

The genus *Nigritella* (Orchidaceae) in the Iberian Peninsula

by

Llorenç Sáez

Unitat de Botànica, Facultat de Ciències, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Barcelona, Spain. llorens.saez@uab.es

Abstract

As a result of the revision of *Nigritella* L.C.M. Richard in the Iberian Peninsula, here we recompile information of its variability, taxonomy, nomenclature and chorology. Two taxa are recognized: *Nigritella austriaca* subsp. *iberica* (Teppner & E. Klein) L. Sáez, comb. nov. and *N. gabasiana* Teppner & Klein, and the presence of *N. corneliana* is excluded. Detailed phytodermologic analysis showed that size of guard cells is useful for species identification.

Key words: chorology, Iberian Peninsula, leaf surface, *Nigritella*, taxonomy.

Resumen

Tras la revisión del género *Nigritella* L.C.M. Richard en la Península Ibérica, se aportan datos sobre la variabilidad, taxonomía, nomenclatura y corología de sus diferentes especies. Se reconocen dos táxones: *Nigritella austriaca* subsp. *iberica* (Teppner & E. Klein) L. Sáez, comb. nov. y *N. gabasiana* Teppner & Klein, y se excluye la presencia de *N. corneliana*. El análisis fitodermológico indica que el tamaño de las células oclusivas es un carácter útil para la identificación de ambas especies.

Palabras clave: corología, *Nigritella*, Península Ibérica, superficie foliar, taxonomía.

Introduction

The genus *Nigritella* L.C.M. Richard (Orchideae, Orchidaceae) includes about a dozen species that are distributed over the mountain areas of central and southern Europe (Alps, Apennines, Carpathians, Balkans, Pyrenean and Cantabrian mountains) and Scandinavia (Teppner, 1996; Teppner & Klein, 1998; Delforge, 2001). This genus constitutes a polyploid complex with a diversity nucleus in the Alps (Hedrén & al., 2000). Studies by Teppner & Klein over the last two decades have greatly contributed to the knowledge of this genus (Teppner & Klein, 1985a, 1985b, 1990, 1993, 1998; Teppner, 1996; Klein & Drescher, 1996; Teppner & Ster, 1996; Hedrén & al., 2000).

Most of the *Nigritella* species have a similar habit and exhibit marked similarities in macromorphological traits, which makes it difficult to identify them in the field (Bournérias & al., 1998; Brüttsch, 2000). Here we clarify some taxonomic and chorologic aspects of the genus *Nigritella*, since there is still information lacking on the identity and the distribution of several species in the Iberian Peninsula.

Material and methods

This revision was based mainly on herbarium material (102 specimens), including types, from the following herbaria (abbreviations according to Holmgren & al., 1990 and Holmgren & Holmgren 1993): ARAN, BC, BCB, BCF, BCN, JACA, and MA. Features of gross morphology were studied under a binocular stereoscopic microscope. Those concerning floral pieces were examined on flowers from the basal 1/3 of the inflorescence. Measurements of flowers were made from fresh plants (18 specimens) or on rehydrated herbarium material (flowers were submerged 5 minutes in boiling water). We have not been able to found significant differences between measures made from fresh plants and those made on rehydrated specimens.

For scanning electron microscopy (SEM) observations, the seeds samples were glued to aluminium stubs, coated with 50 nm gold film and examined in a Hitachi-2300S scanning electron microscope at 15 kV.

For epidermic studies, dried leaves were rehydrated in water (leaves were submerged in water for 24-48 hours, depending on their conservation condition)

and decolored for 3-8 hours in a commercial solution of sodium hypochlorite until totally transparent (Stace, 1965). After washed with distilled water, the adaxial and abaxial epidermic surfaces were separated and stained in an alcoholic solution of Bismarck brown at 2% for 12 hours. They were then dehydrated through an alcohol series, placed in xylol, mounted in Canada Balsam and studied under a Zeiss-Universal microscope equipped with a camera lucida. Twenty measures of stomatal guard cells were done for each accession. Epidermic data on *N. gabasiana* and *N. austriaca* subsp. *iberica* were obtained from 53 and 19 individuals respectively (Table 1).

For the geographical distribution, data is given from herbarium sheets which document the occurrence of each species for each of the provinces of the study area, according to the criteria used in *Flora iberica* (Castroviejo & al., 1986). Nevertheless, additionally reliable chorological data can be consulted in Teppner & Klein (1993).

Results

Taxonomic characters

The main morphological characters studied are listed below. Their potential taxonomical value is discussed.

Macromorphologic features

Inflorescences. All taxa have flowers in a dense terminal spike. In *N. gabasiana*, the inflorescence is subconical in flower and ovate to subcylindrical in fruit. In *N. austriaca* subsp. *iberica* the spikes are subconical or hemispherical in flower, and become subovate in fruit. Nevertheless, the size and shape vary among individuals and populations, and in my opinion this character cannot be used for species identification.

Flower colour. The basic flower colour of these two species is dark red-brown to reddish-violet. Nevertheless, some plants differ, e.g., a yellow-flowered specimen of *N. gabasiana* from the province of Huesca, Central Pyrenees (JACA 142698).

Labellum. The shape and size of the labellum has been used very often as a discriminatory feature in identifying several species of the genus (Teppner & Klein, 1985a, 1985b, 1990; 1993; Brüttsch, 2000; Delforge, 2001). Thus, *Nigritella gabasiana* is characterised by its labellum, which is only slightly open, narrow at its basal third, and has recurved margins that almost touch each other (Fig. 4). Regarding the size of the labellum, *N. austriaca* subsp. *iberica* and *N. gabasiana* show few differences and noticeably overlap. Nevertheless, the labellum of *N. austriaca* subsp. *iberica* can reach 10.2 mm in length, whereas in *N. gabasiana* it is less than 9.1 mm long.

Bracts. The occurrence of denticles (and their size and shape) in the margin of the lowermost bracts of the inflorescence has been used as a diagnostic character (Teppner & Klein, 1990; Bournérias & al., 1998; Brüttsch, 2000; Delforge, 2001). On the basis of my observations, I conclude that bract denticulation is a character that should be used with caution. Smooth basal bracts (sometimes irregularly denticulate) are common in *N. austriaca* subsp. *iberica*. However, some specimens (MA 295050) bear irregularly denticulate lowermost bracts (denticles up to 0.03 mm long). Bracts of *N. gabasiana* are usually denticulate, with subconical denticles up to 0.05 mm long. The presence of this denticulation, at least in the middle of the bract, is a characteristic feature of this species according to several authors (Bournérias & al., 1998; Delforge, 2001). A detailed study of the bracts from herbarium material and 27 live plants revealed that some specimens of *N. gabasiana* bear slightly denticulate (JACA 10097272) or even smooth bracts (MA 622648, JACA 787987, JACA 141097). Furthermore, I found that this character is variable even within a single population. In the localities of Serra d'Ensiija and Coll de la Creueta (Eastern Pre-Pyrenees) plants with denticulate basal bracts, and other specimens with smooth or slightly denticulate ones are found in the same locality. The plants of Coll de la Creueta were cytologically determined as diploids and had a chromosome number of $2n = 40$.

Table 1. Leaf surface features of Iberian *nigritella* species. Minimal (Min.) and maximal (Max.) values, means (\bar{x}) and their standard deviations (sd); n = number of measurements. Intermediate plants were excluded

Taxa	Stomatal Length (μm)		Cells/ mm^2	
	$\bar{x} \pm sd$	Min.-Max.	$\bar{x} \pm sd$	Min.-Max.
<i>N. gabasiana</i> (53 specimens)	54.18 \pm 3.03 ($n = 1060$)	45-60	516.5 \pm 52.3 ($n = 106$)	383.3-641.6
<i>N. austriaca</i> subsp. <i>iberica</i> (19 specimens)	65.68 \pm 3.11 ($n = 380$)	57-75	328.8 \pm 30.2 ($n = 38$)	283.3-383.3

Phyto dermologic features

Main phyto dermologic features of Iberian *Nigritella* leaves are as follows:

Shape of cells. The epidermic cells are, in most cases, polygonal, with straight anticlinal walls on adaxial leaf surface (more or less straight on abaxial face). The thickness of these anticlinal walls varies from 8 to 13 m on the adaxial surface (4 to 10 m on abaxial surface) in *Nigritella gabasiana*. In *N. austriaca* subsp. *iberica* these values range from 7 to 14 m (4-12 m on abaxial surface).

Stomata. The *Nigritella* species bear hypoamphistomatic leafs. All the samples present anomocytic stomata (Fig. 1). On the adaxial surface, stomata are restricted to the apical area of the leaf (5-15 mm from the top) and near the margins on the upper part of the leaf.

Size of guard cells. For each species, the mean values of stomatal guard cell length from the abaxial surface are presented in Table 1. The guard cells of *Nigritella austriaca* subsp. *iberica* are longer than those of *N. gabasiana* (Fig. 2, Tab. 1). The use of guard cell length to infer the ploidy level of specimens has been successfully applied in many groups of plants (Evans, 1955; Barrington & al., 1986; Borrino & Powell, 1988; Viane, 1990). However, to our knowledge, this character has never been used in *Nigritella*. The predictive value of the phyto dermological data to check the ploidy level is useful in the species of *Nigritella* found in the Iberian Peninsula, and probably in the whole genus.

In some Pyrenean specimens (Candanchú, JACA 270272; Sallent de Gallego, ARAN 59604; Añisclo, JACA 610874; Torla, JACA 135578) the results obtained from the stomatal guard cells and cell frequency are difficult to evaluate. These samples bear stoma-

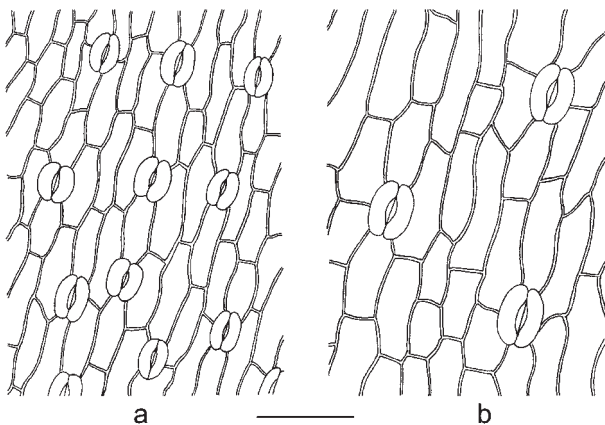


Fig. 1. Light microscopy view of the adaxial leaf epidermis of Iberian species of *Nigritella*. **a**, *N. gabasiana*: Barcelona, Serra d'Ensiya (LS-5955); **b**, *N. nigra* subsp. *iberica*: Huesca, Castanesa (MA 528527). Bar = 100 μ m.

ta with intermediate lengths between those of the two species examined in this study. In addition, cell density also shows intermediate values. Thus, we cannot rule out that some of these specimens may be hybrids, presumably triploids, since the putative parents occur in the same location or at least in neighbouring areas. Partial sexuality in apomictic plants of the *N. nigra* group has been reported by Teppner (1996).

Cell frequency. Cell frequency (cells/mm²) is higher in the diploid *N. gabasiana* (Table 1). This can be explained by the polyploidy nature of *N. austriaca* subsp. *iberica*: polyploids generally present larger cells as the level of ploidy increases. Cell frequency can be used to differentiate between diploid and tetraploid populations, at least in the Pyrenees.

Seeds

The seeds of the two species are very similar in shape and size (Fig. 3, Tab. 2). *Nigritella gabasiana* seeds measure 0.25-0.37 \times 0.15-0.25 mm, with testa cells corresponding to 1/3 or 1/2 of the chalaza side, nearly isodiametrical or up to twice as long as width, with anticlinal walls that are not sinuous. Seeds of *N. austriaca* subsp. *iberica* are slightly smaller (0.23-0.36 \times 0.15-0.22 mm), with testa cells corresponding to 1/3 or 1/2 of the chalaza side, usually twice as long as width, and with sinuous anticlinal walls.

Taxonomic status of Iberian *Nigritella*

Although the Iberian *Nigritella* taxa exhibit marked similarities in macromorphological traits, some morphological and phyto dermological characters can be used to distinguish diploid from tetraploid plants. *Nigritella gabasiana* is a diploid, sexual, species (Teppner & Klein, 1993; Teppner, 1996), closely related to the diploid endemic species of the Balcans *N. rhelli-*

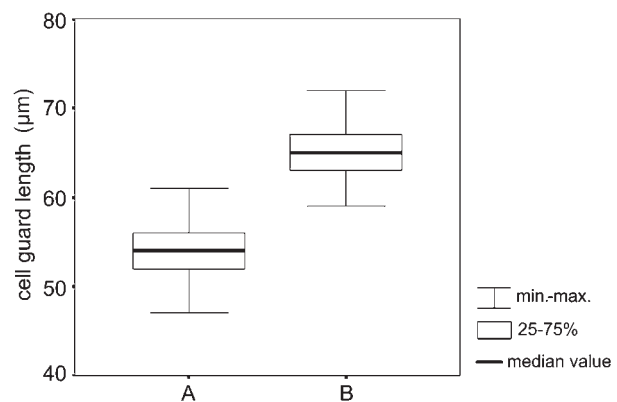


Fig. 2. Boxes plots of cell guard length of *Nigritella gabasiana* (A) and *N. nigra* subsp. *iberica* (B).

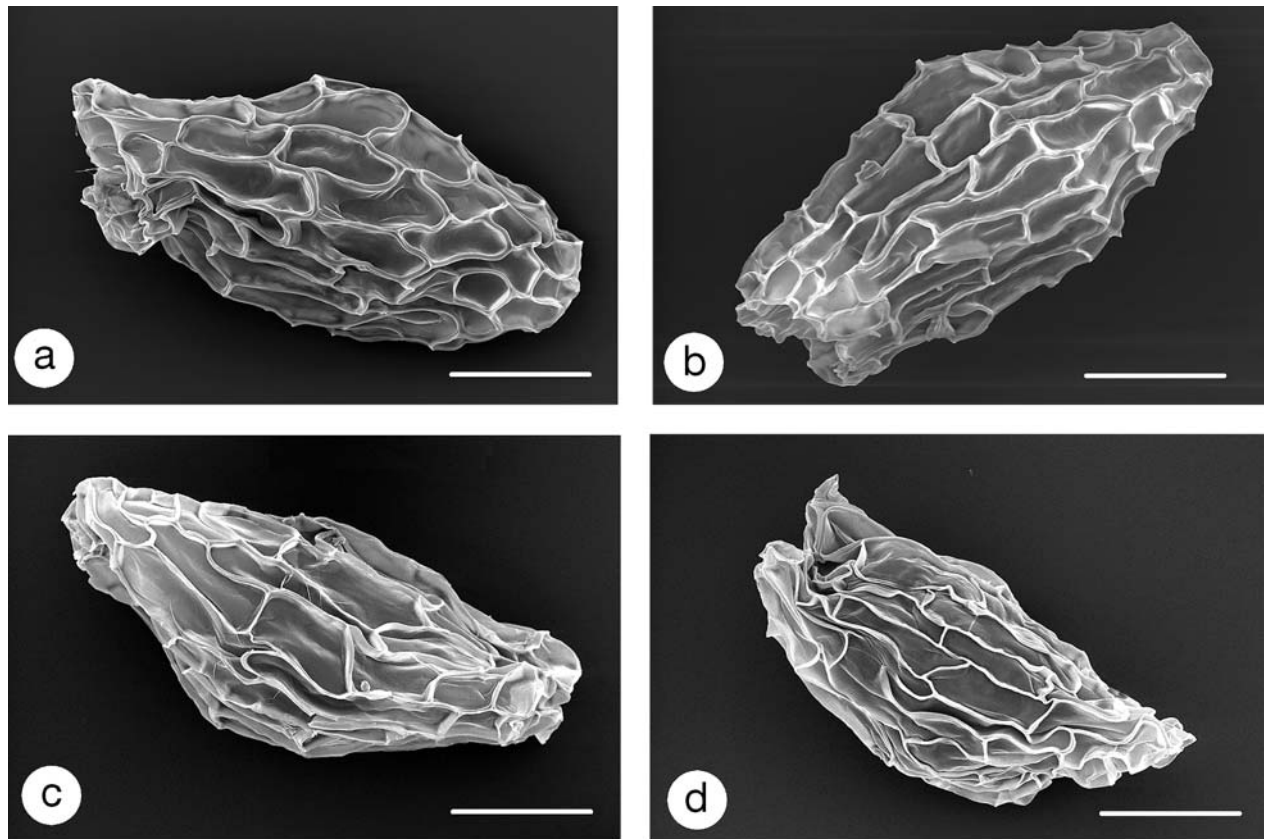


Fig. 3. Scanning electron micrographs of seeds of *Nigritella gabasiana* (**a, b**) and *N. nigra* subsp. *gallica* (**c, d**); **a**: Navarra, Peña Ezkaurre (JACA 539667); **b**: Girona, Vall de Ribes, vessants del Taga (BC 146088); **c**: Huesca, cerca del lago Bacibé, Castanesa (JACA 160382); **d**: Huesca, Torla (JACA 353292). Bars = 100 μ m.

Table 2. Seed features of Iberian *Nigritella* species. Minimal (Min.) and maximal (Max.) values, (\bar{x}) and their standard deviations (sd); n = number of measurements. Measurements are given in mm

Taxa	Seed Length		Seed width	
	$\bar{x} \pm sd$ (n)	Min.-Max.	$\bar{x} \pm sd$ (n)	Min.-Max.
<i>N. gabasiana</i> (12 specimens)	0.31 ± 0.02 ($n = 120$)	0.25-0.37	0.20 ± 0.02 ($n = 120$)	0.15-0.25
<i>N. austriaca</i> subsp. <i>iberica</i> (5 specimens)	0.29 ± 0.03 ($n = 50$)	0.23-0.36	0.19 ± 0.02 ($n = 50$)	0.15-0.22

canii Teppner & Klein (Hedrén & al., 2000). *Nigritella rbellicani* is readily distinguished from *N. gabasiana* by its slightly narrowed labellum in its basal third, which is usually smaller (4.5-7 mm long), and its basal bracts, with denticles 0.05-0.1 mm long.

The *Nigritella nigra* group includes three polyploid taxa that reproduce apomictically by adventitious embryos (Teppner, 1996). *Nigritella nigra* subsp. *nigra* is a triploid restricted to Scandinavia (Teppner & Klein, 1990). On the other hand, *N. nigra* subsp. *austriaca* Teppner & E. Klein and *N. nigra* subsp. *iberica* are tetraploids (Teppner & Klein, 1990, 1993).

The splitting of the *N. nigra* group into discrete geo-

graphical entities (usually recognized at the subspecific level) is supported by constant, karyological, morphological and molecular features (Teppner & Klein, 1993; Hedrén & al., 2000). *Nigritella nigra* subsp. *iberica* is closely related to *N. nigra* subsp. *austriaca*, which occurs in the middle part of the Eastern Alps (Teppner & Klein, 1990). Some authors merged these two tetraploids within a single taxon, *N. austriaca* (Delforge & al., 1991; Bournérias, 1998; Brüttsch, 2000), or recognized them within *N. nigra* at subspecific level (Breiner & Breiner, 1993; Teppner & Klein, 1993) or within *N. austriaca* at varietal rank (Delforge, 2001) Morphological discontinuities (mainly of quan-

titative nature) and the allopatric distribution of *N. nigra* s. str., *N. nigra* subsp. *iberica* and *N. nigra* subsp. *austriaca*, and molecular data on the origin of the tetraploid plants through hybridization between a triploid taxon closely related to *N. nigra* subsp. *nigra* and several diploid species (cf. Hedrén & al., 2000: 263) supports the recognition of a triploid (*N. nigra*) and a tetraploid taxon (*N. austriaca*) at specific rank. I conclude that this division better reflects the phylogenetic relationships of this polyploid complex. The two tetraploid taxa probably originated separately within their present distribution. The western populations of *N. austriaca* were probably the result of hybridization between a triploid species and a western diploid species, whereas in eastern populations, the diploid was genetically different (Hedrén & al., 2000). Accordingly, the western and eastern populations constitute morphologically recognisable groups, despite their putative hybrid origin. In this context, recognition of subspecific taxa within a single species (*N. austriaca*) is a better reflection of the morphological variation according to biogeographic patterns. Therefore, we propose the following change in nomenclature:

Nigritella austriaca* subsp. *iberica (Teppner & E. Klein) L. Sáez, **comb. nov.**
Nigritella nigra subsp. *iberica* Teppner & E. Klein in Phytion (Horn) 33(2): 192. 1993

Taxonomic treatment

Nigritella Rich., Orch. Eur. Annot.: 26. 1817
Gymnadenia subgen. *Nigritella* (Rich.) O. Gerbaud & P. Quentin in Nat. Belg. 80(3): 374. 1999

Perennials, herbaceous, autotrophs. Tubers 2, digitate. Stem erect, simple, slightly angled, green, with basal leaf sheaths, glabrous. Leaves linear to linear-lanceolate, acute (the lowermost are sometimes subobtusate), with denticulate or papillate margins, spirally arranged, sheathing, green, decreasing in size up to stem, the uppermost bract-like, non-sheathing. Inflorescence a dense many-flowered terminal spike, subconical to cylindrical, erect. Bracts lanceolate to linear-lanceolate, acute, green to green-reddish or green-violet. Flowers not resupinate, patent, sessile. Sepals more or less erect, subequal, free. Lateral petals resembling sepals; labellum subtriangular to ovate-lanceolate, entire or slightly crenulate; spur short, saccate, obtuse. Column short; rostellum with an elongated median fold. Fertile stamen 1; anther terminal; pollinia 2, sessile, viscidia small, circular, approximate on a horizontal platform-like structure, without bursicles; pollen

grains in tetrads. Fruit a capsule, erect, oblong or subglobose, 6-keeled. Seeds numerous, minute, reticulate.

Key to taxa of *Nigritella* species in the Iberian Peninsula

1. Labellum slightly open, strongly narrowed in its basal third, and with recurved margins which almost touch each other; lowermost bracts usually denticulate, at least half of its length; flowers slightly aromatic or without fragrance **1. *N. gabasiana***
1. Labellum distinctly open, slightly narrowed in its basal third, not touching each other; lowermost bracts usually smooth or slightly denticulate; flowers vanilla-scented **2. *N. austriaca* subsp. *iberica***

1. *Nigritella gabasiana* Teppner & E. Klein in Phytion (Horn) 33(2): 182. 1993. *Gymnadenia gabasiana* (Teppner & E. Klein) Teppner & E. Klein in Phytion (Horn) 38(1): 221. 1998

Ind. loc.: “Holotypus: Zentral-Pyrenäen, Prov. Huesca, oberstes Rio Gallego-Tal, S.Hänge der Peña Foradada oberhalb El Formigal, ca. 1740 m, Kalk, Rasen ...; 13.7.1992; leg. H. Teppner & E. Klein (P).- Isotypen: GZU, MA”.

Type: Spain. Huesca, Peña Foradada, El Formigal, 13 July 1992, Teppner & Klein s.n. (holotype, P; isotypes, GZU, MA 528528!).

Nigritella nigra auct. [non (L.) Kirschl., Prodr. Fl. Alsace: 159. 1836]

Nigritella angustifolia auct., non Rich.

Illustrations: Fig. 4.

Tubers 12-30(45) × 8-20 mm. Stems 8-35 cm. Leaves 35-180 × 2.5-10 mm. Basal bracts 7-17 × 1.5-3 mm, acute, lanceolate to linear-lanceolate; margin usually with subconical denticles 0.01-0.05 mm long. Inflorescence 12-38 × 15-32 mm, with 16-52 flowers, subconical in flower; ovate to subcylindrical in fruit. Flowers deep red, red-violet to blackish-red, rarely yellow, slightly aromatic or without fragrance. Sepals oblanceolate, elliptical-lanceolate or lanceolate; lateral sepals (6)6.8-8.5(9) × (1)1.4-2(3) mm; middle sepal (5)6.3-8 × 1.1-2.1(2.5) mm. Lateral petals (4.7)6-8.5 × 0.8-1.5(2,3), lanceolate, acute, glabrous; labellum (6.5)7-9.1 × 4-4.5 mm, slightly open, strongly narrowed in its basal third, and with recurved margins which are almost touch, glabrous; spur 0.8-1.4 × 0.8-1 mm. Column (1.3)1.5-1.8 mm. Anther 1-1.6 mm. Gynoecium 2-3 × 1.5-2 mm. Capsule 3-5 × 2-3.3 mm, oblong. Seeds 0.25-0.37 × 0.15-0.25 mm.

Chromosome number: 2n = 40 (Teppner & Klein, 1993).

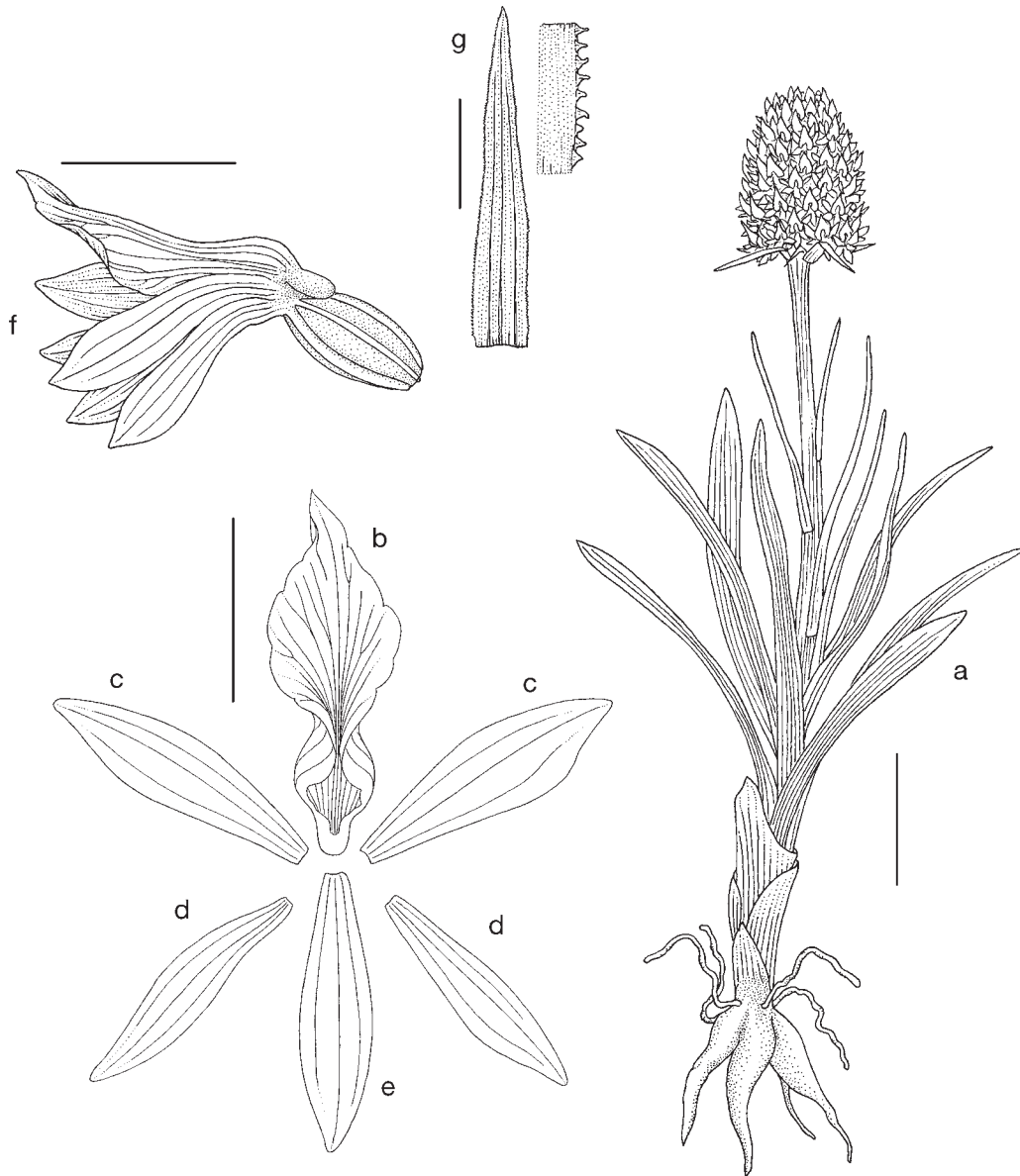


Fig. 4. *Nigritella gabasiana*: a, g (Girona: Fontlletera, pr. Tragurá, BC 60644), b-f (Barcelona, Serra d'Ensija, LS-5955, BCB). **a**, habit; **b**, labellum; **c**, outer sepal; **d**, lateral petal; **e**, middle sepal; **f**, flower; **g**, bract. Bars: a = 2 cm; b, c, d, e, f, g = 5 mm.

Distribution: Endemic to the Pyrenean and Cantabric mountains (Fig. 5).

Habitat: Montane to alpine meadows; clearings in open *Pinus uncinata* and *Betula* woodland, sometimes in rocky limestone sites; 1250 to 2550 m.

Phenology: Flowering June-August.

Representative specimens

ANDORRA. Andorra, VI-1933, *Gallardo s.n.* (BC 125426); Arinsal, coma Llempa, CH7414, 1900 m, 10-VIII-1978, *Fillat & Villar s.n.* (JACA 325678); Llacs dels Pessons, 3-VII-1980, *Soriano s.n.* (BCN 5373). FRANCE. Gèdre, Hautes Pyrénées, V-1872, *Bordère*

s.n. (BC 610293), De Gavarnie a la base del Vignemale, 1820 m, VII-1991, *Sáez s.n.* (BCB); Pic Carlit, 13-VII-2002, *Galbany & al. s.n.* (BCB); vessant NE del Tuc d'Hormiguet, 2000 m, CH4638, 18-VII-1997, *Sáez & al. s.n.* (BCB); Montanha dehs Cauçís, val Peiralada, 2350 m, CH4640, 19-VII-1997, *Sáez & al. s.n.* (BCB); Vall d'Eyne, sota el vessants del Serrat de les Fonts, DG2898, 2280 m VII-2003, *Font s.n.* (BCB); Montroig, vessant N, c. Tuc Laquet, CH5031, 2200 m, VII-2003, *Font. s.n.* (BCB). SPAIN. Asturias: Cabrales, Cuesta Sierra, UN5586, 1700 m, 1-VIII-1980, *García González s.n.* (JACA 520285). Barcelona: Rasos de Peguera, VII-1895, *Costa s.n.* (BC 675935); Pyrénées à Montgrony, pâturages du Puigalt, 2000 m, 9-VII-1914, *Sennen Pl. Espagne 2060* (MA 24326; BCN 5391); Alt Berguedà, Baga dels Serrats, DG17, 1680 m, 3-VII-1975, *Rosell s.n.* (BC 621952); Alt Berguedà, Bagà, Plans de Canells, DG18, 1950 m,

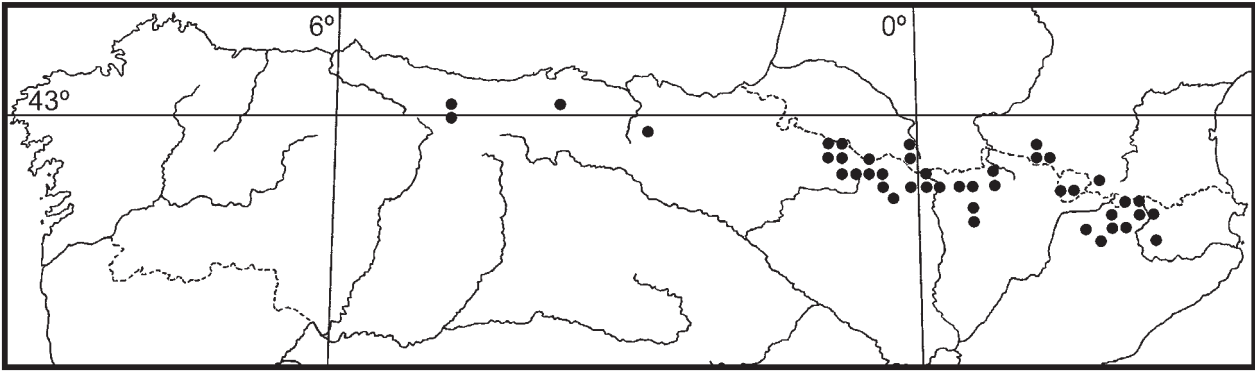


Fig. 5. Distribution map of *Nigritella gabasiana* in the Iberian Peninsula based on herbarium specimens.

10-VII-1982, *Soriano s.n.* (BCN 5380); Serra d'Ensija, CG9571, 2315 m, 7-VII-2002, *Sáez LS-5955* (BCB). **Burgos:** Espinosa de los Monteros, cerca del Portillo de la Lunada, VN469802, 1350 m, *Marín & Domingo s.n.* (MA 639613). **Cantabria:** Portillo de Lunada, San Roque de Río Miera, VN48, 28-VII-1983, *Aedo s.n.* (MA 611466); Fuente de la Begerina, Camaleño, UN5075, 1760 m, 18-VII-1991, *Aedo s.n.* (MA 622248). **Girona:** entre Pla Traver y Puigsacalm, VI-1912, *Bolós s.n.* (BC 145366); Fontlletera, pr. Tragurá, 22-VII-1922, *Cuatrecasas s.n.* (BC 60644); Ribes de Freser, vessants del Taga, al capdamunt de la Vallfèra, 1750 m, 18-VII-1961, *Vigo s.n.* (BC 146088); de Núria a Sant Amadeu, 2100 m, 1-VII-1977, *A. Carrillo & J.M. Ninot s.n.* (BCN 5375); coll de la Creueta, 17-VII-2002, *Galbany & al. s.n.* (BCB). **Huesca:** Aísa, Candanchú, Valle de Aspe, XN9939, 1900 m, 12-VIII-1965, *Montserrat s.n.* (JACA 144665); Aísa, alto hacia Rioseta, YN0138, 1800-1900 m, 11-VII-1967, *Montserrat s.n.* (JACA 397367A); Aísa, Tobazo, Tortiella Alto, YN0037, 1900-2000 m, 12-VII-1970, *Montserrat & Villar s.n.* (JACA 327270); Ansó, Hoya del Solano, XN8254, 1650-1700 m, 17-VIII-1972, *Villar s.n.* (JACA 10146372, JACA 10148572); Canal Roya, Canfranc, YN0641, 1700 m, 22-VI-1973, *Fillat s.n.* (JACA 217373); Siresa, umbría Punta Cuta, XN8939, 1600 m, 11-VII-1974, *Villar s.n.* (JACA 10030974); umbrías de Pico Agüerri, XN8843, 1600 m, 25-VII-1974, *Villar s.n.* (JACA 10076974); Balcón de Pineta, Bielsa, BH5830, 1700-2300 m, 14-VII-1978, *Montserrat s.n.* (JACA 206478); Peña Telera, Biescas, YN1529, 2200-2500 m, 25-VIII-1978, *Villar s.n.* (JACA 391278); Borau, puerto de Arisa, Lecherines, XN9935, 2300 m, 11-VII-1985, *Montserrat & al. s.n.* (JACA 238385); Sallent de Gallego, Formigal, 1450 m, 21-VI-1986, *Garín s.n.* (ARAN 34480); Anso-Fago, Linza-Hiru Erregen Mahaia, XN8453, 2000 m, 22-VII-1986, *Aizpuru & Catalán s.n.* (ARAN 34478); entre el barranco de San Adrián y fuente de la Muria, Bissaurri, BH9601, 1650-1850 m, 5-VII-1987, *Sesé & al. s.n.* (JACA 691787); Turbón, la Plana, BG9499, 1880-2000 m, 25-VI-1987, *Sesé s.n.* (JACA 787987); del refugio Ángel Orus a las crestas del Forcau, BH92, 2150-2450 m, 20-VII-1987, *Galán Cela & al. PG2826b* (MA 632769); Benasque, CH1025, 2300-2550 m, 18-VIII-1995, *Ferrández & Sesé s.n.* (JACA 232295); Fanlo-Nerín, BH5421, 2000 m, 11-VI-1997, *Gómez s.n.* (JACA 120197); Yebra de Basa, YN2311, 1580-1610 m, 22-VI-1997, *Montserrat s.n.* (JACA 141097); Tella-Sin Escuaín, BH6322, 1900-1950 m, 11-VII-2000, *Benito & Goñi s.n.* (JACA 273783); Fanlo, Plana del Descargador, YN4427, 2250-2300 m, 20-VII-2000, *Benito s.n.* (JACA 273515). **León:** Posada de Valdeón, Peña Remoña, UN5079, 2200 m, 16-VII-1978, *García s.n.* (JACA 130285). **Lleida:** Vilamós, Valle de Arán, VII-1909, *M. Llenas s.n.* (BC 60643); Vall d'Aran, Hospital de Viella, 1625 m, 17-VI-1973, *Vigo & Farreny s.n.*

(BC 612039); **Navarra:** Isaba, Peña Ezkaurre, XN7748, 1560-1600 m, 5-VIII-1967, *Montserrat s.n.* (JACA 539667); Isaba, Larra barranco Budoguía, XN8454, 2000-2200 m, 16-VII-1972, *Villar s.n.* (JACA 10097272); Isaba, Ezkaurre, XN7647, 1800 m, 15-VII-1985, *Aizpuru & Catalán s.n.* (ARAN 34481); Isaba, Bazarea, XN7058, 1850 m, 21-VII-1986, *Aizpuru & Catalán s.n.* (ARAN 34479). **Vizcaya:** Aldamin, WN1866, 1362 m, 11-VII-1981, *Aseguinolaza s.n.* (ARAN 9373).

2. *Nigritella austriaca* subsp. *iberica* (Teppner & E. Klein) L. Sáez, **comb. nov.** *Nigritella nigra* subsp. *iberica* Teppner & E. Klein in *Phyton* (Horn) 33(2): 192. 1993. *Gymnadenia nigra* subsp. *iberica* (Teppner & E. Klein) Teppner & E. Klein in *Phyton* (Horn) 38(1): 222. 1998. *Gymnadenia austriaca* var. *iberica* (Teppner & E. Klein) G. Foelsche & W. Foelsche in *J. Eur. Orchideen* 31(4): 803. 1999

Ind. loc.: "Holotypus: Zentral-Pyrenäen, Prov. Huesca, Esera-Tal, Massiv von Castanesa, Umgebung von Cerler, Llanos del Ampriu ca. 1900 m, flach geneigter, NNW exponierter Hang; 12.7.1992; leg. H. Teppner & E. Klein (MA).- Isotypen: GZU, P".

Type: Spain. Huesca, Castanesa, Llanos del Ampriu, 12 July 1992, *Teppner & Klein s.n.* (holotype, P; isotypes, GZU, MA 528527!).

= *Nigritella nigra* subsp. *gallica* E. Breiner & R. Breiner in *Mitt. Arbeitskr. Heim. Orch. Baden-Württemberg* 25(4): 471. 1993. *Gymnadenia austriaca* var. *gallica* (E. Breiner & R. Breiner) P. Delforge in *Nat. Belg.* 79(4): 255. 1998

Ind. loc.: "l'Alpe d'Huez, Gallia, Holotypus 26.06.1993, leg. E. u. R. Breiner, Naturmuseum Augsburg, 93-351/1075".

Type: France. Alpe d'Huez, 26 June 1993, *Breiner s.n.* (holotype, Naturmuseum Augsburg, not seen).

N. nigra subsp. *austriaca* auct., non Teppner & E. Klein
N. nigra auct., non (L.) Kirschl.
N. angustifolia auct., non Rich.

Illustrations: Fig. 6.

Tubers 12-40 × 7-22 mm. Stems 8-30 cm. Leaves 30-180 × 5-6(9) mm. Basal bracts (6)8-18 × 2-4 mm, margin usually entire, sometimes slightly or irregularly denticulate (denticles c. 0,03 mm long). Inflorescence 15-30 × 12-25 mm, with 20-42 flowers, subconical or hemispherical in flower, subovate in fruit. Flowers brownish-red to blackish-red, vanilla scented. Sepals oblanceolate to lanceolate, glabrous; lateral sepals (6)6.8-9(9.5) × (1.5)2-2.3(2.5) mm, middle sepal (6.5)7-

8.4 × (1.3)1.5-2,1 mm. Lateral petals (5.5)6.5-8.2 × 0.9-1.5(2.2) mm, acute, lanceolate; labellum 7-10.2 × 4.5-5 mm, distinctly open, slightly narrowed in its basal third, glabrous; spur (0.9)1-1.4 × 0.9-1 mm. Column 1.8-2.2(2.5) mm. Anther 1-1.9 mm. Gynoecium 2-3.5 × 1.5-2.5 mm. Capsule 3-4.5 × 2.5-3.5 mm, oblong or subglobose. Seeds 0.23-0.36 × 0.15-0.22 mm.

Chromosome number: $2n = 80$ (Teppner & Klein, 1993).

Distribution: Pyrenees, SE France (Massif Central), Western Alps and Jura (Fig. 7).

Habitat: Subalpine and alpine meadows; clearings in open *Pinus uncinata* woodlands: 1270 to 2450 m.

Phenology: Flowering July to August.

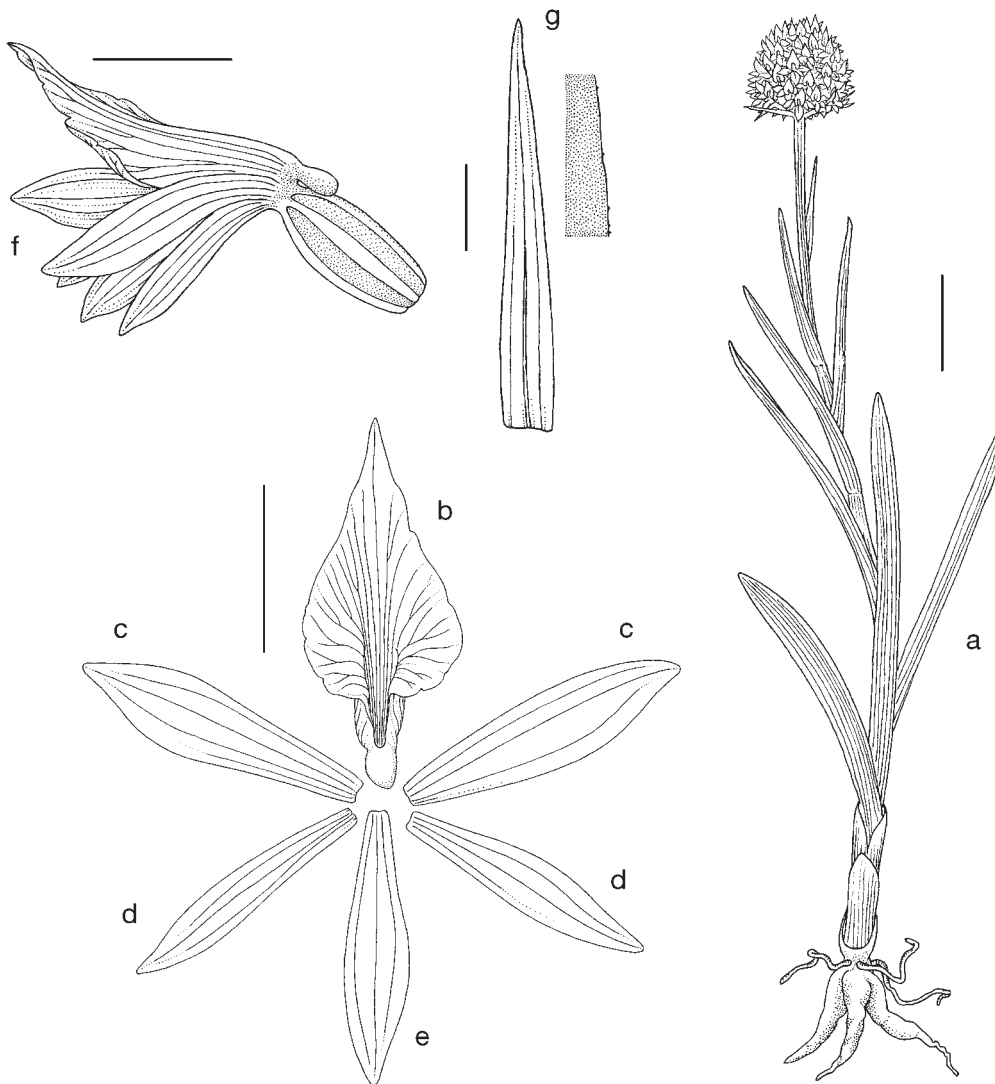


Fig. 6. *Nigritella austriaca* subsp. *iberica* (Lleida: Pas de Roca Plana, Sierra de Cadi, MA 345545). **a**, habit; **b**, labellum; **c**, outer sepal; **d**, lateral petal; **e**, middle sepal; **f**, flower; **g**, bract. Bars: a = 2 cm; b, c, d, e, f, g = 5 mm.

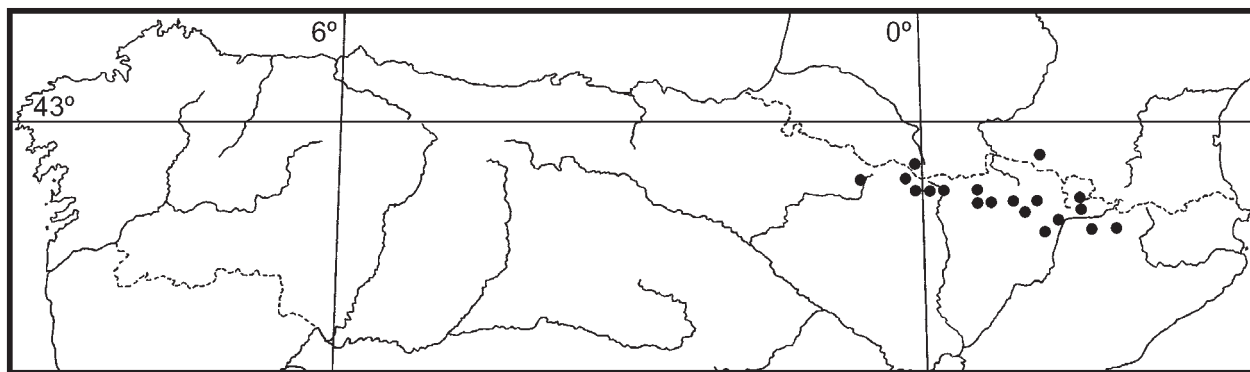


Fig. 7. Distribution map of *Nigritella austriaca* subsp. *iberica* in the Iberian Peninsula on the basis of herbarium specimens.

Remarks: The name *Nigritella nigra* subsp. *iberica* was published as a separate print on July, 1993, as can be seen in *Phyton* 33(2): 179. In December, 1993, *N. nigra* subsp. *gallica* was published. Therefore, despite the opinion of some authors (Galán Cela & Gamarra, 2002; Delforge, 2001), *N. nigra* subsp. *iberica* has priority over subsp. *gallica*.

Representative specimens

ANDORRA. Subiendo al estany Furcat, 2100 m, *Losa*, (BCN 5368); Coll d'Ordino, CH8212, 2000 m, 5-VII-1992, *Nieto & al.* GN 3227 (MA 514786). FRANCE. De Gavarnie a la base del Vignemale, 1820 m, VII-1991, *Sáez s.n.* (BCB); vessant N de la Montanha deth Trapet, val Peiralada, 2300 m, CH4441, 19-VII-1997, *L. Sáez & al. s.n.* (BCB). SPAIN. **Barcelona:** Cadí, 1700 m, 24-VII-1967, *Fernández Casas s.n.* (MA 328776). **Girona:** La Molina, VII-1950, *Montserrat s.n.* (BCN 5361); La Molina, 2000 m, 27-VI-1950, *Montserrat s.n.* (BCN 5359). **Huesca:** Candanchú, 3-VIII-1972, *Ron s.n.* (MA 192736); Canal de Izas, Canfranc, YN0836, 2000 m, 1-VII-1974, *Fanlo s.n.* (JACA 782374); Benasque, valle de Estós, CH9527, 1600-2100 m, 1-VIII-1974, *Villar s.n.* (JACA 525574); Los Lecherines, 3-VIII-1979, *Amich & al. s.n.* (MA 295050); Circo de Añisclo, BH5927, 2000 m, 27-VII-1982, *Luceño s.n.* (MA 392970); c. ago Bacibé, Castanesa, 2230 m, 6-VIII-1982, *Montserrat & Dussaussois s.n.* (JACA 160382); bajo el collado de la Magdalena, XN9935, 1900 m, 20-VII-1984, *Villar & al. s.n.* (JACA 89784); Sierra de Chía, 1700 m, 29-VI-1985, *Carrillo s.n.* (BCN 5387); El Ampriu, Benasque, Cerler, CH0014, 1900-2100 m, 18-VII-1987, *Sesé s.n.* (JACA 762687); Eriste, entre refugio Forcau y Posets, BH92, 2250 m, 21-VII-1987, *Castroviejo & al. SC 10112b* (MA 391214); Forqueta de Gabieto, Torla, 2000-2300 m, 19-VIII-1992, *Montserrat & Villar s.n.* (JACA 353292); Gallinero, supra Cerler, Benasque, 2400 m, VII-1994, *Pallarés s.n.* (MA 546817); Puértolas, Escuaín, Faixa Cazcarra, BH6321, 1265-1390 m, 13-VI-1997, *Benito & Soriano s.n.* (JACA 271301); Torla, Faja de Pelay, YN4324, 1850 m, 12-VII-2000, *Benito & Guzmán s.n.* (JACA 273491). **Lleida:** Pas de Roca Plana, sierra de Cadi 2280 m, 8-VII-1949, *Bolòs & al. s.n.* (MA 345545; MA 164013; BCN 5393); Espot, Estanyets, 2000 m, 9-VII-1934, *Rothmaler s.n.* (BC 78487); Pallerols del Cantó, crta. de Seu a Sort, 31TCG69, 1720 m, 6-VII-1978, *Montserrat s.n.* (JACA 173378); Vessant W del Coma l'Espasa, Vall de Boí, CH21, 1900 m, 17-VII-1978, *Carrillo & Ninot s.n.* (BCN 5383); serra de Taús [CG58], 1900 m, 1-VII-1983, *Vigo & al. s.n.* (BCN 5392); Buigues

de Cosme, Vall Fosca, CH30, 1700 m, 15-VI-1985, *Pineda s.n.* (BCN 5386).

Species which presence is doubtful

Nigritella corneliana (Beauverd) P. Gözl & H.R. Reinhard in *Jahresber. Naturwiss. Ver. Wuppertal* 39: 39. 1986 (*N. nigra* subsp. *corneliana* Beauverd in *Bull. Soc. Bot. Genève ser. 2*, 17: 336. 1926) has been reported in the eastern Pyrenees (Sanz & Nuet, 1997). This is a diploid species endemic of the southwestern Alps, characterised by its red-cinnabar to carmine-pink flowers, generally fading towards the base of the inflorescence, where they can be almost whitish (Bournérias & al., 1998). Because we did not find the species after several visits to the Pyrenean locality where it was cited and photographed, and no appropriate herbarium material from that site is known, we consider the occurrence of this species in the Iberian Peninsula doubtful.

Acknowledgements

The author thanks M. Galbany for her help with cytological work on *Nigritella gabasiana* and J.M. Ninot for linguistic advice. M. Hedrén and H. Teppner offered helpful suggestions and criticism on the manuscript. This work was supported by the research grant "Flora iberica VI" (REN2002-04634-C05-01).

References

- Barrington, D.S., Paris, C.A. & Ranker, T.A. 1986. Systematic inferences from spores and stomate size in the ferns. *American Fern Journal* 76(3): 149-159.
- Borrino, E.M. & Powell, P. 1988. Stomatal guard cell length as an indicator of ploidy in microspore derived plants of barley. *Genome* 30: 158-160.
- Bournérias, M., Demange, M., Démares, M. Engel, R., Gerbaud, O., Melki, F., Quentin, P. & Tyteca, D. 1998. *Nigritella* L.C.M. Richard. In: M. Bournérias (ed.), *Les Orchidées de France, Belgique et Luxembourg*, Parthénop: 143-149. Paris.

- Breiner, E. & Breiner, R. 1993. Beiträge zur Gattung *Nigritella* in den Westalpen. *Mitteilungsblatt Arbeitskreis Heimische Orchideen Baden-Württemberg* 25(4): 467-487.
- Brütsch, J.P. 2000. Die Gattung *Nigritella* Rich. *Baobibia* 14: 21-32.
- Castroviejo, S., Laínz, M., López, G., Montserrat, P., Muñoz, F., Paiva, J. & Villar, L. (eds.). 1986. *Flora iberica* I. Real Jardín Botánico, CSIC. Madrid.
- Delforge, P. 2001. *Guide des Orchidées d'Europe, d'Afrique et du Proche Orient*. Ed. 2. Delachaux et Niestlé. Lausanne-Paris.
- Delforge, P., Devillers-Terschuren, J. & Devillers, P. 1991. Contributions taxonomiques et nomenclaturales aux Orchidées d'Europe (Orchidaceae). *Naturalistes Belges* 72: 99-101.
- Evans, A. 1955. The production and identification of polyploids in red clover, white clover and lucerne. *New Phytologist* 54: 149-162.
- Galán Cela, P. & Gamarra, R. 2002. Check list of the Iberian and Balearic orchids 1. *Aceras* R. Br. – *Nigritella* Rich. *Anales del Jardín Botánico de Madrid* 59: 187-208.
- Hedré, M., Klein, E. & Teppner, H. 2000. Evolution of Polyploids in the European Orchid genus *Nigritella*: Evidence from Allozyme Data. *Phyton (Austria)* 40(2): 239-275.
- Holmgren, P.K. & Holmgren, N.H. 1993. Additions to Index Herbariorum (Herbaria), edition 8 - Second Series. *Taxon* 42: 489-505.
- Holmgren, P.K., Holmgren, N.H. & Banett, L.C. 1990. Index Herbariorum. Part I: The herbaria of the world. 8th ed. *Regnum Vegetabile* 120: 1-163.
- Klein E. & Drescher, A. 1996. *Nigritella nigra* (Orchidaceae-Orchideae) im Massif Central (Frankreich). *Phyton (Austria)* 36(2): 231-250.
- Sanz, H. & Nuet, J. 1997. *Guia de camp de les orquídies de Catalunya*. Montblanc-Martín. Barcelona.
- Stace, C.A. 1965. Cuticular studies as aid to plant taxonomy. *Bulletin of the British Museum (Natural History), Botany* 4: 1-78.
- Teppner, H. 1996. Adventitious embryony in *Nigritella* (Orchidaceae). *Folia Geobotanica Phytotaxonomica* 31(3): 323-331.
- Teppner, H. & Klein, E. 1985a. Karyologie und Fortpflanzungsmodus von *Nigritella* (Orchidaceae - Orchideae), inkl. *N. archiducis-joannis* spec. nov. und zweier Neukombinationen. *Phyton (Austria)* 25(1): 147-176.
- Teppner, H. & Klein, E. 1985b. *Nigritella widderi* spec. nov. (Orchidaceae - Orchideae) - *Phyton (Austria)* 25(2): 317-326.
- Teppner, H. & Klein, E. 1990. *Nigritella rhellicani* spec. nova und *N. nigra* (L.) Rchb. f. s. str. (Orchidaceae - Orchideae). *Phyton (Austria)* 31(1): 5-26.
- Teppner, H. & Klein, E. 1993. *Nigritella gabasiana* spec. nova, *N. nigra* subsp. *iberica* subsp. nova (Orchidaceae-Orchideae) und deren Embryologie. *Phyton (Austria)* 33(2): 179-322.
- Teppner, H. & Klein, E. 1998. Etiam atque etiam: *Nigritella* versus *Gymnadenia*: Neukombinationen und *Gymnadenia dolomitensis* spec. nova (Orchidaceae - Orchideae). *Phyton (Austria)* 38(1): 220-225.
- Teppner H. & Ster, T. 1996. *Nigritella buschmanniae* spec. nova (Orchidaceae-Orchideae) und eine Biographie für Frau Adolfine Buschmann. *Phyton (Austria)* 36(2): 277-294.
- Viane R.L.L. 1990. Epidermology of european ferns. In: Rita, J. (ed.), *Taxonomía, Biogeografía y Conservación de pteridófitos*. Societat d'Història Natural de Balears-Institut Menorquí d'Estudis: 69-89. Palma de Mallorca.

Received: 22-X-2003

Accepted: 13-III-2004