Systematics of the narrow endemic species *Brimeura duvigneaudii* (Hyacinthaceae)

Llorenç Sáez[1], Joan Rita[2], Gabriel Bibiloni[2], Cristina Roquet[3], Javier López Alvarado[4]

1. Universitat Autònoma de Barcelona. Facultat de Biociències. Unitat de Botànica E-08193 Bellaterra (Barcelona). gymnesicum@yahoo.es
2. Universitat de les Illes Balears. Departament de Biologia. E-07122 Palma de Mallorca
3. Université Joseph Fourier. Laboratoire d'Ecologie Alpine, UMR-CNRS 5553 BP 53. 38041 Grenoble Cedex 9
4. Institut Botànic de Barcelona (CSIC-ICUB). Passeig del Migdia, sn. 08038 Barcelona

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Abstract

We carried out a morphological study of *Brimeura duvigneaudii* (Hyacinthaceae), a narrow endemic species limited to northeastern Mallorca (Balearic Islands). Morphological analyses showed noticeably variability which is correlated with geographic distribution and some ecological factors. These data led us to propose a new subspecies of *Brimeura duvigneaudii*, which is described from the middle range of Serra de Tramuntana. The new taxon (*Brimeura duvigneaudii* subsp. *occultata*) differs from *B. duvigneaudii* subsp. *duvigneaudii* in several vegetative (leaf anatomy and leaf width) and flower features (corolla size, corolla lobe length and shape, scape length). Data on the local distribution and ecology of the new taxon are reported. The new subspecies is restricted to a karst gorge and it is in danger of extinction, due to its small population size. In adition, controversial taxonomy of *Brimeura amethystina* and *B. fontqueri* (= *B. amethystina* subsp. *fontqueri*) is clarified, and they are confirmed as synonyms on the basis of morphological analyses.

Key words: Endangered flora; Endemism; Infraspecific variation; Taxonomy.

Resumen. *Sistemática del endemismo de área restringida* Brimeura duvigneaudii *(Hyacinthaceae)*

Se ha realizado un estudio morfológico de *Brimeura duvigneaudii* (Hyacinthaceae), una especie endémica restringida al noreste de Mallorca (Islas Baleares). Los análisis basados en caracteres morfológicos indican que existe una variabilidad destacable que se relaciona con una distribución geográfica y algunos factores ecológicos. Estos datos permiten proponer una nueva subespecie de *Brimeura duvigneaudii*, restringida de la zona central de la Sierra de Tramuntana. El nuevo taxon (*Brimeura duvigneaudii* subsp. *occultata*) difiere de *B. duvigneaudii* subsp. *duvigneaudii* tanto en caracteres vegetativos (anatomía foliar y anchura de las hojas) como florales (tamaño de la corola, longitud y forma de los lóbulos de la corola, longitud del escapo). Se aportan datos de la distribución local y la ecología del nuevo taxon.

La nueva subespecie se encuentra restringida a un profundo barranco cárstico y está en peligro de extinción como consecuencia de su exiguo tamaño poblacional. Por otro lado se clarifica la controversia taxonómica relativa a *Brimeura amethystina* y *B. fontqueri* (= *B. amethystina* subsp. *fontqueri*), y se confirma que se trata de sinónimos, sobre la base de los análisis morfológicos realizados.

Palabras clave: flora amenazada; endemismo; variabilidad infraespecífica; taxonomía.

Introduction

The biology and taxonomy of genus *Brimeura* Salisb. has received attention since long time ago (Chouard, 1931; Garbari 1966 & 1970; Vosa, 1979; Bibiloni et al., 1987) and recently Almedia et al. (2001) presented a detailed review. According to the last authors, *Brimeura* is restricted to the Western Mediterranean region and includes three species: *B. amethystina* (L.) Salisb., endemic to north-eastern Iberian Peninsula (mountains bordering the Ebro basin), *B. fastigiata* (Viv.) Chouard, endemic to Western Mediterranean islands (Corsica, Sardinia, Majorca and Minorca), and the majorcan endemic *Brimeura duvigneaudii* (L. Llorens) Rosselló, Mus & Mayol [see Almeida et al. (2001) for the detailed distribution and a complete synonymy of these species].

Brimeura duvigneaudii was described on the basis of specimens collected in Penyal Fumat, Formentor (northern Majorca) by L. Llorens (Llorens, 1984) as a variety of *B. amethystina*. Shortly afterwards, Rosselló et al. (1992) elevated the new taxon to species level, which has been confirmed subsequently (Alomar et al., 1997; Almeida et al., 2001; Sáez & Rosselló, 2001; Bibiloni & Mus, 2007). Furthermore, intraspecific morphological variation in *B. duvigneaudii* has been noted (Almeida et al., 2001). According to these authors, there are two groups of populations that correspond to separate areas in which plants show morphological differences (scape and flower length). These differences were attributed to phenotypic adaptations to a humid and shady microhabitat in Coma Freda karst gorge. Indeed, variation in flower features in this species, has been observed by us during the last decade, which seems to be clearly related to the geographic origin of the plants.

In this work we have assessed the identity of plants from Coma Freda gorge, referred to *B. duvigneaudii*. A reassessment of the morphological characteristics of individuals of both groups of populations (including visits to all the species' populations and cultivation) suggests that *B. duvigneaudii* individuals occurring in Coma Freda gorge are diagnosably distinct to typical populations from Formentor Peninsula and surrounding areas, and should be adscribed to a new taxon.

Material and methods

Material and morphological studies

Morphometric investigation was conducted mainly on live material (partially in the wild and partially under cultivation). Although the main objective of this study is to establish the variability of *B. duvigneaudii*, we also have included data from the other taxa of the genus, including *B. fontqueri* (Pau) Speta, synonymous with

B. amethystina according to Almeida et al. (2001), but recognized at subspecies level by Bolòs & Vigo (2001). Features of gross morphology were studied under a ZEISS binocular stereoscopic microscope.

Morphometric analysis

A number of 141 individuals from 14 localities were studied (table 1). A total of 19 characters have been chosen and measured for their taxonomic significance and their discriminatory value. For the used characters, 13 were quantitative (of which 12 were quantitative continuous and one was quantitative discrete), two were qualitative and the remaining four were calculated as ratios of some of these quantitative continuous traits. The data were explored with a Principal Component Analysis in order to reduce the overall variability into three new uncorrelated variables to understand the morphological relationships between the studied individuals. Statistical analyses were performed with the software SPSS 15.0 (SPSS, 2006).

Plant DNA extraction and cpDNA trnL-F sequences

DNA was extracted from fresh and silica gel-dried leaves with the NucleoSpin® Plant Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) following the man-

Table 1. Studied specimens of *Brimeura* used in the statistical analyses (vouchers in L. Sáez herb. pers.-BCB).

Taxon	Locality	Number of specimens
B. amethystina	Huesca: San Juan de la Peña, 1200 m	11
B. amethystina	Lleida: c. Collegats, 580 m	12
B. amethystina	Lleida: Boumort, vessant S, 1880 m	14
B. amethystina [«*B. fontqueri*»]	Tarragona: Massís del Port, L'Espina, 1100 m	8
B. amethystina [«*B. fontqueri*»]	Tarragona: Tossal de la Reina, 1050 m	17
B. amethystina [«*B. fontqueri*»]	Tarragona: Pic d'Engrilló, 1070 m	11
B. duvigneaudii	Mallorca: Es Fumat	14
B. duvigneaudii	Mallorca: Sobre Cala Figuera	11
B. duvigneaudii	Mallorca: Sa Roca Blanca	7
B. duvigneaudii	Mallorca: Torrent de Coma Freda, 280 m	12
B. fastigiata	Mallorca: Artà, ermita de Betlem	3
B. fastigiata	Mallorca: Talaia Freda, vessant N, 200 m	11
B. fastigiata	Sardinia, Terramala, 400 m	5
B. fastigiata	Sardinia, Berchida, Monte Limbara, cima del Montalvu	5

ufacturer's instructions for one individual of *B. amethystina*, *B. fastigiata* and *B. duvigneaudii* s. str., and three individuals of *B. duvigneaudii* from Coma Freda gorge. The trnL-F intergenic spacer (cpDNA) was amplified and sequenced with the primers trnL-F c and trnL-F f (Taberlet et al., 1991). The thermal cycling profile consisted of: 1 min 35 s at 95 ºC; 30 cycles of 1 min denaturing at 93 ºC, 1 min annealing at 58 ºC, and 2 min of extension at 72 ºC; with an additional final extension step of 10 min at 72 ºC. The ITS region (nrDNA) was amplified and sequenced with the primers ITS1 and ITS4 (White et al., 1990) and amplified under following conditions: hot start at 94 ºC for 4 min; 30 cycles of 1 min 30 s denaturing at 94 ºC, 2 min annealing at 55 ºC and 3 min of extension at 72 ºC; with an additional final extension step of 15 min at 72 ºC. PCR products were purified and sequenced at the University of Florida ICBR Core Facility on an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA). Chromatograms were edited with Chromas 2.0 (Technelysium Pty Ltd, Tewantin, Australia). For each region, sequences were aligned by eye using BioEdit (Hall 1999). We added one trnL-F sequence of *B. amethystina* obtained from Genbank (accession number FJ423214).

Distribution

All the localities reported for *Brimeura duvigneaudii* were visited, and the most important potential habitats were also checked for new populations; in total, 20 UTM 1 km^2 squares were visited. When the species was detected, we recorded habitat and phenological data (i.e., the presence of vegetative plants, flowering stage, fruiting stage, etc.) and the exact GPS coordinates.

Results

Corolla size and shape

As pointed out already by Almeida et al. (2001), the corolla characters are of key importance in the taxonomy of the genus *Brimeura*. All the examined flowers of *B. duvigneaudii* from northern Majorca (Formentor area) have smaller corollas (7.2-9 mm long) than those of the population of Coma Freda gorge (9.5-12.5 mm long) (fig. 1, fig. 2, table 2). The corolla lobes and the tube are shorter in the northern populations (fig. 1). Moreover, the shape of the corolla lobes shows marked differences between population groups: plants collected in the Formentor area bear lobes ovate or ovate-orbicular (outer lobe length/outer lobe ratio = 0.95-1.20), while plants of the southern population have longer lobes, with a larger outer lobe length/outer lobe ratio =1.20-1.53. In contrast, no significant differences were detected in the morphology of the corolla between populations of *B. amethystina* and *B. fontqueri* (fig. 3).

Corolla colour

Flowers of *B. duvigneaudii* are usually lighter in colour than those of *B. amethystina* (Almeida et al., 2001), although occasionally the latter species can have white

flowers. Range includes from a whitish or even whitish-green perianth, to light violet. The most common colour of both groups of populations of *B. duvigneaudii* is a white or whitish-violet flower, or whitish-greenish. Much of this variation can be detected within a single population.

Leaves

Almeida et al. (2001) provided detailed information about leaf anatomy of the species of *Brimeura*. Our observations are consistent with their data, but the individuals of the Coma Freda gorge population have wider leaves (1.7-3.1 mm) than those of the samples of the populations of Northern Majorca (0.8-2 mm). These characters are retained in cultivation.

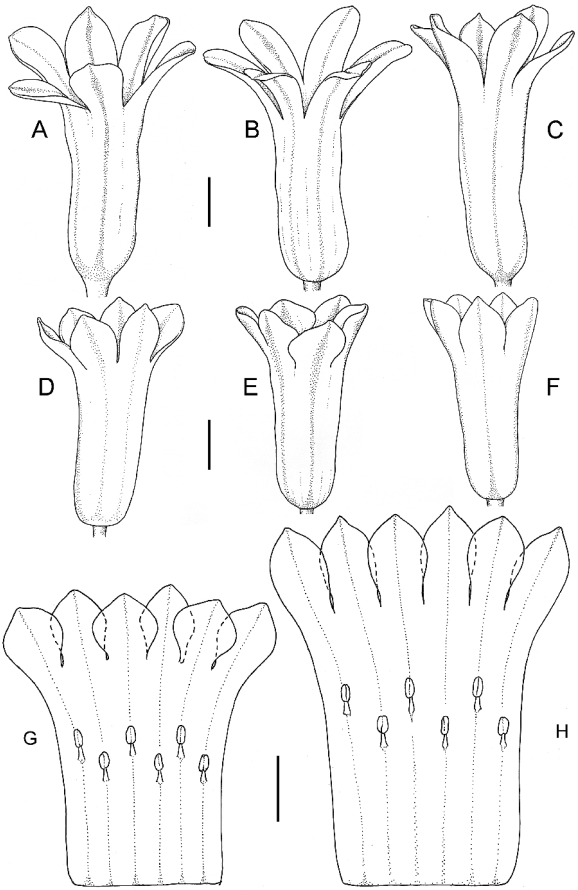

Figure 1. Corollas of *Brimeura duvigneaudii:* **A, B, C, H**: plants from Coma Freda gorge. **D, E, F, G**: plants from Formentor area. Scale: 2 mm.

Pedicels and bracts

Pedicels are erect to erecto-patent in both groups of populations of *B. duvigneaudii*. Lowermost pedicels of *B. duvigneaudii* from Formentor area are shorter (4.5-18 mm long) than those of the population of Coma Freda gorge (20-33 mm long). Bracts are similar in both groups of populations, although plants from the population of Coma Freda gorge bear longer lowermost bracts (3.5-7 mm long) than northern populations (2-4.7 mm long). *Brimeura duvigneaudii* (sensu lato) has smaller lowermost bracts

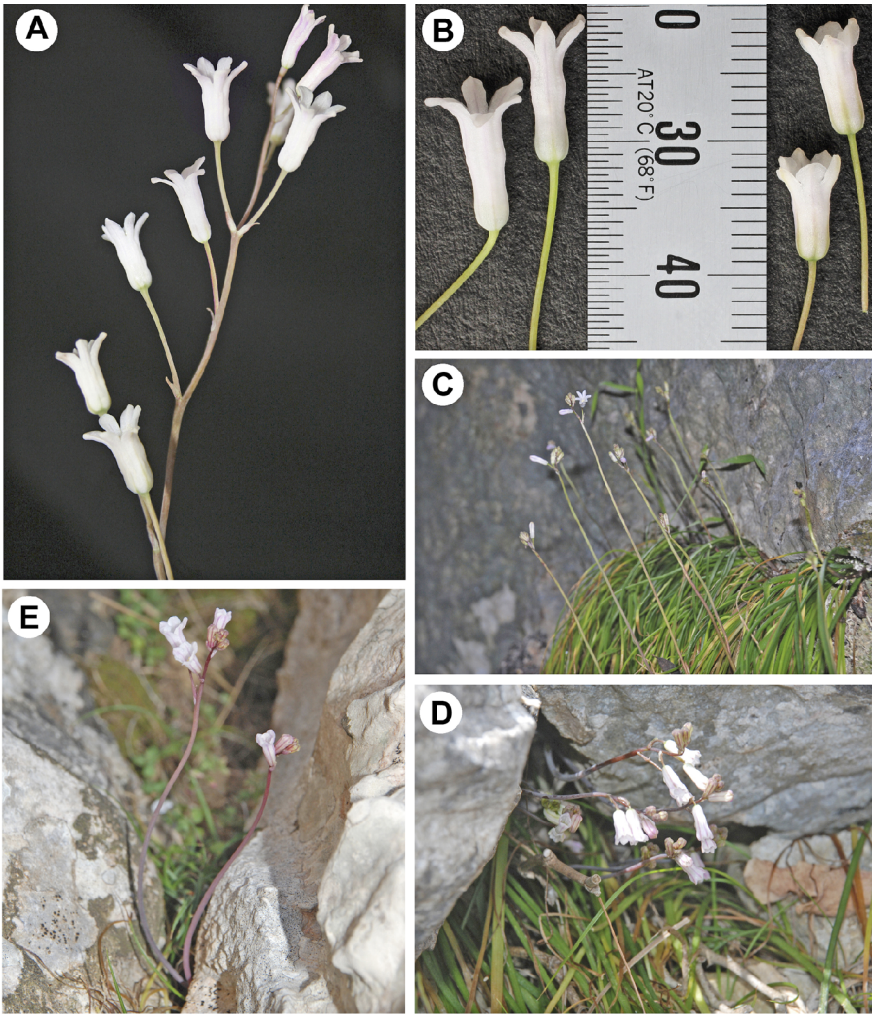

Figure 2. Inflorescence **(A)** and plants **(C)** of *Brimeura duvigneaudii* from Coma Freda gorge (photos, L. Sáez and X. Rotllan respectively). **B**: flowers from Coma Freda gorge (left) and from Formentor area (right) (photo, L. Sáez). **E**, **D**: plants from Formentor area (photos, X. Rotllan).

Table 2. Differences between plants of both groups of populations of *Brimeura duvigneaudii*. Measurements are in mm.

Character	Coma Freda gorge population	Formentor area population
Leaf width	1.7-3.1	0.8-2
Scape length	145-280	60-150
Number of flowers	(7)9-14	5-11
Bract size	3.5-7 × 1.4-2	2-4.7 × 0.6-1.5
Pedicel length	20-33	4.5-18
Corolla length	9.5-12.5	7.2-9
Corolla lobes length	2.7-4.1	1.9-2.7
Corolla lobes width	1.7-2.9	1.8-2.5
Corolla lobes shape	ovate-oblong to oblong-elliptic	broadly ovate to suborbicular
Fruit	3.5-5 × 3.7-5	unknown
Seeds	1.7-2 × 1-1.5	unknown

(2-7 mm long) than *B. amethystina*, including including individuals regarded as *B. fontqueri* (8.8-15 mm long). Both groups of populations of *B. duvigneaudii* can also be separated by the ratio length of the lower pedicel/length of lower bract (2-3.83 in northern populations, 4.4-6.28 in the population of Coma Freda gorge). We have not found differences in the lower pedicel/length of lower bract ratio between populations of *B. amethystina* and *B. fontqueri* (fig. 3).

Fruits and seeds

Each flower produces a fruit that bears 6 seeds. The fruit of *B. duvigneaudii* is a subglobose glabrous capsule of 3.5-5 × 3.7-5 mm. Plants from Coma Freda gorge population develop fruits and subsphaerical-subtrigonous viable seeds (1.5-2 × 0.9-1.6 mm); however, we have not observed the development of fruits in the population of Formentor area. Seeds from Coma Freda gorge population exhibit the same hypogeous type germination system than *Brimeura amethystina* (Chouard, 1931). *Brimeura amethystina* seeds are subsphaerical, of 2.3-3 × 1.8-2 mm, whereas *B. fastigiata* presents subtrigonous seeds of 1.8-2 × 1.6-1.8 mm.

Principal Component Analysis

The first three principal components have been selected. All three components explains the 82.52% of the total variance (PC1=37.92%; PC2=26.19%; PC3=18.41%) (fig. 4). The factors that contribute most to the first component are the maximum length of the corolla, followed by the number of flowers, the length of the scape and the leaf width as a positive values, and the arrangement of stamens the ratio between length and width of the external lobe, the ratio between length and width of the internal lobe, the length of the internal lobe and the length

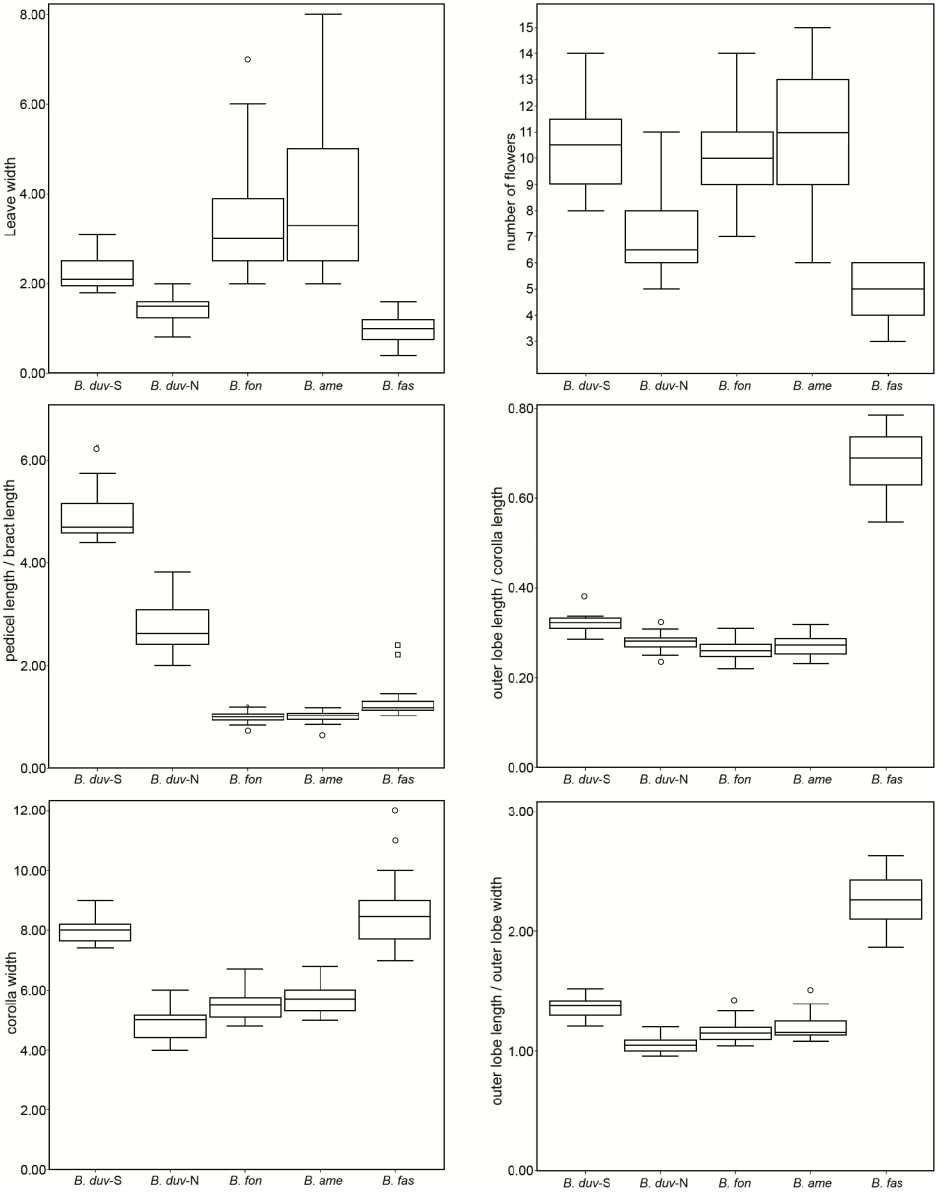

Figure 3. Box-plots for 6 morphological traits. The whiskers represents the maximum and minimum values, the box signifies the upper and lower quartiles and the mean value is depicted by a black horizontal line within the box. *B. duv-S: Brimeura duvigneaudii,* population of Coma Freda gorge; *B. duv-N: Brimeura duvigneaudii,* populations of Formentor area; *B. fon: Brimeura fontqueri; B. ame: Brimeura amethystina* (Pyrenean populations); *B. fas: Brimeura fastigiata.* ○: Outliers; □ extreme values.

of the outer lobe as negative ones. The factors that contribute most to the second component are the lowermost bract width, the length of the lowermost bract and the presence/absence of bulb's cataphylls as positive values, and the ratio between pedicel length and bract length as negative ones. Finally, the third component is contributed positively by the ratio between pedicel length and bract length and the lowermost pedicel length, while is contributed negatively for the presence/absence of cataphylls and the length of the lowermost bract. The scatterplot allows for the visualization of the sampled individuals against the first three principal components, showing the taxa clustered into different groups except for *B. fontqueri* and *B. amethystina*, which presents a large overlap.

TrnL-F sequences

Partial sequences of 362 base pairs (bp) of the the *trnL-F* intergenic spacer for each individual were obtained. We detected 8 polymorphic sites (table 3), all of them specific for the individuals of one species: 2 changes in the sequences of *B. amethystina* individuals compared to the other species, 3 changes in *B. fastigiata*, and 2 changes in the individuals of *B. duvigneaudii*. Apart from that, we detected two deletion events, of 4 and 2 bp, for *B. fastigiata*. The region was not variable enough to detect intra-specific variation.

Taxonomic treatment of the new subspecies

Based on the morphometric study of *B. duvigneaudii* specimens, we propose to recognize as a new subspecies the plants from the Coma Freda karst gorge (fig. 4).

Brimeura duvigneaudii subsp. ***occultata*** L. Sáez, Rita, Bibiloni, Roquet & López Alvarado ***subsp. nov.***

Diagnosis
A Brimeura duvigneaudii subsp. duvigneaudii similis, sed foliis latioribus et corolla longiora (9.5-12.5 mm) cum perianthii segmentis longioribus (2.7-4.1 mm) differt.

Table 3. Condensed alignment of variable positions (column) in a partial sequence of the *trnL-F* region of the species of *Brimeura*

Taxon	106	113	154	158	171	204	324	354
B. amethystina	T	A	T	T	C	T	T	C
B. fastigiata	C	A	T	–	T	C	C	T
B. duvigneaudii (Formentor area population)	T	C	C	C	C	T	C	T
B. duvigneaudii (Coma Freda gorge population)	T	C	C	C	C	T	C	T

Holotypus
Balearic Islands: Mallorca, torrent de Coma Freda, 31SDE9105 270 m, calcareous shady cliffs, 26-V-2003, *L. Guàrdia Valle & L. Sáez* LS-6109 (BC 904039).
Isotypus: (ibidem, *L. Sáez*, herb. pers.-BCB)

Etymology
The name refers to the hidden locality of Coma Freda gorge, which is the only known locality of the new subspecies.

Description
Perennial herb, glabrous. Bulb ovoid to subglobose, 0.5-3.1 cm long, 0.3-2 cm wide, tunicated, whitish, naked or covered by evanescent cataphylls. Leaves 150-600 × 1.7-3.1 mm, plane-convex in section. Scape 14.5-28 cm long; inflorescence 7-13.5 cm, usually in unilateral raceme, (7)9-14 flowered. Lower bract 3.5-7 mm long, 1.4-2 mm wide, lanceolate to narrowly lanceolate, membranous. Lower pedicel 20-33 mm long, erect to erecto-patent in flower and fruit; ratio length lower pedicel/length lower bract = 4.4-6.8. Corolla tubular-campanulate, white to rose, 9.5-12.5 mm long, tube 6.5-8.7 mm long, 2.3-2.6 mm wide; lobes 2.7-4.1 mm long, 1.7-2.9 mm wide (ratio length/width = 1.08-1.64), ovate-oblong to oblong-

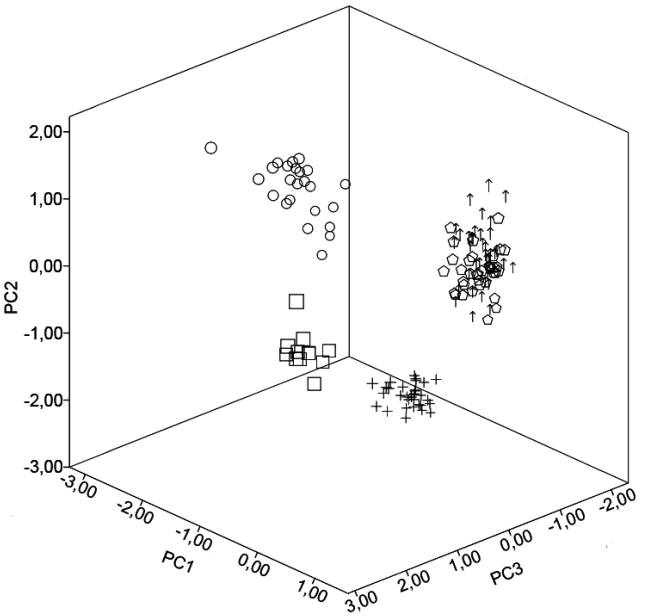

Figure 4. The Principal Component Analysis scatter plot shows the grouping of *Brimeura* individuals against the three principal components which together explains the 82.52% of the total variance. Taxon: □ *B. duvigneaudii* (Southern population), + *B. duvigneaudii* (Northern population), ⬠ *B. Fontqueri,* ↑ *B. amethystina*, ○ *B. Fastigiata*.

elliptic, equally coloured on both sides, patent or sligthly recurved. Filaments inserted at two levels, 0.7-1 mm long, 0.5-0.7 mm wide, whitish to rose; anthers 0.7-1.1 mm long, yellow. Ovary subglobose, 1.5-2.2 mm long, 1.6-2 mm wide, usually pale green, with a rough protuberance on each carpel; style 3-4.4 mm long, whitish, narrowly cylindrical, broadened to the base and gradually merging into the ovary. Capsule subglobose-subtrigonous, 3.5-5 × 3.7-5 mm, glabrous. Seeds 1.5-2 × 0.9-1.6 mm, deep dark brown, subsphaerical-subtrigonous, somewhat irregular, usually with a more or less flat or plano-convex face.

Habitat and distribution

The new subspecies is restricted to a single karst canyon located in the middle ranges of the Serra de Tramuntana (Majorca). It grows in humid shady Liassic calcareus cliffs close to the water course, at ca. 270 m a.s.l. Very few vascular plants were found near the cliffs were the new taxon grows: *Adiantum capillus-veneris* L., *Allium triquetrum* L. and *Pistacia terebinthus* L. In this locality several hygrophilous species of bryophytes can be found, such as *Eucladium verticillatum* (Brid.) Bruch & Schimp., *Fissidens viridulus* (Sw.) Wahlenb. and *Plagiochasma appendiculatum* Lehm. & Lindenb.

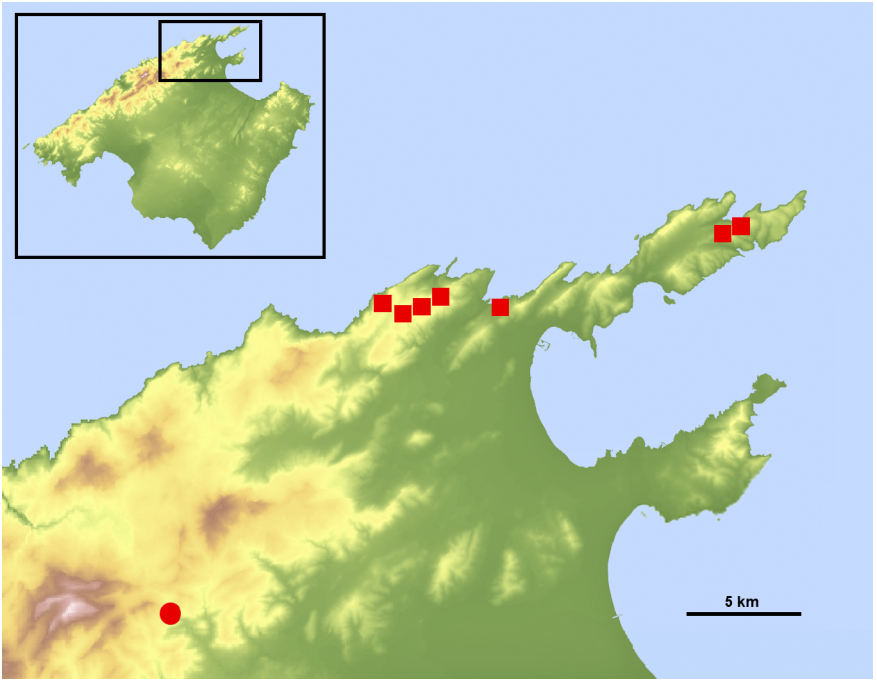

Figure 5. Distribution map of *Brimeura duvigneaudii* subsp. *occultata* (circle) and *B. duvigneaudii* subsp. *duvigneaudii* (squares).

Brimeura duvigneaudii subsp. *duvigneaudii* grows in rocky slopes and limestone rock crevices, usually in little shady meadows, together with tyrrhenian or endemic elements such as *Bellium bellidioides* L., *Carex rorulenta* Porta, *Cyclamen balearicum* Willk., *Micromeria filiformis* (Aiton) Benth., *Sibthorpia africana* L. and *Sonchus willkommii* (Burnat & Barbey) Rosselló & L. Sáez, usually in sunny areas, relatively close to the sea, at 150-560 m. *Brimeura duvigneaudii* subsp. *duvigneaudii* occurs in small patches and as isolated individuals. Several subpopulations show dense patches containing up to hundreds of individuals.

Conservation

Brimeura duvigneaudii has been included in the IUCN (2001) category «Critically Endangered» (Bibiloni & Mus, 2007). *Brimeura duvigneaudii* has been found in 9 UTM 1 km^2 squares. The new subspecies can be also categorized as Critically Endangered according to the IUCN Red List Criteria: B1ab(iii)+2ab(iii); D, since it is only known from a single site, with a very restricted extent of occurrence and area of occupancy (the unique population covers a very small area of about 50 square meters). The number of reproductive individuals is very small (less than 50). On the basis of our field observations, there is no evidence of a decline in the number of individuals in the population, although seed production and the establishment of seedlings is highly variable. In addition, the adventure sport of canyoning nowadays attracts large numbers of visitors and threatens to overwhelm the loading capacity of this fragile ecosystem (Ginés & Mayol, 1995). There is also some removal of plants by collectors. Grazing by livestock is negligible at the Coma Freda locality, given the inaccessibility of the site, contrary to *Brimeura duvigneaudii* subsp. *duvigneaudii*, which is greatly affected by grazing pressures at Formentor area.

Key to species and subspecies of *Brimeura*

This is an amendment for the key in Almeida et al. (2001) to accommodate the new subspecies.

1. Flower lobes as long as the tube of corolla or longer; staminal filaments arranged nearly at same level, inserted close to the tube gorge ..... ***B. fastigiata***
   – Flower lobes smaller than the tube of corolla; staminal filaments arranged in two levels, inserted under the tube gorge 2
2. Lowermost bract subequal or slightly smaller than the peduncle; flowers blue or violet, rarely white; bulbs covered by dry cataphylls ..... ***B. amethystina***
   – Lowermost bract minor than 1/2 of peduncle; flowers white or rose; bulbs naked 3
3. Corolla 8.4-9.5 mm long; lobes of corolla as long as width, erect; lowermost peduncle until 4 times longer than the bract, usually shorter; leaves usually less than 2 mm width ..... ***B. duvigneaudii*** subsp. ***duvigneaudii***
   – Corolla 9.5-12.5 mm long; lobes of corolla longer than width, patent o erecto-patent; lowermost peduncle more than 4 times longer than the bract, usually longer; leaves usually more than 2 mm width ..... ***B. duvigneaudii*** subsp. ***occultata***

Discussion

Our results indicate that the population of *B. duvigneaudii* from Coma Freda karst gorge (subsp. *occultata*) is morphologically consistent, and is clearly delimited compared to populations of *B. duvigneaudii* subsp. *duvigneaudii*, as well as the other species of the genus. The new subspecies has the closest morphological affinities to typical *B. duvigneaudii* with which it shares the character of the bulb, and not to *B. amethystina* or *B. fastigiata*. Both groups of *B. duvigneaudii* populations share just those features of outstanding specific value (cf. Chouard, 1931), especially the bulb morphology, which is not covered by dry cataphylls. However, since the corolla shape and size and other minor diagnostic morphological characters (table 1) are correlated with a particular geographic distribution and ecological conditions, and the Coma Freda gorge population is spatially separated from typical populations of *B. duvigneaudii* (which grow in drier habitats and relatively close to the sea), it seems justified to recognize such variation at the subspecific rank. These diagnostic features are maintained after 10 years of nursery cultivation, so that the morphological differences between both subspecies would not be attributable to variations associated with a more humid and shady habitats. The karst canyon where the new taxon occurs has been considered as a refuge for rare or endemic plant species in the Balearic Islands (Cros et al., 2005). It is remarkable that only the population of *B. duvigneaudii* subsp. *occultata* produces fruits and seeds whereas those of *B. duvigneaudii* subsp. *duvigneaudii* seem to reproduce asexually by bulb division, although grazing pressure by goats should not be neglected. Both subspecies of *B. duvigneaudii* are also very close regarding cytogenetic grounds. According to Almeida et al. (2001) plants of *B. duvigneaudii* from the typical locality [subsp. *duvigneaudii*] and those from the Coma Freda gorge [subsp. *occultata*] are diploid with 2n=28 chromosomes (although one or two accessory chromosomes were found in both groups of populations). Karyotypes of *B. duvigneaudii* and *B. amethystina* are very similar (Almeida et al., 2001) and, in fact, these authors also reported a close relationship between *B. duvigneaudii* and *B. amethystina* based on several floral features. In relation to molecular data, although very preliminary, results indicate that tRNA-Leu gene from *trnL-trnF* cpDNA region seems to test the validity of proposed taxonomic classification since all the species have their own defining polymorphic sites. In any case it is clear that a more extensive sampling is needed since the presence of different haplotypes within the same population is feasible. Insular differentiation has been reported for several majorcan endemic taxa from mountain areas [*Arenaria grandiflora* subsp. *glabrescens* (Willk.) G. López & Nieto Fel. and *A. grandiflora* subsp. *bolosii* (Cañig.) Küpfer; *Euphorbia maresii* Knoche subsp. *maresii* and *E. maresii* subsp. *balearica* (Willk.) Malag. ex Molero & al.]. According to Rosselló & Castro (2008) such differentiation has mostly affected morphological characters, and involved ecological shifts that allowed the colonization of new habitats in peripheral areas. However, available data do not suggest that karyological changes have been involved in the genesis of most of these lineages. In relation to molecular data obtained in this study, although very preliminary, these show that the tRNA-Leu gene available data do not allow to build a hypothesis on the genesis of *B. duvigneaudii* subsp.

occultata; in any case it would be another example of differentiation of a Balearic endemic that did not entail a karyological differentiation.

So far, the recognition of infraspecific taxa in *Brimeura* have not been supported by detailed systematic studies. Bolòs & Vigo (2001) and Bolòs et al. (2005) have recognized the southern populations of *B. amethystina* (Tarragona Province) at subspecific level (*B. amethystina* subsp. *fontqueri*), although they were included within the synonymy of *B. amethystina* by Almeida et al. (2001). The former authors gave several diagnostic features allegedly separating it from northern [Pyrenean] populations of *B. amethystina*. However, our study indicate that all the features claimed to be exclusive to *B. fontqueri* are also found from northern populations of *B. amethystina* (fig. 3 and 4), as also demonstrated in the review of Almeida et al. (2001). Furthermore, in the case of southern populations of *B. amethystina* [*B. fontqueri*], there is no evidence of ecological specialization and plants grow in similar habitats respect to Pyrenean populations.

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Appendix. Material Examined: See Almeida & al. (2001)

Additional specimens

Brimeura amethystina: Spain, Huesca: San Juan de la Peña, 30TXN9109, 1200 m, 26-VI-2008, *C. Roquet & L. Sáez* (L. Sáez, herb. pers.-BCB); Lleida, massís de Boumort, vessant S, 31TCG4676, 1880 m, 26-VI-2001, *L. Sáez & al.* (L. Sáez, herb. pers.-BCB); Lleida: Boumort, pastura pedregosa, 13-VI-2008, *M. Guardiola* (L. Sáez, herb. pers.-BCB); Tarragona: Massís del Port, Tossal de la Reina, 31TBF7722, 1050 m, 18-V-2009, *L. Sáez & al.* (L. Sáez, herb. pers.-BCB).

Brimeura duvigneaudii subsp. *duvigneaudii*: Mallorca, Castell del Rei, Pollensa, 4-VI-2010, *X. Rotllan* (L. Sáez, herb. pers.-BCB); Mallorca: Cala Carbó, Pollensa, 5-VI-2010, *X. Rotllan* (L. Sáez, herb. pers.-BCB); Mallorca, península de Formentor, Es Fumat, Pollensa, 8-VI-2010, *X. Rotllan* (L. Sáez, herb. pers.-BCB); Mallorca, península de Formentor, Pollensa, 8-VI-2010, *X. Rotllan* (L. Sáez, herb. pers.-BCB); Mallorca, Es Fumat, 28-VI-2010, *X. Rotllan* (L. Sáez, herb. pers.-BCB); per sobre de Cala Figuera, sota carretera Formentor, 28-VI-2010, *X. Rotllan* (L. Sáez, herb. pers.-BCB).

Brimeura duvigneaudii subsp. *occultata*: Balearic Islands: Mallorca, torrent de Coma Freda, 31SDE9105 270 m, calcareous shady cliffs, 26-V-2003, *L. Guàrdia Valle & L. Sáez* LS-6109 (BC 904039); Mallorca, torrent des Guix o de Coma Freda, 28-V-2010, *X. Rotllan* (L. Sáez, herb. pers.-BCB).

Brimeura fastigiata: Balearic Islands, Mallorca: Talaia Freda, vessant N, c. Torrent de Sa Parada, 31SEE2800, 200 m, 21-IV-2003, *L. Guàrdia Valle & L. Sáez* LS-6103 (L. Sáez, herb. pers.-BCB); Mallorca: penyals per sobre de l'ermita de Betlem, Artà, 31SED2798, 340 m, V-2010, *M.A. Conesa* (L. Sáez, herb. pers.-BCB); Sardinia, Berchidda, Terramala cascata N di Piscale, 400-450 m, 25-V-2010, *G. Calvia* (L. Sáez, herb. pers.-BCB); Berchidda, Terramala boscaglie presso il torrente, 400 m, 25-V-2010, *G. Calvia* (L. Sáez, herb. pers.-BCB); Berchidda, Limbara sud, «Sas Solanas» Rocce presso las cascatella, 650 m, 24-V-2010, *G. Calvia* (L. Sáez, herb. pers.-BCB); Berchidda, Limbara sud, versante orientale M. su Fraile Rupi granitiche, 400-500 m, 24-V-2010, *G. Calvia* (L. Sáez, herb. pers.-BCB); Strada Berchidda-Vallcciola, prima di su ponte e su Fraile, macchie, 400-450 m, 24-V-2010, *G. Calvia* (L. Sáez, herb. pers.-BCB).