 – 5,9	3,5 – 5,2	3,8 – 6,1	2,7 – 4,9
30	spur L	0,9 – 1,5	1,0 – 1,3	0,9 – 1,6	1,0 – 1,4	0,9 – 1,7	0,8 – 1,1

Overall, the investigated populations of *Nigritella nigra* s.l. from the Southern Carpathians, the Eastern Alps, the Dolomites, the Jura Mountains and the Pyrenees show only slight differences between the single provenances, especially between *N. nigra* subsp. *austriaca* and *N. nigra* subsp. *iberica*. (Tables 5-6). These findings are in agreement with TEPPNER & KLEIN (1993: 205), who reported a large biometric overlap of the tepals and lips between the two subspecies, yet claiming that the higher maximal values, attributed to the Pyrenean *N. nigra,* were sufficient to separate it as *N. nigra* subsp. *iberica*. Our new data show slightly higher mean values for the length of tepals and lip of *N. nigra* subsp. *iberica*, even if not fully confirming significantly higher max values for these floral elements.

In contrast, the Carpathian population does not fit well into this picture of overlapping values between the several provenances for most traits, and reveals a visible differentiation due to its significantly larger petals (Fig. 19) and shorter lip (Fig. 21), supported by slightly shorter sepals (Fig. 20). This relation becomes even more evident by consistently lower values of the ratio length to width of these floral traits (Fig. 22; Table 7, char. 81-83, 93). Other traits like bracts, ovary and gynostemium show no differences from the other subspecies of *N. nigra*.

These relations are confirmed by discriminant analysis (DA) using the whole data set of 17 traits. However, they become more evident when using the data from only 11 traits, i.e., petals, sepals, lip and spur, since data from bracts, ovary and column do not contribute to the differentiation of the investigated taxa. The values of factors 1 and 2 are highest for lip length (F1: 0,832/F2: 0,305), petal length (0,719/0,633), petal width (-0,588/0,544), median sepal length (0,691/0,367), and lateral sepal length (0,624/0,508). The resulting diagram based on the first and strongest factors F1 and F2 (Fig. 17) shows a slight differentiation of Carpathian *Nigritella nigra* from *N. nigra* subsp. *austriaca* (both Stiria and South Tyrol) and subsp. *iberica* (both Jura and Pyrenees), the latter two strongly overlapping. *N. rhellicani* takes a slightly more separated position. On the whole, these outcomes reflect very well the morphological differences between the three groups, even if at a low level.

The dendrogram obtained by Agglomerative Hierarchical Clustering (AHC) based on Mahalanobis distances reflects well the grouping of the single entities/taxa by differentiating at a relatively low level the Bucegi-population from the central and western *N. nigra*-populations (Fig. 18). *N. rhellicani* clusters have similarly shorter petals, sepals and lip to the Bucegi-plants, but differentiate from them by their narrower petals.

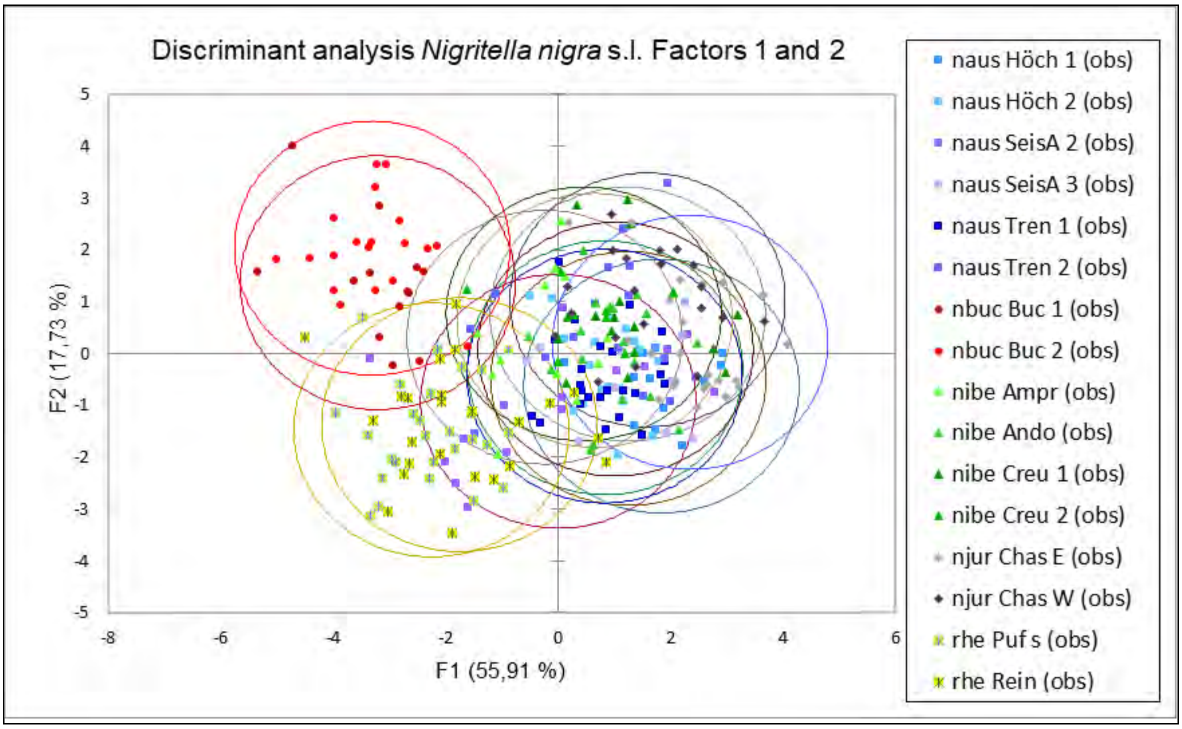


Fig. 17: Discriminant analysis based on 11 traits (L/W of petals, lat. sepals, med. sepal, lip, spur). The slight differentiation of Carpathian *N. nigra*, western *N. nigra* and *N. rhellicani* corresponds to differences in length of lip and width of petals and sepals.

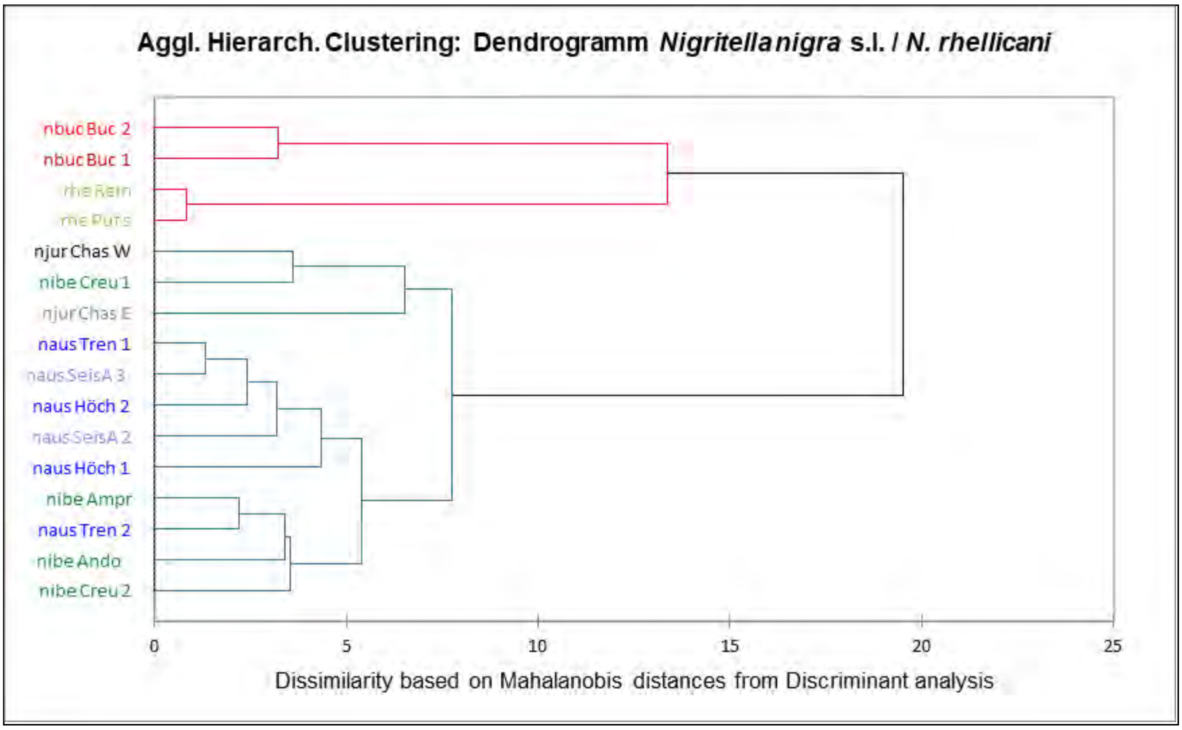


Fig. 18: Agglomerative Hierarchical Clustering (AHC) of Mahalanobis-distances from discriminant analysis (Fig. 17) gives a slight differentiation at low level of Carpathian *N. nigra* together with *N. rhellicani* from central-western *N. nigra* s.l., corresponding to their differences in length of lip and width of petals, sepals.

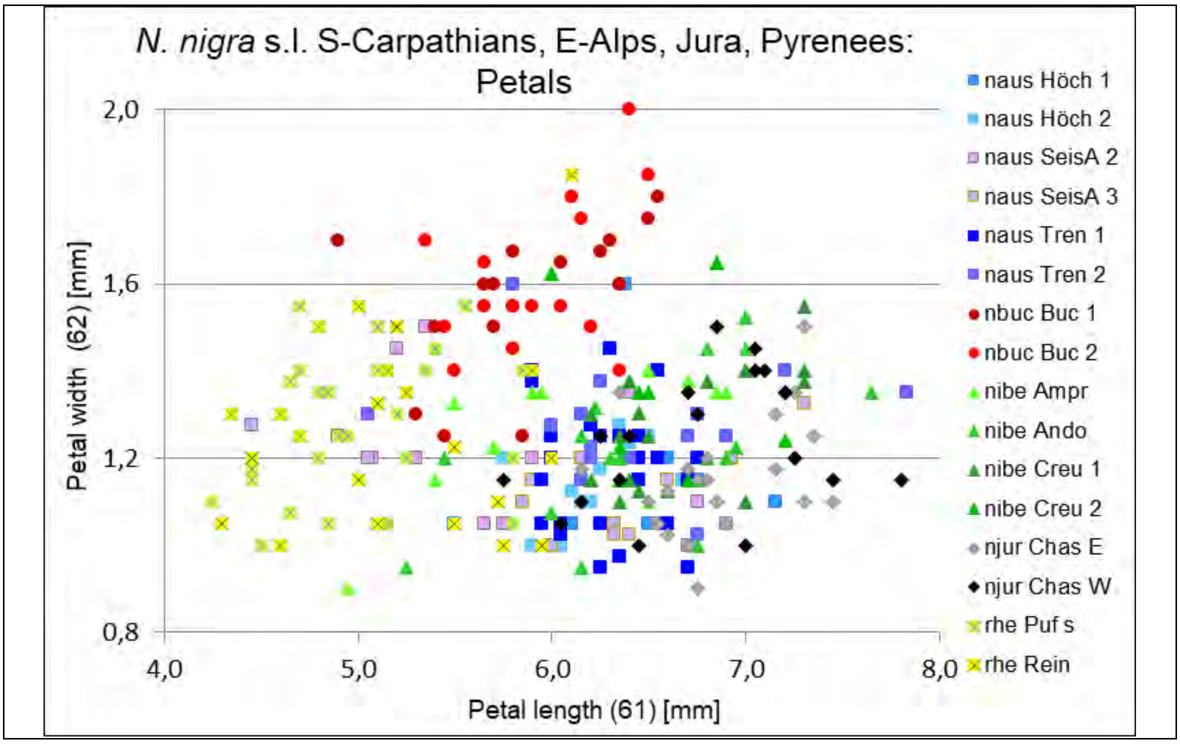


Fig. 19: Diagram of Petal length = f (Petal width). The Carpathian samples of *N. nigra* from Bucegi show a clear trend to larger and slightly shorter petals than western provenances, whereas no significant differences can be seen between the Alps, the Jura and the Pyrenees. The petals of *N. rhellicani* are even shorter.

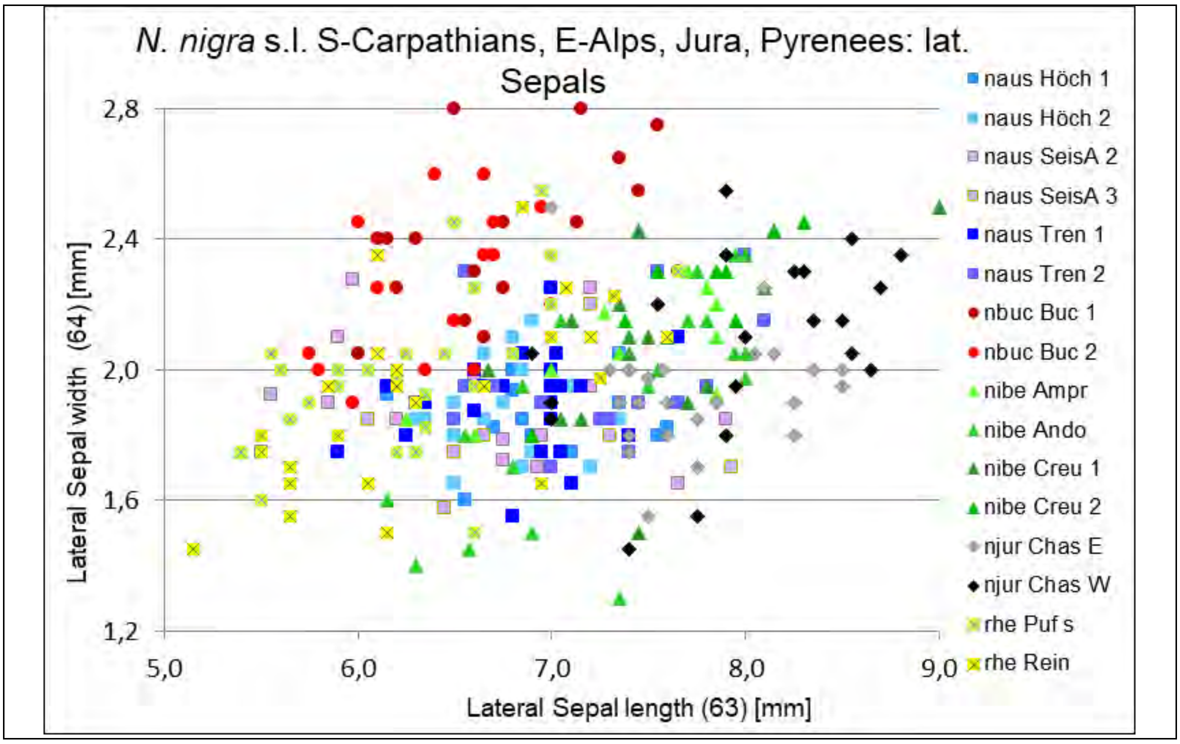


Fig. 20: Diagram of Lateral Sepal length = f (Lat. Sepal width). The Carpathian samples of *N. nigra* from Bucegi show slightly larger and shorter lateral sepals than western *N. nigra*. *N. rhellicani*, once again, shows lower length/width than *N. nigra* s.l.

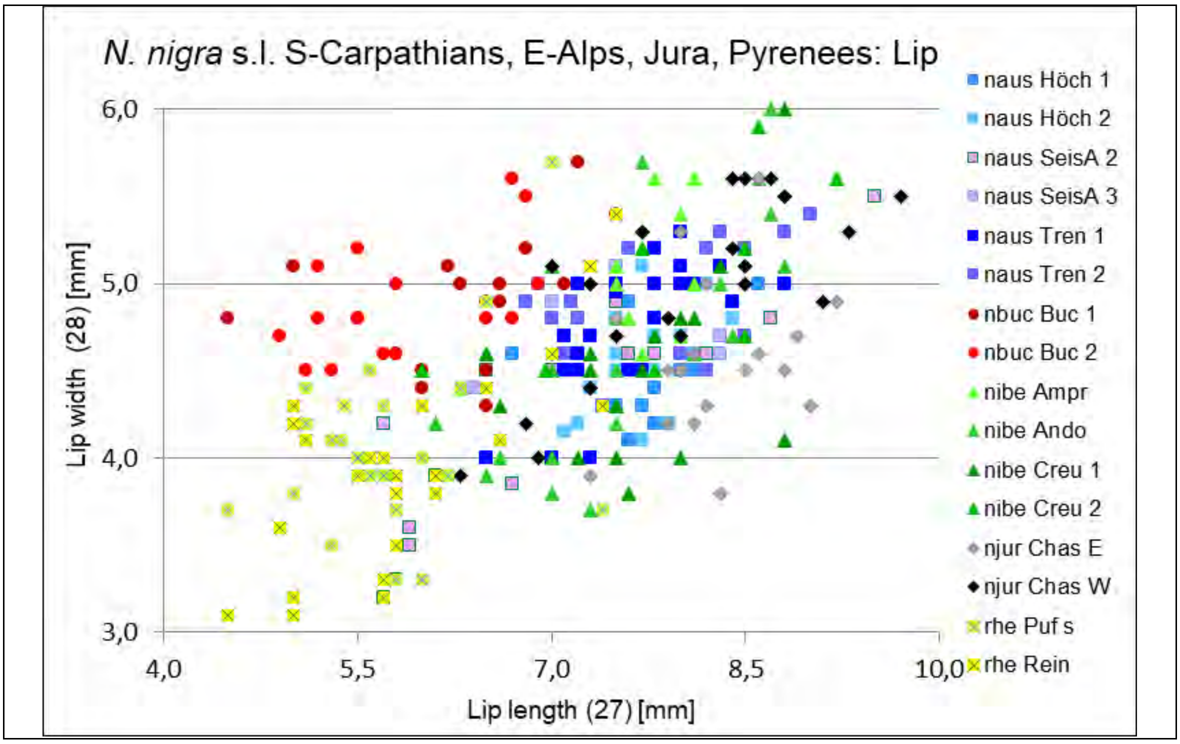


Fig. 21: Diagram of Lip length = f (Lip width). The Carpathian samples of *N. nigra* from Bucegi show shorter lips than western *N. nigra*. The lip of *N. rhellicani* reveals to be narrower than Carpathian *N. nigra* and shorter/narrower than western *N. nigra* s.l.

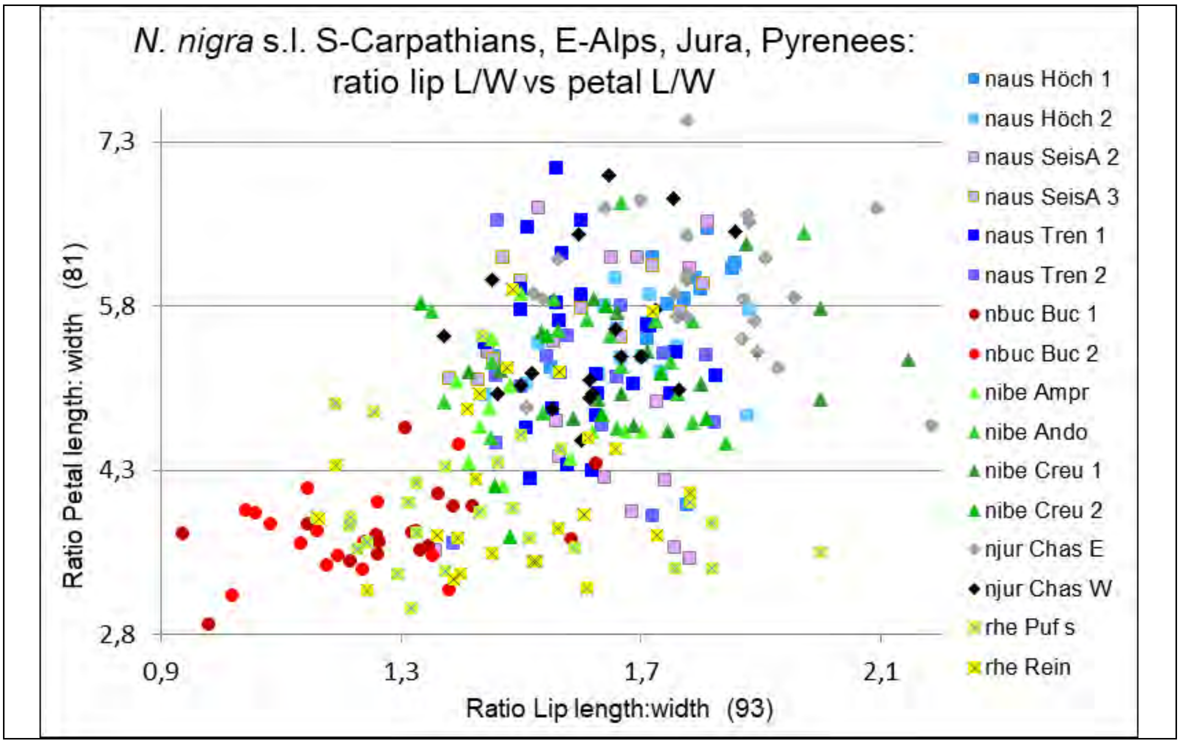


Fig. 22: Diagram of the Ratio Lip length/width = f (Ratio Petal length/width).
The differences shown in Fig. 19-21 become even more evident when the ratios are compared. The Carpathian samples clearly separate from western *N. nigra*.
N. rhellicani takes an intermediate position.

Central and western *N. nigra* show a weak clustering at a very low level into three groups, however, either not including all of its subpopulations or, vice versa, including one alien subpopulation. So, first *N. nigra* subsp. *iberica* from Jura clusters with one Pyrenean population, whereas all others give one cluster divided into two subclusters, one with five of six groups of *N. nigra* subsp. *austriaca* and a second with three of four groups of *N. nigra* subsp. *iberica* with the sixth group of *N. nigra* subsp. *austriaca*. Cross-validation results of DA with the five main groups *N. nigra* subsp. *austriaca*, subsp. *iberica* Jura, subsp. *iberica* Pyrenees, subsp. *bucegiana* and *N. rhellicani* give a high percentage of correct attribution for *N. rhellicani* (82%) and the Bucegi-population (87%); only one sample of the latter is attributed to subsp. *austriaca* and three to *N. rhellicani*. Contrary, *N. nigra* subsp. *austriaca* (63%) and subsp. *iberica* (Pyrenees: 53%; Jura: 70%) have significantly lower scores of correct attributions, caused by multiple mixup(s) within subsp. *austriaca* and subsp. *iberica* themselves. Overall, this pattern confirms the close morphological relationship of *N. nigra* subsp. *austriaca* with *N. nigra* subsp. *iberica*.

5. Discussion

TEPPNER & KLEIN (1990) studied dark-coloured members of *Nigritella* from the Central European mountain regions and compared those to the *N. nigra* known from Scandinavia. They confirmed that the Scandinavian *N. nigra* is reproducing asexually by means of nucellar embryony, and they showed that it is a triploid with 2n = 3x = 60 (not 2n = 64 as originally reported in AFZELIUS (1932: 367)). The Central European dark-coloured *Nigritella* was shown to be composed of two entities, one diploid (2n = 2x= 40) reproducing sexually, and one tetraploid (2n = 4x = 80) reproducing asexually. The former was described as a new species, *N. rhellicani,* whereas the latter was described as a new subspecies of *N. nigra*, *N. nigra* subsp. *austriaca* Teppner & E.Klein, differing not only in chromosome number and distribution area from the Scandinavian nominate subspecies, *N. nigra* subsp. *nigra*, but also in a shorter lip and shorter tepals as well as in a slightly longer spur.

A third subspecies of *N. nigra* from the Pyrenees and Western Alps was added by TEPPNER & KLEIN (1993) as *N. nigra* subsp. *iberica* Teppner & E.Klein. This subspecies was characterised by the same chromosome number and mode of reproduction (2n = 4x = 80; nucellar embryony) as subsp. *austriaca*, but it differed in slightly larger floral organs, which together with its western distribution motivated the segregation as a separate subspecies. Other dark-coloured populations from the Pyrenees and the Cantabrian Mountains were found to be diploid (2n = 2x= 40) and sexual, and were recognised as

N. gabasiana Teppner & E.Klein. This species was found to be similar to the alpine *N. rhellicani* in the papillose margins of the lower bracts and the overall floral morphology, but differed in the distinctly larger flowers with longer tepals and lip.

As other tetraploid populations of *N. nigra* were discovered in the Massif Central, the Western Alps, and the Jura Mountains, the relationships of these regional populations to the previously recognized subspecies were debated, and yet a subspecies was described by BREINER & BREINER (1993: 471) as *N. nigra* subsp. *gallica* E.Breiner & R.Breiner. Following morphometric analyses by KLEIN & DRESCHER (1996), the populations from the Pyrenees and the Massif Central were amalgamated with populations from the Eastern Alps as *N. nigra* subsp. *austriaca*, but subsequently, based on findings from allozyme analysis, populations from the western regions were finally all recognized as *N. nigra* subsp. *iberica* (TEPPNER & KLEIN 1998; see below).

Variation patterns obtained by the use of molecular markers largely agree with those described in morphometric and embryological studies. An allozyme analysis by HEDRÉN et al. (2000) showed that the various polyploid subspecies of *N. nigra* were characterised by patterns of fixed heterozygosity in agreement with an asexual mode of reproduction, whereas the diploids *N. rhellicani* and *N. gabasiana* were genetically variable within populations and showed patterns of genetic segregation, consistent with sexual reproduction and outcrossing. Moreover, although the polyploid subspecies of *N. nigra* shared the same alleles, they still differed consistently from each other in expression patterns and consequently in the number of allele copies at the investigated allozyme loci.

DNA-based studies provided further details on the relationships of dark-coloured *Nigritella* taxa. STÅHLBERG (1999) used AFLP fingerprinting (Amplified Fragment Length Polymorphisms) to describe overall differentiation in the nuclear genome. He observed a high degree of similarity between the asexual subspecies of *N. nigra*, subsp. *nigra*, subsp. *austriaca* and subsp. *iberica*, and a lower degree of similarity between *N. nigra*, *N. rhellicani* and *N. gabasiana*. Regarding the latter two species, he found that *N. gabasiana* was relatively homogenous, whereas *N. rhellicani* was more variable and difficult to separate from other diploid members of *Nigritella* using AFLP markers. In congruence with results obtained from allozymes, analyses based on nuclear SSR loci showed restricted variation within asexual *N. nigra* and a much higher variation within diploid *N. rhellicani* and *N. gabasiana* (HEDRÉN et al. 2017). Still, some more detail was obtained for *N. nigra* in which it was found that populations from the northern and southern regions of the Eastern Alps carried slightly different genotypes.

Analysing nuclear SNP (Single Nucleotide Polymorphisms) data, a high degree of similarity was observed between *N. nigra* subsp. *nigra* and *N. nigra* subsp. *austriaca*, but these taxa differed more clearly from diploid *N. rhellicani* and even more so from *N. gabasiana* (BRANDRUD et al. 2019).

As revealed by analysis of repeat regions in the plastid DNA, *N. gabasiana* was clearly divergent from the other *Nigritella* species. Furthermore, the Scandinavian *N. nigra* subsp. *nigra* was somewhat differentiated from the Central European subspecies, but *N. rhellicani* was not clearly differentiated from the latter as they partly shared the same plastid haplotypes (HEDRÉN et al. 2017).

In this study, we obtained more comprehensive data on the molecular and morphological differentiation among regional populations of *Nigritella nigra* s.l. As for the molecular data, we chose not to include any data on the diploid *N. rhellicani* and *N. gabasiana* for comparison with *N. nigra*, as the diploids are easily separated from the asexual polyploids (see above) and their position in relation to the asexual *N. nigra* is not the focus here.

No additional accessions of *N. nigra* from Scandinavia were analysed in the present study. However, also with a large number of additional accessions from other parts of Europe to compare with, it is still evident that the genotypes identified in the Scandinavian regional population are unique to this region, although the genotypes are still similar to the ones found in the Alps. In agreement with TEPPNER & KLEIN (1993: 205), we also show that the lip of the Scandinavian material is relatively long in comparison to material from other regions, albeit with a high degree of overlap (Table 2). We do not find the spur of the Scandinavian *N. nigra* (Table 2) to be as short as previously stated by TEPPNER & KLEIN (1990: 15; 0,8-1.1 mm, measured only on herbarium material), but as supported by the triploid ploidy level and in agreement with its northern distribution, we agree that the Scandinavian population deserves recognition as a separate subspecies.

In continental European mountains, the genotypes found in the Pyrenees and the Western Alps were different from, but still similar to, the genotypes found in the Eastern Alps (Fig. 15 - 16; PCO). Nearly all plants from the western regions had fragments of either 110 bp or 112 bp at the Gc51 locus, whereas in most plants from the Eastern Alps these fragments were replaced by fragments of 120 bp, 124 bp, and/or 128 bp at this locus. In agreement with TEPPNER & KLEIN (1993), we also found the tepals to be, on average, slightly longer in the western plants than in the eastern plants. Combined with the previously described differences in allozyme expression patterns (HEDRÉN et al. 2000), we agree that it is justified to treat the western and eastern regional populations as different subspecies of *N. nigra* subsp. *iberica* and *N. nigra* subsp. *austriaca*, respectively.

Two dominant SSR genotypes were found in the western *N. nigra* subsp. *iberica* and both were present in the Pyrenees and the Jura Mountains. They had divergent banding patterns at the two analysed SSR loci, and they probably represent old genotypes that have been present in the area for a long time. Their shared presence in the Pyrenees to the southwest and in the Jura to the northeast suggests that *N. nigra* in the combined western area survived the last ice age in a single refugium from which the extant populations were recruited. Similar conclusions have been drawn on other alpine plant species with disjunct distributions in the Pyrenees and the Western Alps, for example, *Hypericum nummularium* (GAUDEUL 2006).

Our morphometric data show that populations from the Pyrenees and the Jura agree closely with each other in the length of floral organs including the lip and the other tepals (Figs. 19-22). Given the presence of the two dominant SSR genotypes, both in the Pyrenees and in the Jura Mountains and the presence of a single allozyme genotype in the Pyrenees, in the Massif Central and in the Western Alps (HEDRÉN et al. 2000), we agree with TEPPNER and KLEIN (1998) that the combined western population is best treated as a single subspecies, *N. nigra* subsp. *iberica.* Consequently, no further subdivision is motivated.

Two closely similar SSR genotypes, differing only in the position of one fragment at locus Gc51 (Table 1) dominated in the Eastern Alps. Genotype 33 was exclusively found in the Northern Calcareous Alps. Genotype 26 was dominant in the Southern Calcareous Alps, but was also found at one sampling site in the north. The difference in the main distribution of these two genotypes may be taken as evidence for some degree of differentiation between the northern and southern populations in the Eastern Alps. It could be speculated, for example, that *N. nigra* was split into a northern and a southern refugium during the last ice age, and that the presence of genotype 26 in the north is an indication of a more recent dispersal event across the Central Alps from the south. Nevertheless, since the divergence between these genotypes is minimal and since a split between northern and southern populations is only partially supported by the morphometric data (Table 2), we consider that the entire population in the Eastern Alps is best accommodated within a single subspecies of *N. nigra*, *N. nigra* subsp. *austriaca*.

Plants of the *N. nigra* complex from the Southern Carpathians have not been analysed for molecular markers before. Here, we analysed 86 samples from various subsites in the Bucegi Natural Park, situated in the Bucegi Mountains of the Southern Carpathians, Central Romania. We found one dominant genotype in this material and eight minor genotypes which were all closely similar to the dominant one. All these genotypes were different from genotypes discovered in other areas. From the PCO (Fig. 15 - 16) it appears that the Carpathian genotypes are strongly divergent from the rest, but the difference mostly comes back to the lack of 80 bp and 92 bp fragments at locus Gc51 in the Carpathian samples. Otherwise, the rest of the fragments expressed at this locus are within the same range as fragments expressed in other samples.

The fact that most plants from the Southern Carpathians had exactly the same genotype and the rest were closely similar to this one, differing only for one or two fragments, shows that the Carpathian plants reproduce asexually, just as other members of *Nigritella nigra*. We have no information on the exact chromosome number of the Carpathian plants, but the presence of four, or in some plants five, bands at locus Gc51, suggests that they are tetraploid. Interestingly, BALTISBERGER & WIDMER (2009: 358) report about a finding of *Nigritella rhellicani* (i.e., a "black" coloured entity) from Piatra Craiului near Zărnești a mountain range of about 25 km, situated in the North-western Bucegi area, whose ploidy had been determined as 2n = 4x = 80. These tetraploid black *Nigritella* most presumably may be attributed to the plants from Bucegi.

Morphologically, the Southern Carpathian *Nigritella* populations are also slightly different from the other European populations. The Carpathian plants have short tepals and lips, but these organs are at least as wide as in plants from other regions, which results in low length to width ratios (Fig. 22).

6. Taxonomic conclusions

Altogether, we find it appropriate to recognize the Southern Carpathian plants of the Black Vanilla Orchid as a separate subspecies of *N. nigra*. The formal description follows below.

***Nigritella nigra* subsp. *bucegiana* Hedrén, Anghel. & R.Lorenz, subsp. nov.**

Diagnosis: Subspeciebus *nigra*, *austriaca* Teppner & E.Klein et *iberica* Teppner & E.Klein valde similis, sed ab eis petalis, sepalis et labio leviter brevioribus, sed praesertim petalis latioribus differt.

Holotypus: România, South Carpathians, Dambovita, Moroeni, Bucegi, Muntele Cocora, alpine grassland, calcareous conglomerate, leg. Mikael Hedrén & Richard Lorenz sub No. 14-17 ex specimen typorum: UTMWGS84 35T 380537 5026893, 1979 m asl [m ü.d.M.], 4.7.2018, FO Lo 18.073b, Nnbu10.

conservatur in herbario Heidelbergensis sub no. HEID 807889.

Paratypi: România, South Carpathians, alpine grassland, calcareous conglomerate. Nnbu01, 02, Prahova, Sinaia, Bucegi, Muntele Furnica and Vârful Piatra Arsă; Nnbu 11, 12, 13 and 14, Dâmbovița, Moroeni, Bucegi, Muntele Cocora, leg. Mikael Hedrén & Richard Lorenz, conservantur in herbario Heidelbergensis sub no. HEID 807889.

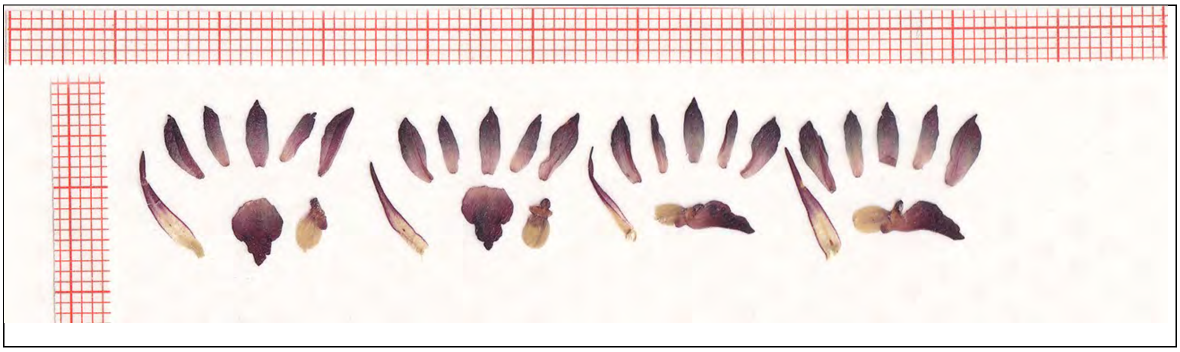


Fig. 23: Holotypus *Nigritella nigra* subsp. *bucegiana*, România, South Carpathians, Dâmbovița, Moroeni, Bucegi, Muntele Cocora, alpiner Magerrasen, kalkkaltiges Konglomerat, leg. Mikael Hedrén & Richard Lorenz, Nr. 14-17.

Icon. hoc loco: Fig. 23 (Holotypus), Fig. 45 (Holotypus, paratypi), Figs. 1-6, 11-14, 24-33, 34-37.

Icon. altera: De Angelli & Anghelescu 2020: 100-101, 4 Figs. s. ind. loc., sub *N. rhellicani*; De Angelli 2022: 241, Fig. 28, sub *N. rhellicani.*

Flower analyses: Fig. 46.

Etymology: The epithet *bucegiana* chosen for the new subspecies is derived from the name *Bucegi*, *ad litteram* meaning *of Bucegi*, a reference to the mountain massif where the subspecies has been discovered.

Description: Hypogeal part of the stem (underground part) forming an extremely short, compressed rhizome. Rhizome produces flattened, digitate root tubers and adventitious roots. Root tubers, of ca. 4-11 mm diameter, deeply divided for at least half of their length, thickened at the base and tapering. Adventitious roots, 2-6, 25-55 × 1-1,3 mm, cylindrical, thick and covered in mycotrophic root hairs (rhizoids). Height of plants about 6-18 cm, stem 3-5 mm wide, ridged/angular (with streaks), entirely light-green. 5-7 (10) basal leaves forming a rosette, green lanceolate, canaliculate, acute, 35-85 mm long and 3-7 mm wide, erect to spreading, to a subtended angle of ca. 40°-45° relative to the stem; 4-7 (8) narrow lanceolate cauline leaves, 5-7 (8) × 2-3 (5) mm, erect, acute, sheathing the stem, green with reddish brown margin.

Flower spike dense, pyramidal at beginning of flowering, becoming spherical to ovate in full flower, 15-25 mm long, 15-20 (25) mm wide, in fruit prolonging up to 30 (35) mm; bracts green to brownish, dark purple, smooth-edged, lower ones 8-14 mm long, 1,1-2,3 mm wide at base, acute, sometimes also with small papillae at the margin. Flowers not resupinated, ± funnel-shaped, tepals divergent, petals and median sepal stretched straight forward. Flower colour red-brown to dark brown-red, scent of faint chocolate to strong and sweet vanilla.

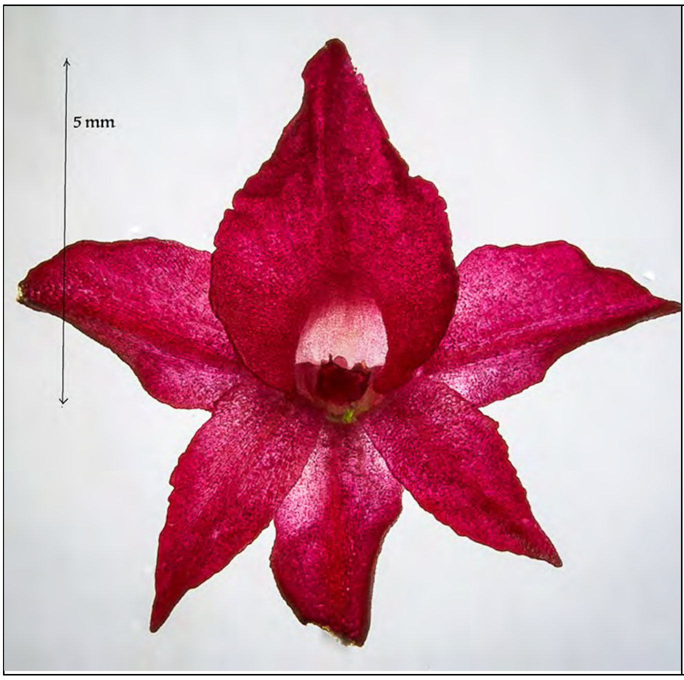
Lateral sepals elliptic to obovate, acute, 5,8-7,6 × 1,9-2,8 mm, median sepal elliptic, acute, 5,0-7,3 × 1,4-2,5 mm, mostly slightly narrower than the lateral sepals. Petals lanceolate, 4,9-6,5 × 1,3-2,0 mm, significantly narrower than lateral sepals. Lip 4,5-7,5 mm long, with small, bulbous base, above base at about 1/4 of total length saddle-shaped with narrowed edges, but not themselves touching, then widely opening to a maximum of approx. 4,3-5,7 mm; apical part of the lip widening with forthcoming flowering, bent upwards, heart-shaped when flattened. Spur 0,9-1,5 mm long, 0,8-1,4 mm wide, saccate, translucent-white to pinkish with a narrow, glabrous entrance, nectariferous.

Ovary 2-3 mm long, about 1,5 mm wide, sessile, glabrous. Gynostemium 1,5-2 mm long, erect, horizontal; anther unique, bithecal, elongated, thecae parallel, rostellar fold not protruding beyond anther, when seen from the side. Auricles 2, lateral to the anther, verrucose, translucent-white, non-pigmented. Stigma concave, wet, viscous, with two elliptic, lateral lobes and a small median lobe adjacent to the spur entrance. Fruit elongate, ovoidal, erect 5,5-7 mm long, 2-3 mm wide, ripe end July-August; fruit set 30-55 (70) %. Seeds 0,3-0,4 (0,6) × 0,1 mm, numerous (microspermy), mature in August-September.

Flowering period: June-July.

Biology: Tetraploid with asexual reproduction. As in other members of *Nigritella*, including the polyploid, apomictic members, *N. nigra* subsp. *bucegiana* secretes small amounts of nectar contained in the spur and it emits several chemical compounds including vanillin and vanillyl ethyl ether. Although pollination is not needed for reproduction, the pleasant vanilla scent attracts various small insects (Figs. 34-37) such as bees, bumblebees, flies, butterflies and moths (De Angelli & Anghelescu 2020: 101, 103).

Variability: The Bucegi-population does not show any significant visible variation apart from slight differences in flower coloration from brighter brownish red to darker reddish brown and in spike length. Most floral characters have a low variability with variation coefficients varying between 5-6% and 11-12%; somewhat higher values up to 20% can be seen in bracts length and width.



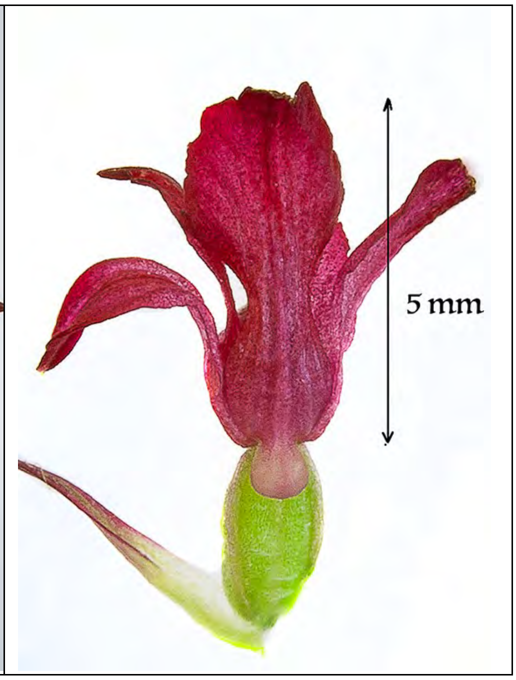


Fig. 24

Fig. 25

Fig. 24-27: *Nigritella nigra* subsp. *bucegiana,* România, South Carpathians, Dâmbovița, Bucegi, Plaiul lui Păcală, 15.7.2018, phot. NA; Fig. 24: Flower detail - The tepals are divergent, petals and median sepal stretched straight forward; Fig. 25: Side view – the nectariferous spur is short, roundish, ca. ¼ ovary length; the ovary is not twisted due to non-resupination; Fig. 26: Top view of the flower; Scale bar 5 mm; Fig. 27: Ovaries, transversal section - The apomictic flowers have swollen ovaries developing into fruits, while still bearing fresh, unpollinated flowers.

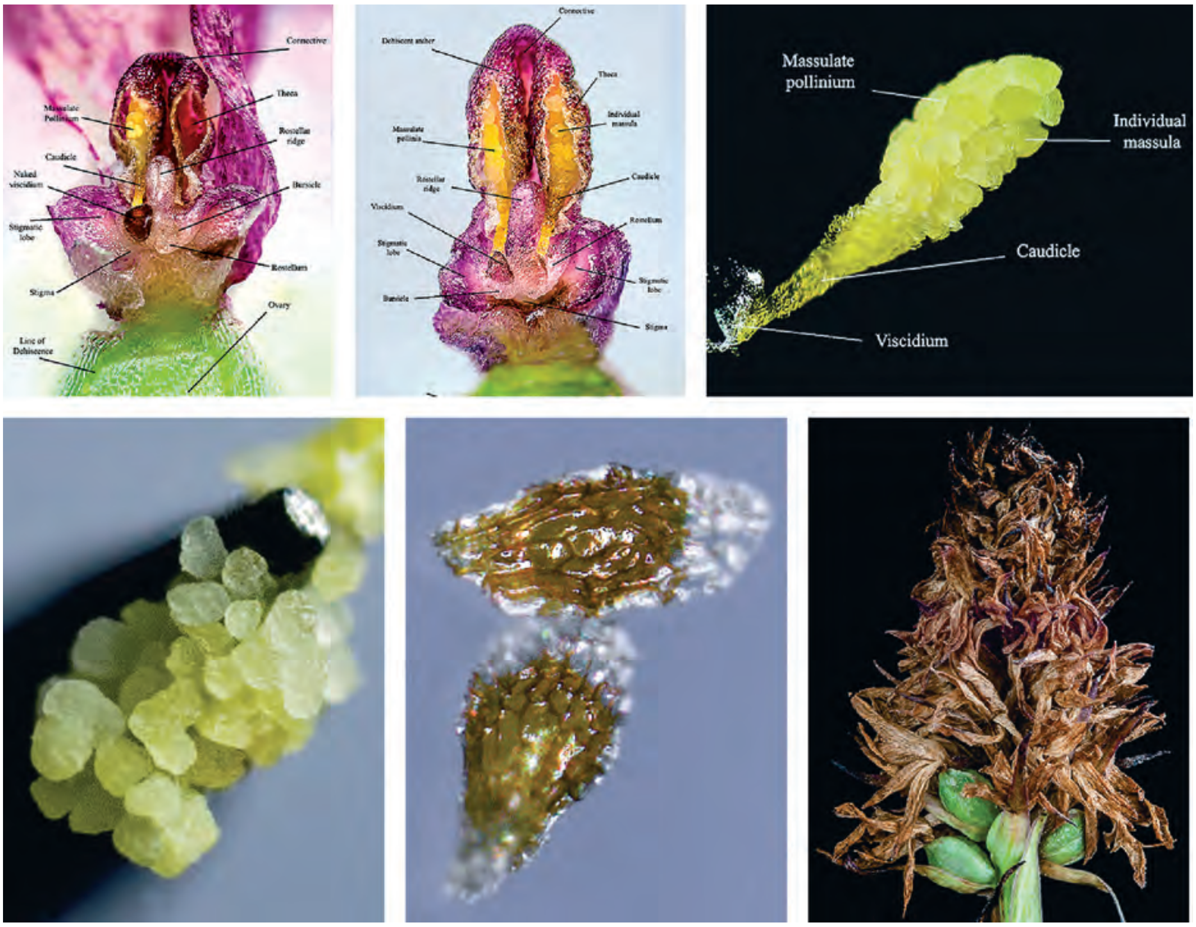


Fig. 28-33 (left above to bottom right): *Nigritella nigra* subsp. *bucegiana* - Gynostemium, Romania, Bucegi, Plaiul lui Păcală, 30.6.2022, phot. NA;

Fig. 28: Gynostemium is pinkish-purple, presenting significant traces of anthocyanins; Fig. 29: Detail of the bilobed stigmatic cavity situated below the unique, dehiscent, bithecal anther containing two parallel pollinaria;
Fig. 30: Pollinarium - It consists of one massulate pollinium, caudicle and translucent viscidium enclosed in a rudimentary bursicle; Fig. 31: Pollinium - It consists of compacted blocks of pollen, edge-shaped, termed as masulae;
Fig. 32: Seeds, Romania, Bucegi, Plaiul lui Păcală, 15.8.2022, phot. NA. Wind-borne, mature in August-September; Fig. 33: Fruit, Romania, Bucegi, Plaiul lui Păcală, 19.7.2016, phot. NA. The swollen, green capsules mature in July-August.

Habitat: *Nigritella nigra* subsp. *bucegiana* grows in subalpine to alpine grassland on calcareous nutrient-poor (oligotrophic to mesotrophic) substrates, occurring sympatrically with various alpine species such as *Carex sempervirens*, *Sesleria* spp., *Pinguicula vulgaris*, *Festuca* spp., *Gentiana verna*, *Pilosella aurantiaca*, *Thymus pulegioides*, *Pedicularis verticillata*, *Leontodon hispidus*, *Erigeron neglectus*, *Cerastium alpinum*, *Cerastium fontanum* subsp. *vulgare*, *Acinos alpinus*, *Antennaria dioica*, *Alchemilla flabellata,*




Fig. 34-37: Insects visiting *Nigritella nigra* subsp. *bucegiana* to collect nectar, Romania, Bucegi, Plaiul lui Păcală, 30.6.2022, phot. NA. Fig. 34 (above left): *Zygaena loti* (*Zygaenida*), 29.6.2022, phot. NA; Fig. 35 (above right): (cf.) *Adia cinerella* (*Anthomyiidae*), 4.7.2021, phot. NA; Fig. 36 (below left): (cf.) *Episyrphus balteatus* (*Syrphidae*), 29.6.2022, phot. NA; Fig. 37 (below right): *Aglais io* (*Nymphalidae*), Vârful Piatra Arsă, 3.7.2018, phot. RL.

Biscutella laevigata, *Arabis alpina*, *Dianthus glacialis*, and with other orchid species such as *Nigritella miniata*, *Coeloglossum viride*, *Chamorchis alpina*, *Gymnadenia conopsea* and *Pseudorchis albida* subsp. *tricuspis*.

Distribution: the known distribution of *N. nigra* subsp. *bucegiana* is currently restricted to the above tree line area of the Bucegi Mountains Natural Park from 1650 – 2300 m asl.

Notes: In their description of *N. rhellicani* (TEPPNER & KLEIN 1993), the authors listed the accessions that were analysed for chromosome number. Among these plants was also a specimen cultivated in a pot and originating from the Bucegi mountains, Sinaia. This plant must have been collected close to the populations investigated by us. The authors obtained 2n = 40 for this specimen, indicating that it is a diploid and that it must have been reproducing sexually. However, apart from the *Nigritella* plant described here, which is obviously an asexual polyploid, we have only found the red-flowered *N. miniata* in this area (Figs. 38-40).

Diploid, sexual species of *Nigritella* are also known from the Eastern Carpathians in Romania. These species include the pale coloured *Nigritella carpatica* (Zapal.) Teppner, E.Klein & M.Zagulskij (TEPPNER et al. 1994) and the dark brown *Nigritella suceveana* Kreutz, Bobocea & O.Gerbaud (BOBOCEA et al. 2021).

7. Bucegi Natural Park

Since ancient times, Bucegi Mountains have exerted an irresistible attraction for those who have ventured to their heights (Figs. 41-42).

7.1 History of Bucegi Natural Park

The first documents attesting to the presence of humans in Bucegi date from the 15th century, but recent research has concluded that human presence is much older. Nevertheless, for the next four hundred years, the mountains remained a secluded wild place, covered with virgin secular forests, haunted by robbers/bandits and populated by hermits and monks.

It was only at the beginning of the 19th century when the first mention of a trip to the Bucegi Mountains was attested by a Frenchman, Jean Alexandre Vaillant (1804-1886), a teacher, political activist and historian.

Fig. 38-40: *Nigritella miniata*, habitat, flower spike and habitus with visitor *Aglais io*. Romania, Bucegi, Muntele Cocora, 4.7.2018, phot. RL. Fig. 38 shows a cow, one of increasing herds, partially substituting flocks of sheep. This may lead to severe threats of the flora of Bucegi as well as increasing touristic impact.

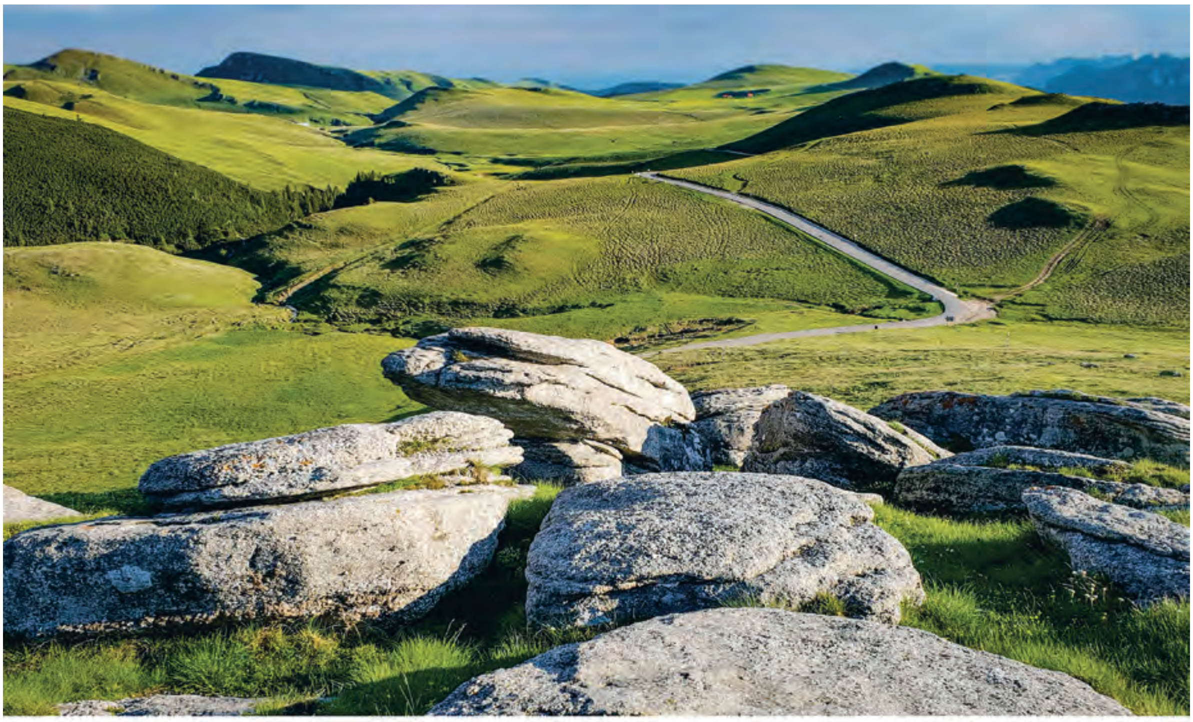


Fig. 41: Plaiul lui Păcală, Dămbovița, Romania, Bucegi NP, 28.6.2021, phot. NA.

Fig. 42: Bușteni, Romania, Prahova, Bucegi NP, 20.7.2016, phot. NA.

In his impressive book, '*La Roumanie'* (Paris, 1844), Vaillant recounts the trip he made in 1839, accompanied by a Romanian friend and two guides, peasants from the surroundings. Besides detailed historical and political accounts, the exquisite, wild beauty of the Bucegi Mountains, 'framing Transylvania', was first mentioned.

7.2 Protection measures of Bucegi Natural Park

Bucegi massif, in a shape of a 'horseshoe', has a length of ca. 25 km, and a width of ca. 12 km. It is bordered to the east by Valea Prahovei, to the north by Valea Râşnoavei, to the west by the Bran-Rucăr corridor, while to the south it is gradually framed by the Subcarpathian hills of the Ialomiţa river (CRISTEA 1960). In 1935, due to the extraordinary beauty, the great diversity of the wildlife and the complexity of its orographic forms, the region has been included in the list of protected areas in Romania and proposed to be given a national park status. On March 6th 2000, the Bucegi Natural Park was finally declared a protected area by law (Law Number 5 of March 6, 2000) and given the status of 'IUCN category V (protected landscape).

7.3 Bucegi Mountains Natural Park, a former orchid paradise, today a sheepfolds and cattle farming paradise.

Unlike other European countries where some species are very rare or have very low populations, in Romania they are still well represented by numerous populations that, fortunately, quite often remain in relatively constant numbers. Such examples are *Cypripedium calceolus*, a species widely distributed throughout Romania, and/or the rare and elusive *Epipogium aphyllum*, which can be found in several locations throughout Romania, the latter being very frequent in Bucegi Natural Park.

The Bucegi Natural Park, included within Natura 2000 site ROSCI0013 (PLANUL DE MANAGEMENT 2011, 2018) is particularly famous for its floristic diversity, from a total of 1108 species, 120 of which are included in the "Red List of Higher Plants from Romania" as rare, endemic, vulnerable or endangered species (Annex 1-41, Ministry Order, 2018). While in other countries certain species have become strictly protected, in Romania, even within the national and natural parks, as well as in the protected areas, orchids are continuously under attack.


Fig. 43: Cattle grazing in Bucegi NP, Romania, Dâmbovița, Plaiul lui Păcală, 28.6.2017, phot. NA.


Fig. 44: Flock of sheep grazing in Bucegi NP, Romania, Prahova, Sinaia, Cota 2000, 10.8.2015, phot. NA.

The complex dynamics of these ecosystems are especially disturbed by human intervention. Currently, especially the eastern plateau of Bucegi Natural Park, can easily be qualified as a true boulevard, due to the multiplication cabins, tourist trails and the construction of a network of roads up to the Cota 1400, Babele and Peştera, Piatra Arsă and of the Sinaia–Vf 2000 and Bușteni cable cars. Various anthropogenic activities such as disordered tourism, chaotic collection and illegal removal of plants, illegal deforestation, disturbance of fauna species, abandonment of waste, and water and soil pollution had a great impact and lead to the modification of habitats and ecosystems.

Additionally, during the last years, the number of sheepfolds and cattle farms increased significantly, covering the alpine grasslands. From early spring to late autumn, hundreds of herds of cattle graze without interruption, on the alpine plains, erasing almost everything in their path. In a single day, they can eat thousands of plants, destroying not only orchids, but also several other rare species of plants. It is this vulnerability and the dangers the orchids face, which caused their reduction and, in some cases, even their extinction from areas where they once used to occur in abundance (De Angelli & Anghelescu 2020). The uncontrolled, intensive grazing affects the entire ecosystem, causing chemical and compositional changes in the soil together with soil compaction and erosion.

It is commending to take this opportunity to draw readers' attention to the vulnerability of these delicate plants and the numerous dangers threatening their existence in the habitats where they occur. In some cases, there has already been a regression and even a disappearance of orchids from areas where they were abundant in the past. There are many species whose populations have few specimens and grow in very restricted areas. Protecting these wonderful yet extremely vulnerable plants, which are part of highly complex ecosystems is imperative.

The pictures (Figs. 43-44) show an overview of the invaded alpine plateau, where a staggering number of sheep and cattle farms suddenly appeared in recent years. Scattered throughout the park, they transformed this protected area into a parcelled, bare land. Overgrazing is a major problem and that is why it must be controlled.

7.4 Geography of Bucegi Natural Park

The Park covers the administrative territory of counties Prahova, Dâmbovița and Brașov, spreading over an area of approximately 32.663 hectares. Today, the Bucegi Natural Park constitutes one of the most representative geographical and touristic regions in the Southern Carpathians, Romania.

The Park includes distinct peaks, Omu Peak (2507 m), the highest in Bucegi, Bucura Peak (2.503 m), Babele (2.294 m), Dichiu Peak (1.719 m), Coștila (2.490 m), Bucșoiu (2.492 m), Caraiman (2.384 m), Jepii Mici (2.143 m), Piatra Arsă (2.044 m) and Lespezi Peak (1.685 m). They surround the Bucegi Plateau, a flat, wide area of about 40 km^2, situated at ca. 2000-2200 m asl, undulating and gradually sloping from north to south. The Bucegi Plateau is covered by vast alpine meadows/plains, with a specific steppe aspect (URECHIA 1979).

7.5 Geology of Bucegi Natural Park

From a geological point of view, the massif is made up of calcareous conglomerates, the "Bucegi conglomerates", sedimentary deposits and rock fragments consisting of limestone, sandstone, crystalline schist, quartz and even blocks of granite (VELCEA 1974).
A characteristic of the Park is the presence of the unusual *megaliths*, which, as a result of gradual erosion, got the bizarre shapes of pillars, columns, mushrooms and arches, some of them supposedly resembling old ladies (The Babele *megaliths*,) or human faces (The Sphinx).

7.6 Climate at Bucegi Natural Park

In general, it presents a capricious climate, with low temperatures and strong winds. The Northern area (between 2500-2300 m) is dominated by a cold, wet and often overcast climate. Snow and frost last 7-8 months and the average annual temperature is -3°C (Omu Peak). The rains are frequent and cold and often accompanied by hail. The Southern area (between 2300-1800 m) is characterized by a warmer and drier climate, sunnier and less windy, with an average annual temperature of 0°C.

7.7 Flora in Bucegi Natural Park

In the Bucegi Natural Park, the vegetation is characterised by 'altitudinal layering', represented by a change in both the landscape and the vegetation, as the altitude increases (AVANU & ANGHELESCU 2013). These zones are roughly demarcated as:

1. The lower zone (700 m asl, deciduous forests);

2. The medium zone (700-1400 m asl, mixed, deciduous and conifers forests);
3. The superior zone (1400-1700 m asl, coniferous forests).

The vegetation characteristic for the first three zones is represented by forests (deciduous, coniferous, mixtures of the two), the habitat of most of the spring species found in the Park: *Galanthus nivalis*, *Corydalis cava*, *Corydalis solida*, *Gagea lutea*, *Allium ursinum*, *Anemone nemorosa*, *Anemone ranunculoides*, *Maianthemum bifolium*, *Erythronium dens-canis*, but also to summer species such as *Telekia speciosa*, *Lunaria rediviva*, *Petasites kablikianus*, *Petasites hybridus*, *Ranunculus carpaticus*, etc.

4. The subalpine zone (1750-2000 m asl, spruces and bushes).

The vegetation present in mountain meadows and clearings has an extremely diverse floristic composition: *Leucanthemum vulgare*, *Centaurea melanocalathia*, *Centaurea nigrescens*, *Silene viscaria*, *Silene flos-cuculi*, *Cruciata laevipes*, *Trollius europaeus*, *Aconitum vulparia*, *Hypericum maculatum*, *Hypericum perforatum*, *Astrantia major*, etc.

5. The alpine zone (2000-2300 m asl, alpine meadows and junipers).

Various species of junipers and dwarf ericaceous bushes are *Rhododendron myrtifolium*, *Vaccinium vitis-idaea*, *V. myrtillus*, *V. uliginosum*, *Loiseleuria procumbens*, *Erica spiculifolia*, *Lycopodium selago* or *Selaginella selaginoides*.

The alpine meadows are the habitat of some of the most spectacular alpine flowers, most protected by law: *Leontopodium nivale* subsp. *alpinum*, *Gentiana verna*, *Gentiana acaulis*, *Gentiana utriculosa*, *Campanula transsilvanica*, *Campanula glomerata*, *Lomatogonium carinthiacum*, *Arnica montana* and many others.

6. The alpine superior zone (2300-2500 m asl, alpine steppe and dwarf plants).

The alpine superior zone is rich in rare or endemic species such as: *Saxifraga mutata* subsp. *demissa*, *Saxifraga oppositifolia*, *Saxifraga luteoviridis*, *Gagea serotina*, *Eritrichium nanum*, *Senecio atratus*, *Sedum annuum*, *Sedum alpestre*, *Draba haynaldii*, *Draba kotschyi*, *Draba fladnizensis*, *Dianthus spiculifolius*, *Gypsophila petraea*, *Viola alpina*, *Polygala amara*, *Pinguicula alpina*.

The alpine steppe with dwarf vegetation includes: *Silene acaulis*, *Hornungia alpina* ssp. *brevicaulis*, *Cerastium alpinum* var. *lanatum*, *Cerastium cerastoides*, *Minuartia sedoides*, *Minuartia recurva*, *Minuartia gerardii*, *Arenaria biflora*, *Arenaria ciliata*, *Scleranthus perennis subsp. marginatus*, *Salix herbacea*, *Salix reticulata*, *Papaver alpinum* ssp. *corona-sancti-stephani*, *Linaria alpina*, *Sedum roseum*, *Hesperis oblongifolia*, *Salix alpina*, *Aster alpinus*, etc.

It is important to note that Bucegi Natural Park is the habitat of approximately 45 spontaneous wild orchid species.

8. Supporting information

In the following supplementary data to this article on *Nigritella nigra* s.l. are given, concerning material examined for variation at the two nuclear SSR loci Gc51 and Gc77 (Table 3), biometric data (Tables 4-7), and reproductions of flower analyses of *N. nigra* subsp. *nigra*, *N. nigra* subsp. *bucegiana*, *N. nigra* subsp. *austriaca*, *N. nigra* subsp. *iberica* and *N. rhellicani* (Figs. 46-49).

Table 3: Material of *Nigritella nigra* s.l. examined for variation at the two nuclear SSR loci Gc51 and Gc77 and the observed genotypes describing the combined fragment pattern at the two loci (see Table 1). Collectors: BD – Branko Dolinar; CG – Cesario Giotta; DS – David Ståhlberg; EK – Erich Klein; GP – Giorgio Perazza; JML – Jean-Marc Lewin; KR – Karl Redl; MH – Mikael Hedrén; NA – Nora Anghelescu; NG – Norbert Griebl; OG – Olivier Gerbaud; RL – Richard Lorenz; SH – Sven Hansson; UH – Ulrich Heidtke; WF – Wolfram Foelsche.

Accession number DNA bank	Region	Locality	Collector(s)	Collection date (Year/Month/Day)	Genotype
9337	Nalp	Austria, Steiermark, Aflenzer Bürgeralm	NG	090626	33
9338	Nalp	Austria, Steiermark, Aflenzer Bürgeralm	NG	090626	33
9339	Nalp	Austria, Steiermark, Aflenzer Bürgeralm	NG	090626	33
9377	SAlp	Italy, Südtirol, Grödner Joch	UH	090708	26
9379	SAlp	Italy, Südtirol, Grödner Joch	UH	090708	26
9380	SAlp	Italy, Südtirol, Grödner Joch	UH	090708	26
9381	SAlp	Italy, Südtirol, Grödner Joch	UH	090708	26

9711	SAlp	Italy, Südtirol, Seiseralm, Grunser Bichl	RL	060717	26
9712	SAlp	Italy, Südtirol, Seiseralm, Grunser Bichl	RL	060717	26
9713	SAlp	Italy, Südtirol, Seiseralm, Grunser Bichl	RL	060717	26
9714	SAlp	Italy, Südtirol, Seiseralm, Grunser Bichl	RL	060717	26
9715	SAlp	Italy, Südtirol, Seiseralm, Grunser Bichl	RL	060717	26
9716	SAlp	Italy, Südtirol, Seiseralm, Grunser Bichl	RL	060717	42
9717	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9718	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9719	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9720	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9721	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9722	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9723	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9724	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9725	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9726	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9727	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9728	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9729	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9730	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9731	SAlp	Italy, Südtirol, Lungiarü, Medalgesalm	RL	060719	26
9735	SAlp	Italy, Südtirol, Fanes, Col Bechei	RL	060719	26
9762	SAlp	Italy, Trentino, Passo Podoi	MH/SH	030704	37
9776	Scand	Sweden, Härjedalen, Ljusnedal, Klinken	MH	980709	47
9777	Scand	Sweden, Härjedalen, Ljusnedal, Klinken	MH	980709	47
9778	Scand	Sweden, Jämtland, Oviken	MH	980708	48
9779	Scand	Sweden, Härjedalen, Ljusnedal, Klinken	MH	980709	47
9803	SAlp	Italy, Südtirol, Seiseralm, Grunser Bichl	RL	060717	26
9806	SAlp	Italy, Südtirol, Seiseralm, Grunser Bichl	RL	060717	35
9807	SAlp	Italy, Südtirol, Seiseralm, Grunser Bichl	RL	060717	35
9808	SAlp	Italy, Südtirol, Seiseralm, Grunser Bichl	RL	060717	26
9826	SAlp	Italy, Südtirol, Fanes, Col Bechei	RL	060717	26
9976	Scand	Sweden, Härjedalen, Ljusnedal, Klinken	MH	980709	47
9977	Scand	Sweden, Härjedalen, Ljusnedal, Klinken	MH	980709	47
9978	Nalp	Austria, Steiermark, Aflenzer Bürgeralm	DS/EK	980705	33
9979	Nalp	Austria, Steiermark, Aflenzer Bürgeralm	DS/EK	980705	33
9989	Nalp	Austria, Steiermark, Aflenzer Bürgeralm	EK	980620	33

9992	Nalp	Austria, Salzburg, Schafberg, SE ridge	KR	980630	33
9994	NAlp	Germany, Bayern, Chiemgauer Berge, Feichtenalm	KR	980628	26
9995	NAlp	Germany, Bayern, Chiemgauer Berge, Feichtenalm	KR	980628	26
9996	WAlp	France, Isère, Mt. de Lans	OG	980614	10
9997	Walp	France, Savoie, Croix de Nivolet	OG	980614	13
9999	Pyr	France, Pyrénées-Orientales, Porté-Puymorens	JML	980711	4
15003	Pyr	France, Pyrénées, Val de Galbe	JML	980713	5
15069	SAlp	Italy, Trentino, Monti Lessini	MH/RL/GP	130707	25
15070	SAlp	Italy, Trentino, Monti Lessini	MH/RL/GP	130707	26
15071	SAlp	Italy, Trentino, Monte Baldo	MH/RL/GP	130708	26
15072	SAlp	Italy, Trentino, Monte Baldo	MH/RL/GP	130708	26
15073	SAlp	Italy, Trentino, Monte Baldo	MH/RL/GP	130708	43
15074	SAlp	Italy, Trentino, Monte Baldo	MH/RL/GP	130708	26
15075	SAlp	Italy, Trentino, Monte Baldo	MH/RL/GP	130708	26
15076	SAlp	Italy, Trentino, Monte Baldo	MH/RL/GP	130708	26
15077	SAlp	Italy, Veneto, Pordoi, Ossario del Pordoi	MH/RL/CG	130712	26
15078	SAlp	Italy, Veneto, Pordoi, Ossario del Pordoi	MH/RL/CG	130712	53
15079	SAlp	Italy, Veneto, Pordoi, Ossario del Pordoi	MH/RL/CG	130712	26
15080	SAlp	Italy, Veneto, Pordoi, Ossario del Pordoi	MH/RL/CG	130712	52
15081	SAlp	Italy, Veneto, Pordoi, Ossario del Pordoi	MH/RL/CG	130712	26
15082	SAlp	Italy, Veneto, Pordoi, Ossario del Pordoi	MH/RL/CG	130712	26
15083	SAlp	Italy, Südtirol, Pra de Störes	MH/RL/CG	130713	26
15084	SAlp	Italy, Südtirol, Pra de Störes	MH/RL/CG	130713	26
15085	SAlp	Italy, Südtirol, Pra de Störes	MH/RL/CG	130713	43
15086	SAlp	Italy, Südtirol, Pra de Störes	MH/RL/CG	130713	26
15087	SAlp	Italy, Südtirol, Pra de Störes	MH/RL/CG	130713	26
15088	SAlp	Italy, Südtirol, Pra de Störes	MH/RL/CG	130713	26
15089	SAlp	Italy, Südtirol, Fanes, Ju de Limo	MH/RL/CG	130714	26
15090	SAlp	Italy, Südtirol, Fanes, Ju de Limo	MH/RL/CG	130714	42
15091	SAlp	Italy, Südtirol, Fanes, Ju de Limo	MH/RL/CG	130714	38
15092	SAlp	Italy, Südtirol, Fanes, Ju de Limo	MH/RL/CG	130714	26
15093	SAlp	Italy, Südtirol, Fanes, Ju de Limo	MH/RL/CG	130714	26
15095	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	24
15096	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	26
15097	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	44
15098	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	26
15100	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	26

15104	SAlp	Italy, Südtirol, Dürrenstein	MH/RL/CG	130717	35
15547	Nalp	Austria, Steiermark, Tragöß, Trenchtling	WF	130705	33
15548	Nalp	Austria, Steiermark, Tragöß, Trenchtling	WF	130705	41
15549	Nalp	Austria, Steiermark, Tragöß, Trenchtling	WF	130626	33
15550	Nalp	Austria, Steiermark, Tragöß, Trenchtling	WF	130626	33
15551	Nalp	Austria, Steiermark, Tragöß, Trenchtling	WF	130626	33
15552	Nalp	Austria, Steiermark, Tragöß, Trenchtling	WF	130626	33
15553	Nalp	Austria, Steiermark, Tragöß, Trenchtling	WF	130626	33
15554	Nalp	Austria, Steiermark, Tragöß, Trenchtling	WF	130626	33
15836	SAlp	Italy, Friuli-Venezia Giulia, Monte Grappa	BD	120614	26
16568	SAlp	Italy, Südtirol, Vals, above Stin-Alm	RL	130719	26
16569	SAlp	Italy, Südtirol, Vals, above Stin-Alm	RL	130719	26
16570	SAlp	Italy, Südtirol, Vals, above Stin-Alm	RL	130719	31
16571	SAlp	Italy, Südtirol, Vals, above Stin-Alm	RL	130719	26
16572	SAlp	Italy, Südtirol, Vals, above Stin-Alm	RL	130719	31
16573	SAlp	Italy, Südtirol, Vals, above Stin-Alm	RL	130719	28
16574	SAlp	Italy, Südtirol, Vals, below Stin-Alm	RL	130719	26
16575	SAlp	Italy, Südtirol, Vals, below Stin-Alm	RL	130719	26
16576	SAlp	Italy, Südtirol, Vals, below Stin-Alm	RL	130719	26
16577	SAlp	Italy, Südtirol, Vals, below Stin-Alm	RL	130719	26
16578	SAlp	Italy, Südtirol, Vals, below Stin-Alm	RL	130719	26
16579	SAlp	Italy, Südtirol, Vals, below Stin-Alm	RL	130719	26
20643	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Cabana Dichiu	RL/MH	180627	58
20644	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Vârful Dichiu	RL/MH	180627	58
20692	Carp	Romania, Prahova, Sinaia, Bucegi, Muntele Furnica	RL/MH	180628	58
20693	Carp	Romania, Prahova, Bucegi, Vârful Piatra Arsă	RL/MH	180703	58
20694	Carp	Romania, Prahova, Bucegi, Vârful Piatra Arsă	RL/MH	180703	58
20695	Carp	Romania, Prahova, Bucegi, Vârful Piatra Arsă	RL/MH	180703	58
20696	Carp	Romania, Prahova, Bucegi, Vârful Piatra Arsă	RL/MH	180703	58
20697	Carp	Romania, Prahova, Bucegi, Vârful Piatra Arsă	RL/MH	180703	62
20698	Carp	Romania, Prahova, Bucegi, Vârful Piatra Arsă	RL/MH	180703	58
20699	Carp	Romania, Prahova, Bucegi, Vârful Piatra Arsă	RL/MH	180703	62
20700	Carp	Romania, Prahova, Bucegi, Vârful Piatra Arsă	RL/MH	180703	58
20701	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	60
20702	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20703	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	60
20706	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	61

20707	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20711	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	60
20712	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20713	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20714	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20715	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20718	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20719	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20720	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20721	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20722	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20723	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20724	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20725	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20726	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	55
20728	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20729	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20730	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20731	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20732	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	60
20733	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	55
20734	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	55
20735	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20736	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20743	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Cabana Dichiu	RL/MH	180627	58
20744	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Cabana Dichiu	RL/MH	180627	58
20746	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Cabana Dichiu	RL/MH	180627	58
20747	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Cabana Dichiu	RL/MH	180627	58
20748	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Vârful Dichiu	RL/MH	180627	58
20786	Carp	Romania, Bucegi, Vârful Piatra Arsă	RL/MH	180703	58
20787	Carp	Romania, Bucegi, Vârful Piatra Arsă	RL/MH	180703	58
20788	Carp	Romania, Bucegi, Vârful Piatra Arsă	RL/MH	180703	58
20789	Carp	Romania, Bucegi, Vârful Piatra Arsă	RL/MH	180703	58
20790	Carp	Romania, Bucegi, Vârful Piatra Arsă	RL/MH	180703	58
20791	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	60
20792	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20793	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58

20794	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	60
20795	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20796	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20797	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	61
20798	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	60
20799	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	54
20800	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20802	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20803	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20804	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20805	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20806	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20807	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20808	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20809	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20810	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20811	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20812	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20813	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20814	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20815	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20816	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20817	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20818	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
20822	Carp	Romania, Bucegi, Vârful Piatra Arsă	RL/MH	180703	58
20823	Carp	Romania, Bucegi, Vârful Piatra Arsă	RL/MH	180703	58
20830	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Muntele Cocora	RL/MH	180704	58
23524	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	4
23526	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	4
23530	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	6
23531	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	6
23535	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	13
23536	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	13
23537	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	13
23538	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	4
23539	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	4
23540	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	4

23541	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	4
23542	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	14
23545	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	21
23546	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	12
23547	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	12
23548	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	13
23549	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	12
23550	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	13
23551	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	13
23566	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	49
23568	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	2
23569	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	11
23570	Pyr	Spain, Catalunya, Pyrenees, Col de la Creueta	RL	190703	4
23571	Pyr	Andorra, Pyrenees, Port d’Envaliria	RL	190704	17
23572	Pyr	Andorra, Pyrenees, Port d’Envaliria	RL	190704	17
23573	Pyr	Andorra, Pyrenees, Port d’Envaliria	RL	190704	17
23574	Pyr	Andorra, Pyrenees, Col Botella - Port de Cabus	RL	191705	17
23581	Pyr	Andorra, Pyrenees, Col Botella - Port de Cabus	RL	191705	4
23582	Pyr	Andorra, Pyrenees, Col Botella - Port de Cabus	RL	191705	4
23583	Pyr	Andorra, Pyrenees, Col Botella - Port de Cabus	RL	191705	19
23584	Pyr	Andorra, Pyrenees, Col Botella - Port de Cabus	RL	191705	20
23585	Pyr	Andorra, Pyrenees, Port d’Envaliria	RL	190704	4
23586	Pyr	Andorra, Pyrenees, Port d’Envaliria	RL	190704	4
23587	Pyr	Andorra, Pyrenees, Port d’Envaliria	RL	190704	4
23588	Pyr	Andorra, Pyrenees, Port d’Envaliria	RL	190704	4
23589	Pyr	Andorra, Pyrenees, Col Botella - Port de Cabus	RL	190705	4
23590	Pyr	Andorra, Pyrenees, Col Botella - Port de Cabus	RL	190705	13
23591	Pyr	Andorra, Pyrenees, Col Botella - Port de Cabus	RL	190705	18
23592	Pyr	Andorra, Pyrenees, Col Botella - Port de Cabus	RL	190705	13
23593	Pyr	Andorra, Pyrenees, Col Botella - Port de Cabus	RL	190705	4
23594	Pyr	Andorra, Pyrenees, Col Botella - Port de Cabus	RL	190705	4
23595	Pyr	Andorra, Pyrenees, Col Botella - Port de Cabus	RL	190705	4
23596	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	4
23597	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	4
23601	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	4
23603	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	4
23604	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	4

23605	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	4
23608	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	4
23609	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	4
23610	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	7
23611	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	4
23617	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	4
23618	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	4
23619	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	1
23620	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	4
23621	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	3
23622	Pyr	Spain, Aragon, Pyrenees, Cerler Ampriu	RL	190708	8
23624	Pyr	Spain, Aragon, Pyrenees, Bujaruelo, Sandaruelo	RL	190710	4
23625	Pyr	Spain, Aragon, Pyrenees, Bujaruelo, Sandaruelo	RL	190710	17
23626	Pyr	Spain, Aragon, Pyrenees, Col Portalet	RL	190711	17
23627	Pyr	Spain, Aragon, Pyrenees, Bujaruelo, Puerto Viejo	RL	190712	4
23629	Pyr	Spain, Aragon, Pyrenees, Bujaruelo, Sandaruelo	RL	190710	17
23631	Pyr	Spain, Aragon, Pyrenees, Bujaruelo, Puerto Viejo	RL	190712	4
23651	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23652	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23653	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23654	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23655	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23656	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23657	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23660	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	4
23661	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23662	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23663	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23666	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23667	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	13
23668	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23669	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	13
23670	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	13
23671	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	13
23672	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23673	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23674	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	15

23677	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	13
23678	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	9
23679	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23680	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23681	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23683	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23684	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23685	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23686	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23687	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23688	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23689	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	13
23690	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190628	16
23692	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23693	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23694	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23695	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23696	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	13
23697	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	13
23698	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	13
23699	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	13
23700	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23701	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23702	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23703	Jura	Switzerland, Bern Jura, Col de Chasseral	RL	190629	4
23711	NAlp	Germany, Bayern, Chiemgauer Berge, Feichtenalm	RL	170626	26
23712	NAlp	Germany, Bayern, Chiemgauer Berge, Feichtenalm	RL	170626	26
23713	Nalp	Austria, Steiermark, Trenchtling, Edelweißboden	RL	170629	33
23715	NAlp	Germany, Bayern, Chiemgauer Berge, Feichtenalm	RL	170626	23
23716	Nalp	Austria, Steiermark, Trenchtling, Edelweißboden	RL	170629	33
23717	Nalp	Austria, Steiermark, Trenchtling, Edelweißboden	RL	170629	33
23718	Nalp	Austria, Steiermark, Trenchtling, Edelweißboden	RL	170629	33
23719	Nalp	Austria, Steiermark, Trenchtling, Edelweißboden	RL	170629	33
23720	Nalp	Austria, Steiermark, Trenchtling, Edelweißboden	RL	170629	33
23721	Nalp	Austria, Steiermark, Trenchtling, Edelweißboden	RL	170629	33
23722	Nalp	Austria, Steiermark, Trenchtling, Edelweißboden	RL	170629	34
23724	Nalp	Austria, Steiermark, Trenchtling, Edelweißboden	RL	170629	33

23727	SAlp	Italy, Trentino, Monte Baldo	MH/RL/GP	130708	26
23728	SAlp	Italy, Trentino, Monte Baldo	MH/RL/GP	130708	26
23729	SAlp	Italy, Trentino, Monte Baldo	MH/RL/GP	130708	45
23730	SAlp	Italy, Veneto, Pordoi, Ossario del Pordoi	MH/RL/CG	130712	22
23731	SAlp	Italy, Veneto, Pordoi, Ossario del Pordoi	MH/RL/CG	130712	45
23733	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	26
23734	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	26
23735	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	26
23736	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	32
23739	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	50
23740	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	31
23741	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	26
23742	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	26
23744	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	31
23745	SAlp	Italy, Südtirol, above Ütia Munt de Senes	MH/RL/CG	130715	26
23746	SAlp	Italy, Südtirol, Ütia Munt de Senes	MH/RL/CG	130715	31
23747	SAlp	Italy, Südtirol, Ütia Munt de Senes	MH/RL/CG	130715	30
23750	SAlp	Italy, Südtirol, Lungiarü, Peitler Wiesen	MH/RL/CG	130716	26
23753	SAlp	Italy, Südtirol, Lungiarü, Peitler Wiesen	MH/RL/CG	130716	26
23754	SAlp	Italy, Südtirol, Lungiarü, Peitler Wiesen	MH/RL/CG	130716	27
23755	SAlp	Italy, Südtirol, Lungiarü, Peitler Wiesen	MH/RL/CG	130716	36
23756	SAlp	Italy, Südtirol, Lungiarü, Peitler Wiesen	MH/RL/CG	130716	26
23772	Pyr	Andorra, Pyrenees, Col Botella - Port de Cabus	RL	190705	4
23892	SAlp	Italy, Südtirol, Senes airfield	MH/RL/CG	130715	51
23900	SAlp	Italy, Südtirol, Ütia Munt de Senes	MH/RL/CG	130715	29
23901	SAlp	Italy, Südtirol, Ütia Munt de Senes	MH/RL/CG	130715	40
23902	SAlp	Italy, Südtirol, Ütia Munt de Senes	MH/RL/CG	130715	39
23903	SAlp	Italy, Südtirol, Ütia Munt de Senes	MH/RL/CG	130715	46
24098	Carp	Romania, Dâmbovița, Moroeni, Bucegi Plaiul lui Păcală	NA	210630	58
24100	Carp	Romania, Dâmbovița, Moroeni, Bucegi Plaiul lui Păcală	NA	210630	58
24101	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Plaiul lui Păcală	NA	210630	58
24102	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Plaiul lui Păcală	NA	210630	57
24107	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Plaiul lui Păcală	NA	210630	59
24111	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Plaiul lui Păcală	NA	210630	56
24113	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Plaiul lui Păcală	NA	210630	58
24114	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Plaiul lui Păcală	NA	210630	58
24115	Carp	Romania, Dâmbovița, Moroeni, Bucegi, Plaiul lui Păcală	NA	210630	58

Table 4: Biometric data of *Nigritella nigra* subsp. *bucegiana* from Southern Carpathians and subsp. *iberica* from Jura. Length (L) and width (W) of 17 flower traits with mean value (mv), standard deviation (s) of measures [mm] of the grouped populations, 4 ratios and presence (1) / absence (0) of papillae at the bract border of n samples of single flowers (traits 27-93) and inflorescences (traits 14-15).

Species		*Nigritella nigra*							
subspecies		*bucegiana*				*iberica*			
provenance		Bucegi Prahova		Bucegi Dambovita		Jura Chasseral			
sample name		nbuc Buc 1		nbuc Buc 2		njur Chas W		njur Chas E	
no. samples [n flowers/spikes]		13	11	19	17	20	20	24	24
no.	character	mv	*s*	mv	*s*	mv	*s*	mv	*s*
14	inflorescence L	23,7	*1,8*	20,9	*3,8*	20,6	*2,9*	20,6	*2,9*
15	inflorescence W	20,5	*1,3*	18,6	*1,8*	21,0	*1,7*	20,9	*1,5*
61	petals L	5,9	*0,5*	6,0	*0,3*	6,8	*0,5*	6,9	*0,3*
62	petals W	1,6	*0,2*	1,6	*0,2*	1,2	*0,1*	1,2	*0,1*
63	lat. sepals L	6,6	*0,6*	6,6	*0,5*	8,0	*0,6*	7,7	*0,4*
64	lat. sepals W	2,4	*0,2*	2,3	*0,3*	2,1	*0,3*	1,9	*0,2*
23	med. sepal L	6,1	*0,6*	6,2	*0,5*	7,0	*0,5*	7,3	*0,5*
24	med. sepal W	2,1	*0,2*	1,9	*0,2*	1,6	*0,1*	1,6	*0,2*
27	lip L	6,1	*0,8*	6,0	*0,8*	8,0	*0,9*	8,2	*0,5*
28	lip W	4,8	*0,4*	5,0	*0,3*	5,0	*0,5*	4,6	*0,4*
29	Δ lip base-max.width	2,2	0,3	1,8	*0,3*	2,9	*0,4*	2,4	*0,3*
30	spur L	1,2	*0,1*	1,1	*0,1*	1,3	*0,1*	1,2	*0,1*
31	spur W	1,1	*0,1*	1,0	*0,2*	1,2	*0,1*	1,1	*0,2*
32	bract L	10,7	*1,4*	9,7	*1,6*	12,2	*0,9*	11,5	*1,3*
33	bract W	1,8	*0,3*	1,6	*0,3*	1,9	*0,3*	1,8	*0,2*
35	ovar L	3,9	*0,3*	3,5	*0,4*	4,6	*0,6*	4,5	*0,5*
36	ovar W	3,0	*0,5*	2,6	*0,3*	3,0	*0,4*	2,9	*0,3*
37	gynostemium L	1,9	*0,1*	1,8	*0,2*	2,0	*0,3*	1,8	*0,1*
38	gynostemium W	1,2	*0,2*	1,3	*0,2*	1,5	*0,1*	1,1	*0,2*
81	ratio petal L : W	3,7	*0,3*	3,8	*0,4*	5,5	*0,7*	6,0	*0,6*
82	ratio lat. sepal L : W	2,8	*0,3*	2,9	*0,2*	3,9	*0,5*	4,0	*0,4*
83	ratio med.sepal L:W	2,9	*0,3*	3,3	*0,3*	4,3	*0,5*	4,5	*0,6*
93	ratio lip L : W	1,3	*0,2*	1,2	*0,1*	1,6	*0,1*	1,8	*0,2*
39	papillae bract	0,17	*0,28*	0,13	*0,27*	0,01	*0,02*	0,00	*0,00*

Table 5: Biometric data of *Nigritella nigra* subsp. *iberica* from the Pyrenees (Catalunya, Andorra, Aragon). Length (L) and width (W) of 17 flower traits with mean value (mv), standard deviation (s) of measures [mm] of the grouped populations, 4 ratios and presence (1) / absence (0) of papillae at the bract border of n samples of single flowers (traits 27-93) and inflorescences (traits 14-15).

Species		*Nigritella nigra*							
subspecies		*iberica*							
provenance		Col de Creueta				Port Cabus, P. d'Envaliria		Cerler Ampriu	
sample name		nibe Creu 1		nibe Creu 2		nibe Ando		nibe Ampr	
no. samples [n flower/spike]		16	5	8	0	19	11	12	0
no.	character	mv	*s*	mv	*s*	mv	*s*	mv	*s*
14	inflorescence L	22,9	*2,3*	s.d.		22,4	*5,1*	s.d.	
15	inflorescence W	19,9	*0,5*	s.d.		20,8	*2,0*	s.d.	
61	petals L	6,7	*0,4*	6,6	*0,4*	6,5	*0,6*	6,1	*0,6*
62	petals W	1,3	*0,1*	1,3	*0,2*	1,2	*0,2*	1,3	*0,2*
63	lat. sepals L	7,5	*0,5*	7,7	*0,5*	7,3	*0,6*	7,2	*0,8*
64	lat. sepals W	2,1	*0,2*	2,2	*0,2*	1,9	*0,3*	2,0	*0,2*
23	med. sepal L	7,1	*0,6*	7,1	*0,6*	6,7	*0,6*	6,3	*0,5*
24	med. sepal W	1,8	*0,3*	1,9	*0,1*	1,8	*0,3*	1,9	*0,2*
27	lip L	7,7	*0,7*	7,9	*1,0*	7,6	*0,8*	7,4	*0,7*
28	lip W	4,5	*0,5*	5,0	*0,5*	4,7	*0,7*	4,9	*0,5*
29	Δ lip base-max.width	2,8	*0,4*	2,4	*0,3*	2,4	*0,4*	2,3	*0,4*
30	spur L	1,2	*0,2*	1,3	*0,1*	1,3	*0,2*	1,2	*0,1*
31	spur W	1,1	*0,1*	1,2	*0,2*	1,1	*0,2*	1,1	*0,1*
32	bract L	11,7	*1,1*	12,4	*1,2*	11,3	*1,4*	11,0	*1,1*
33	bract W	1,8	*0,4*	1,8	*0,5*	1,8	*0,4*	1,8	*0,3*
35	ovar L	4,9	*0,6*	4,5	*0,4*	4,4	*0,4*	3,9	*0,6*
36	ovar W	3,2	*0,5*	3,0	*0,5*	2,9	*0,4*	2,6	*0,5*
37	gynostemium L	1,8	*0,3*	2,0	*0,2*	1,8	*0,1*	1,7	*0,1*
38	gynostemium W	1,2	*0,2*	1,6	*0,2*	1,2	*0,3*	1,6	*0,4*
81	ratio petal L : W	5,3	*0,5*	5,0	*0,8*	5,4	*0,6*	4,8	*0,5*
82	ratio lat. sepal L : W	3,7	*0,4*	3,5	*0,2*	3,9	*0,6*	3,5	*0,3*
83	ratio med.sepal L: W	4,0	*0,5*	3,7	*0,4*	3,9	*0,6*	3,4	*0,3*
93	ratio lip L : W	1,7	*0,2*	1,6	*0,2*	1,6	*0,2*	1,5	*0,1*
39	papillae bract	0,01	*0,03*	0,03	*0,05*	0,02	*0,04*	0,01	*0,03*

Table 6: Biometric data of *Nigritella nigra* subsp. *austriaca* from the Eastern Alps and *N. rhellicani* from the Central Alps. Length (L) and width (W) of 17 flower traits with mean value (mv), standard deviation (s) of measures [mm] of the grouped populations, 4 ratios and presence (1) / absence (0) of papillae at the bract border of n samples of single flowers (traits 27-93) and inflorescences (traits 14-15).

Species	*Nigritella*									
subspecies	*nigra austriaca*						*rhellicani*			
provenance	Trenchtling		Bürgeralm Höchstein		Seiseralm Grunser B.		Rein Ochsenlenke		Puflatsch	
sample name	naus Tren 1, 2		naus Höch 1,2		naus SeisA 3		rhe Rein		rhe Puf s	
no. samples [n fl./spike]	39	24	30	30	38	28	24	24	27	27
no. character	mv	*s*	mv	*s*	mv	*s*	mv	*s*	mv	*s*
14 inflorescence L	19,4	*2,1*	18,8	*2,0*	19,4	*2,3*	19,6	*3,2*	22,5	*3,6*
15 inflorescence W	20,5	*1,7*	19,6	*1,6*	18,9	*1,7*	17,0	*2,4*	18,1	*1,7*
61 petals L	6,3	*0,4*	6,3	*0,3*	6,1	*0,7*	5,2	*0,6*	5,0	*0,4*
62 petals W	1,2	*0,1*	1,1	*0,1*	1,2	*0,1*	1,3	*0,2*	1,3	*0,2*
63 lat. sepals L	7,0	*0,5*	6,9	*0,4*	6,9	*0,7*	6,3	*0,7*	6,3	*0,5*
64 lat. sepals W	1,9	*0,2*	1,9	*0,1*	1,9	*0,2*	1,9	*0,3*	2,0	*0,2*
23 med. sepal L	6,8	*0,4*	6,8	*0,5*	6,6	*0,8*	5,9	*0,6*	5,7	*0,6*
24 med. sepal W	1,7	*0,3*	1,8	*0,2*	1,7	*0,2*	1,8	*0,3*	1,8	*0,2*
27 lip L	7,7	*0,6*	7,8	*0,4*	7,6	*1,2*	5,9	*0,8*	5,7	*0,6*
28 lip W	4,8	*0,4*	4,6	*0,3*	4,6	*0,6*	4,0	*0,6*	4,0	*0,5*
29 Δ lip base-max.w	2,3	*0,5*	2,6	*0,3*	2,4	*0,5*	1,8	*0,3*	1,8	*0,2*
30 spur L	1,2	*0,2*	1,2	*0,1*	1,2	*0,1*	1,3	*0,2*	1,3	*0,2*
31 spur W	1,0	*0,1*	1,0	*0,1*	1,1	*0,1*	1,0	*0,1*	1,0	*0,1*
32 bract L	11,6	*1,0*	11,1	*0,9*	10,6	*1,3*	9,5	*1,9*	10,1	*1,6*
33 bract W	1,9	*0,3*	1,7	*0,2*	1,8	*0,3*	1,8	*0,4*	1,8	*0,3*
35 ovar L	4,4	*0,4*	4,2	*0,6*	3,9	*0,4*	3,5	*0,4*	3,6	*0,5*
36 ovar W	2,8	*0,4*	2,5	*0,3*	2,7	*0,3*	2,6	*0,4*	2,9	*0,4*
37 gynostemium L	2,0	*0,2*	2,0	*0,1*	1,9	*0,2*	1,7	*0,2*	1,7	*0,2*
38 gynostemium W	1,2	*0,2*	1,2	*0,1*	1,1	*0,1*	1,0	*0,1*	1,1	*0,2*
81 ratio petal L : W	5,3	*0,7*	5,5	*0,5*	5,2	*0,9*	4,2	*0,8*	4,0	*0,6*
82 rat. lat.sepal L:W	3,6	*0,3*	3,7	*0,3*	3,6	*0,5*	3,3	*0,4*	3,2	*0,4*
83 rat. med.sep L:W	3,7	*1,0*	3,9	*0,6*	3,9	*0,6*	3,4	*0,6*	3,3	*0,5*
93 ratio lip L : W	1,6	*0,1*	1,7	*0,1*	1,7	*0,2*	1,5	*0,2*	1,4	*0,2*
39 papillae bract	0,02	*0,05*	0,14	*0,28*	0,12	*0,20*	0,63	*0,27*	0,70	*0,27*

Table 7: Summary of biometric data of *Nigritella nigra* s.l. from the Southern Carpathians, Eastern Alps, Jura, Pyrenees and *N. rhellicani* from the Central Alps. Means and SD of complete data of table 3, 4 and 5 for the single regions. Smaller values in comparison to subsp. *bucegiana* are marked in blue, greater values in red.

Species		*Nigritella nigra*								*Nigritella*	
subspecies		*bucegiana*		*austriaca*		*iberica*		*iberica*		*rhellicani*	
provenance		Carpathians Bucegi		East. Alps Stiria S-Tyrol		Jura Chasseral		Pyrenees Cat-And-Ara		Central Alps	
sample name		nbuc Buc		naus Tren , Höch , SeisA		njur Chas		nibe Creu, Ando, Ampr		rhe Rein, Puf s	
no. samples [n fl./spike]		32	28	107	58	44	44	55	16	51	51
no.	character	mv	s	mv	s	mv		mv	s	mv	s
14	inflorescence L	22,0	*3,4*	19,2	*2,2*	20,6	*2,9*	22,6	*4,3*	21,1	*3,7*
15	inflorescence W	19,3	*1,9*	19,6	*1,8*	21,0	*1,5*	20,5	*1,7*	17,6	*2,1*
61	petals L	5,9	*0,4*	6,3	*0,5*	6,8	*0,4*	6,5	*0,5*	5,1	*0,5*
62	petals W	1,6	*0,2*	1,2	*0,1*	1,2	*0,1*	1,3	*0,2*	1,3	*0,2*
63	lat. sepals L	6,6	*0,5*	6,9	*0,5*	7,9	*0,5*	7,4	*0,6*	6,3	*0,6*
64	lat. sepals W	2,3	*0,2*	1,9	*0,2*	2,0	*0,2*	2,0	*0,3*	2,0	*0,3*
23	med. sepal L	6,1	*0,6*	6,7	*0,6*	7,1	*0,5*	6,8	*0,6*	5,8	*0,6*
24	med. sepal W	2,0	*0,2*	1,7	*0,2*	1,6	*0,2*	1,8	*0,3*	1,8	*0,3*
27	lip L	6,1	*0,8*	7,7	*0,8*	8,1	*0,7*	7,6	*0,8*	5,8	*0,7*
28	lip W	4,9	*0,3*	4,7	*0,4*	4,8	*0,5*	4,7	*0,6*	4,0	*0,6*
29	Δ lip base-max.w	2,0	*0,3*	2,4	*0,5*	2,6	*0,4*	2,5	*0,4*	1,8	*0,3*
30	spur L	1,1	*0,1*	1,2	*0,1*	1,3	*0,1*	1,2	*0,2*	1,3	*0,2*
31	spur W	1,0	*0,1*	1,0	*0,1*	1,1	*0,2*	1,1	*0,1*	1,0	*0,1*
32	bract L	10,1	*1,6*	11,1	*1,2*	11,8	*1,2*	11,5	*1,3*	9,8	*1,7*
33	bract W	1,7	*0,3*	1,8	*0,3*	1,8	*0,3*	1,8	*0,4*	1,8	*0,3*
35	ovar L	3,7	*0,4*	4,2	*0,5*	4,5	*0,5*	4,5	*0,6*	3,6	*0,5*
36	ovar W	2,7	*0,4*	2,7	*0,4*	2,9	*0,4*	2,9	*0,5*	2,7	*0,4*
37	gynostemium L	1,8	*0,2*	2,0	*0,2*	1,9	*0,2*	1,8	*0,2*	1,7	*0,2*
38	gynostemium W	1,2	*0,2*	1,1	*0,2*	1,3	*0,2*	1,3	*0,3*	1,1	*0,1*
81	ratio petal L : W	3,7	*0,4*	5,3	*0,7*	5,8	*0,7*	5,2	*0,6*	4,1	*0,7*
82	rat. lat.sepal L:W	2,8	*0,3*	3,6	*0,4*	4,0	*0,5*	3,7	*0,5*	3,2	*0,4*
83	rat. med.sep L:W	3,1	*0,4*	3,8	*0,7*	4,4	*0,6*	3,8	*0,5*	3,4	*0,6*
93	ratio lip L : W	1,2	*0,1*	1,7	*0,1*	1,7	*0,2*	1,6	*0,2*	1,5	*0,2*
39	papillae bract	0,15	*0,27*	0,09	*0,20*	0,00	*0,02*	0,01	*0,03*	0,66	*0,27*

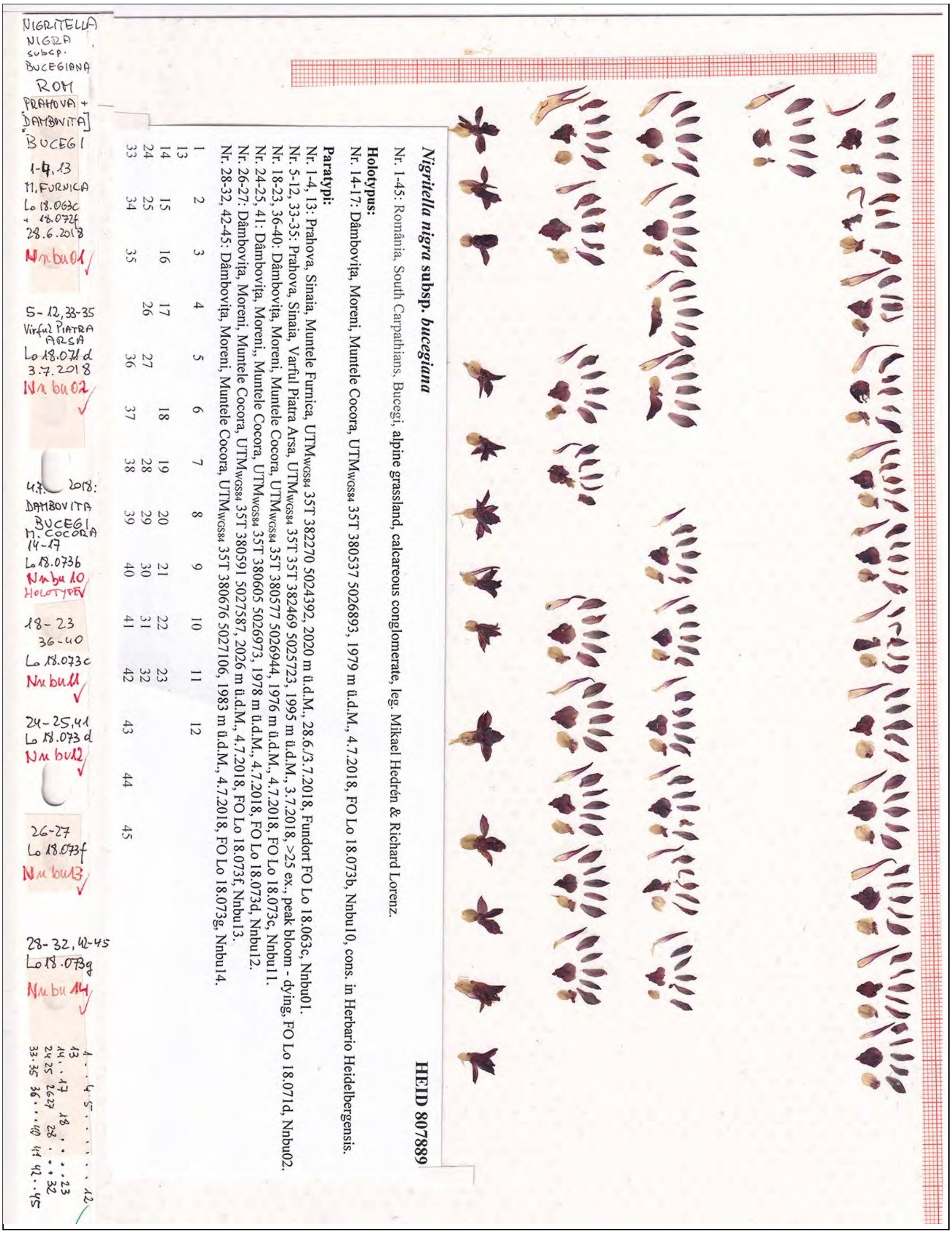


Fig. 45: Type specimen of *Nigritella nigra* subsp. *bucegiana* with Holo- and Paratypes. România, South Carpathians, Dambovita, Moroeni, Bucegi mountains, leg. Mikael Hedrén & Richard Lorenz. No. 14-17 represents the holotype, all others paratypes. Conservantur in herbario Heidelbergensis sub no. HEID 807889.

Fig. 46: Flower analyses of *N. nigra* subsp. *bucegiana*: Line 1-5, Bucegi (Nnbu1-02, 10-14). *N. nigra* subsp. *austriaca.* Line 6-8, Seiseralm (Naus30a-30b).

Fig. 47: Flower analyses of *N. nigra* subsp. *austriaca*: Line 1-3, Trenchtling, Terra typica (Naus02a-02b); line 4-8, Höchstein (Naus 11a-11a2).

Fig. 48: Flower analyses of *N. nigra* subsp. *iberica*: Line 1-4, Chasseral (Nnjur01-03). *N. rhellicani*: Line 5-8, Südtirol, Rein in Taufers (Nrhe10-10a).

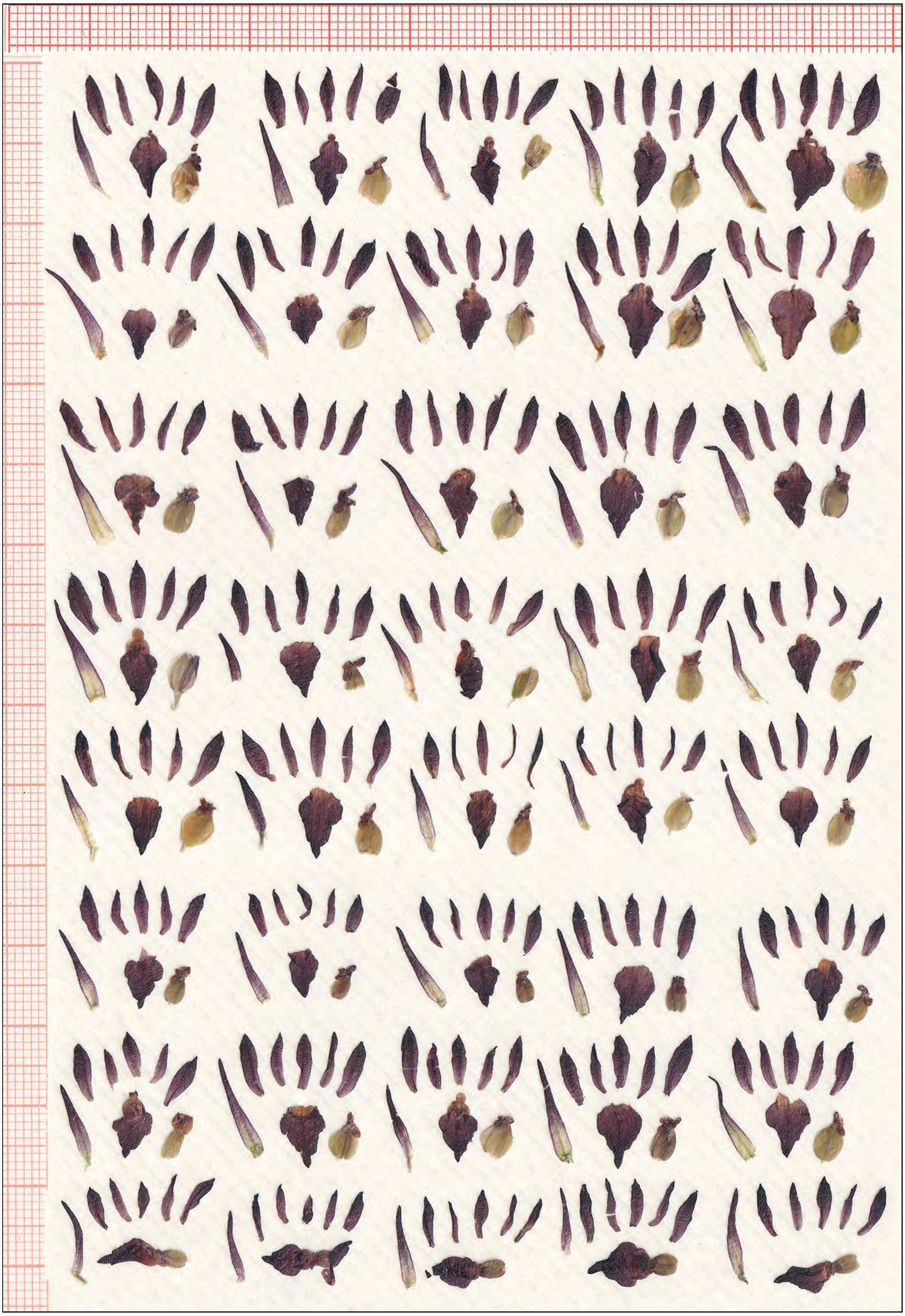


Fig. 49: Flower analyses of *N. nigra* subsp. *iberica*: Line 1-2, Col Creueta (Nibe 10-10b); line 3-5, Andorra (Nibe 15-17); line 6-8, Ampriu, Terra typica (Nibe 25).

Acknowledgements

We thank Vincent and Christoph Boillat, Heinz Heltmann, Rudolf Rösler, Hermann Voelckel, Helmuth Zelesny for providing information on *Nigritella* sites, Karl Redl, David Ståhlberg, Erich Klein†, Ulrich Heidtke, Norbert Griebl, Jean-Mark Lewin, Olivier Gerbaud, and Branko Dolinar for providing valuable comments and/or supplementary samples used for molecular analysis.
We would like to thank Mr. Colțoiu Alexandru, Director Bucegi Natural Park Administration, National Directorate of Forests – Romsilva, for permission to investigate the orchid flora present in the Park (Research Permit no. 1887/CAN/22.07.2021). We would also like to express our gratitude to Adrian Ruicănescu, Klaus von der Dunk, Corneliu Pârvu, Melania Stan and Szekely Levente for helping with insect identification.

Author contributions

In this study, MH carried out DNA extractions, performed laboratory analyses, and summarized the genetic data. RL compiled morphometric data and contributed summary statistics on morphological differentiation. NA contributed material and provided general knowledge about the populations from the Southern Carpathians. All authors evaluated the results, contributed to the text and figures and approved on the final manuscript.

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