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ORIGINAL ARTICLE

Morphological analysis of the *Silene gigantea* complex (Caryophyllaceae) across the Balkan Peninsula, south-western Turkey and Cyprus

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Abstract We investigated the morphology of the *Silene gigantea* complex across its distribution range in the Balkan Peninsula, south-western Turkey and Cyprus. We performed a factorial analysis for mixed quantitative and qualitative variables followed by *k*-means analyses to determine the most probable morphological clustering within the species. A linear discriminant analysis was then computed using the DISQUAL method to test the a priori classification. The results indicate that three entities, which are consistent with the previously published taxonomic treatment, can be recognised. Moreover, our results show that the distribution of *S. gigantea* subsp. *hellenica* can be extended to Turkey, and that *S. gigantea* subsp. *rhodopea* is actually absent from this country, in contradiction to previous assessments. The global distribution of the *S. gigantea* complex is established and the morphological characteristics of each subspecies are discussed.

Keywords Eastern Mediterranean Basin · DISQUAL · LDA · Mixed data · Multivariate analyses · Taxonomy

Introduction

The genus *Silene* L. (Caryophyllaceae) is very well-represented in the Mediterranean Basin, especially in the eastern part where Greece shows 38 % species endemism (Greuter 1997; Trigas et al. 2007), Turkey 45 % (Coode and Cullen 1967; Davis 1971; Yıldız et al. 2009; Yıldız and Çırpıcı 2013) and Cyprus 15 % (calculated from Meikle 1977). *Silene gigantea* (L.) L. is a biennial (or monocarpic perennial) and gynodioecious species, that grows mainly on calcareous substrates and is endemic to the Balkan Peninsula and western Asian area, including Cyprus (Greuter et al. 1984). Its taxonomic position within the genus *Silene* is not clearly established, although Greuter (1995a) suggested placing it in *Silene* sect. *Brachypodae* (Boiss.) Chowdhuri, which is close to *Silene* section *Siphonomorpha* Otth. In the latest classification of *Sileneae*, Oxelman et al. (2013) included it in *Silene* serie *Italicae* Rohrb., which mostly overlaps with section *Siphonomorpha*. A possible relationship with the Asian *Silene* sect. *Holopetalae* Chowdhuri was also hypothesised by Ghazanfar and Nasir (1986).

Silene gigantea forms a controversial complex across its distribution range, as shown by the numerous divergent taxonomic treatments found in the literature (see Grisebach 1843; Boissier 1867; Rohrbach 1868; Pančić 1884; Formánek 1899; Velenovsky 1891; Halácsy 1894; Williams 1896; Stojanov and Stefanov 1924; Bornmüller 1924; Bouloumoy 1930; Rechinger 1943; Chowdhuri 1957; Chater et al. 1993; Jordanov 1966; Mouterde 1966; Josifović 1970; Strid 1980; Delipavlov et al. 1983; Demitri 1983; Paparisto et al. 1988; Micevski 1993; Tan et al. 2001; Vangjeli 2003; Yıldız 2006; Yıldız and Çırpıcı 2013). Within *Silene gigantea*, Greuter (1995b) suggested that three vicarious subspecies should be recognised in

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Greece: *S. gigantea* subsp. *gigantea*, *S. gigantea* subsp. *hellenica* Greuter and *S. gigantea* subsp. *rhodopea* (Janka) Greuter (=*S. rhodopea* Janka). This concept has been applied in Turkey where two subspecies, *S. gigantea* subsp. *gigantea* and *S. gigantea* subsp. *rhodopea,* are presently recognised (Coode and Cullen 1967; Yıldız and Çırpıcı 2013). Since the publication of *Flora Orientalis* (Boissier 1867), several authors have reported *S. gigantea* in Lebanon, north-western Syria or Mount Amanus, located in the Turkish Hatay province (Post 1896, 1932; Bouloumoy 1930; Thiébaut 1936; Coode and Cullen 1967; Yıldız and Çırpıcı 2013). However, all specimens we could observe from these countries or localities, and identified as *S. gigantea*, actually correspond to *S. confertiflora* Chowdhuri belonging to section *Holopetalae* (Chowdhuri 1957). Accordingly, Mouterde (1966) and Greuter et al. (1984) mentioned *S. gigantea* as being absent from Syria and Lebanon and Thomé and Thomé (2007) did not include it in their flora of Lebanon.

Silene gigantea is, however, reported from Cyprus (Coode and Cullen 1967; Meikle 1977; Greuter 1995b, 1997; Yıldız 2006; Yıldız and Çırpıcı 2013) and in the countries neighbouring northern Greece (Stojanov and Stefanov 1924, 1948; Jordanov 1966; Demitri 1983; Paparisto et al. 1988; Micevski 1993; Vangjeli 2003; Goranova et al. 2013). However, despite the former numerous reports of this species, the precise distribution of it, as well as the taxonomic concept of it outside of Greece, are unclear, especially in Turkey, Bulgaria and Macedonia. In these areas, two subspecies, with ambiguous morphological differences, are systematically reported (see Jordanov 1966; Coode and Cullen 1967; Micevski 1993; Yıldız and Çırpıcı 2013).

Silene gigantea, which occurs on both the mainland and islands, is a morphologically polymorphic species with $2n = 24$ chromosomes (Ghazanfar 1983; Strid and Andersson 1985; Yıldız et al. 2008). The inflorescence organisation is very variable and, as mentioned by Greuter (1995b), morphological differences are easily found within the species while it remains "difficult to describe and quantify these differences and, if one tries, they tend to break down as soon as further material is added". The type of indumentum on the calyx is, however, a criterion of high taxonomic value for subspecies recognition (Greuter 1997). The latter author also reports on a possible introgression zone in Greece between *S. gigantea* subsp. *gigantea* and *S. gigantea* subsp. *rhodopea,* whereas Ghazanfar (1989) states that the different subspecies of *S. gigantea* do not hybridise in nature due to their different flowering periods. Greuter (1995b) also suggests that an introgression zone might exist in Turkey, which, incidentally, is not mentioned in Yıldız and Çırpıcı (2013).

Therefore, *Silene gigantea* represents a typical case of an unsolved taxonomic complex that we investigated with a morphological approach. In this study, we have analysed 178 collections of *S. gigantea* from several herbaria in order to cover the whole species distribution range. Additional material was collected in Greece (mainland and islands) and several specimens per population were analysed in order to take into account intra- and inter-population morphological variation. The material in hand allowed us to (1) investigate the morphological diversity, taking into account possible hybrid zones, and (2) give the global geographic distribution of the species.

Materials and methods

Plant material

One hundred seventy-eight (178) collections from AIX, ATH, ATHU, G, G-BOIS, G-BU, G-DC, PAL-Greuter, P, SO, W and Z herbaria, as well as from the personal collection of J. Zaffran (Greece, Kriti, Chania), were examined in the present study. Among them, 92 individuals from 62 localities covering the whole species distribution range could be measured due to their good conservation status. Most of these specimens were collected during field trips in Greece in May–June 2011 and May–June 2012 (Online Resource 1). A *specimina visa* list is given in Online Resource 2.

Distribution range

The distribution map was drawn using the Quantum GIS software Version 1.8.0 (http://qgis.org/fr/site/). Occurrences were based on the herbarium specimens observed. In figures and text, a code is given for each floristic region in Greece, Turkey and Cyprus. The code used for Greece corresponds to the phytogeographic areas described by Rechinger (1943, 1950, 1951) and modified from *Flora Hellenica* (Strid 1996, 2000; Strid and Tan 1997). The code for Turkish phytogeographic areas was extracted from the Flora of Turkey (Coode and Cullen 1967), whereas the Cypriot areas were taken from the Flora of Cyprus (Meikle 1977).

Morphological analyses

The morphological characters to be measured were chosen following Jeanmonod and Mascherpa (1982). However, additional characters were taken into account, especially those related to the inflorescence, which seems to present a great taxonomic value within the *S. gigantea* complex. Indeed, the main inflorescence of *S. gigantea* is a thyrsoid, composed by several opposite dichasia with decussate arrangement. The main inflorescence is usually preceded by sub-thyrsoids with a similar decussate arrangement. This results in a pyramidal (or conical) diplothyrsoid as defined by Weberling (1989). Furthermore, a preliminary analysis showed that some individuals present metatopic displacements of buds on flowering branches (Fig. 1) as well as accessory bud developments (Fig. 2) that increase the number of flowers at nodes. Consequently, only the main inflorescence, as described previously, was taken into account in the present study, in order to compare homologous organs.

In total, 84 characters (43 quantitative and 41 qualitative) were recorded on 92 individuals (Table 1). The numerical coding of qualitative characters (2, 4, 8, 16, 24, etc.) permitted the specification of unequivocal medium positions resulting from the addition of two different states (for more details see Jeanmonod and Mascherpa 1982). All quantitative characters were measured from dried specimens under a Leica/Wild M3Z stereomicroscope (6.5× to 40× magnification) using a caliper with a 0.05 mm precision. Petals were boiled before being measured (characters nos. 68–73).

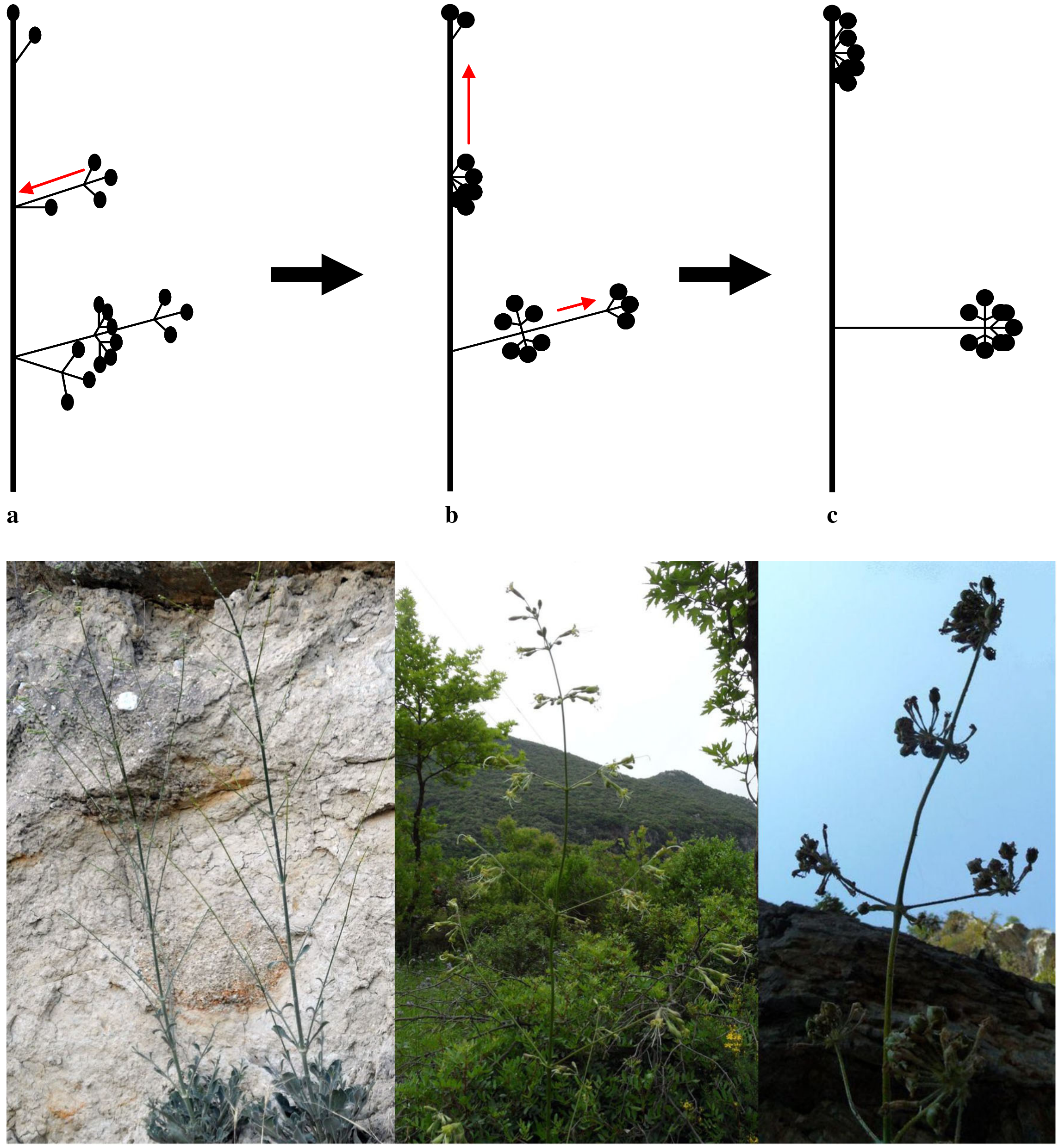

Fig. 1 The different inflorescence forms found in the *Silene gigantea* complex: **a** lax form, **b** intermediate form, **c** condensed verticillasters form. Pictures *below* correspond to *S. gigantea* subsp. *rhodopea*, *S. gigantea* subsp. *hellenica* and *S. gigantea* subsp. *gigantea*, respectively

Fig. 2 Illustration of accessory flower buds located at the dichasia axils

Characters used for multivariate analyses

Variables that appeared to be constant (highlighted in Table 1) or non-informative, as well as variables or individuals with missing data (highlighted in Online Resource 1), were not used in multivariate analyses, reducing the number of individuals to 84.

The use of both quantitative and qualitative characters in multivariate analyses is a sensitive issue. However, several different methods can accommodate for that. One of the most appropriate analyses for our dataset is the Factorial Analysis for Mixed Data (FAMD; Hill and Smith 1976; Escofier 1979; Saporta 1990; Pagès 2013). This analysis performs a Principal Component Analysis (PCA) on both quantitative and qualitative characters, in which the latter ones are transformed into quantitative characters before use, each character state being divided by its respective frequency (see Pagès 2013). Therefore, FAMD takes into account quantitative characters as a normalised PCA and qualitative characters as a Multiple Correspondence Analysis (MCA, see Benzécri 1973 for more details). The results can be interpreted with the usual rules of PCA and MCA (Pagès 2013).

In order to correctly compute the correlation coefficients in multivariate analyses, the number of individuals should be at least four times the number of characters (pers. comm. M. Baylac, MNHN). A preliminary FAMD that took into account all characters was used to assess the most informative ones. The 12 characters, which contributed more than 0.2 on the first three FAMD axes, were selected for further analyses. These characters included all those used by Greuter in his identification key of *S. gigantea* (1995b, 1997), i.e. T.BA, FLRE, FLMO, C.L., C.GL and NBGL (Table 1). The other six characters (T.NB, I.NB, DNPF, C.LO, C.LA and G.LO; Table 1) are of high taxonomic value in the genus *Silene* according to Melzheimer (1981), Greuter (1995a) and Jürgens (2006).

In order to determine the best clustering from multivariate analysis results, *k*-means analyses were performed on individuals' coordinates on all the FAMD axes (84 individuals and 12 characters). Different values of *k* (2–5) were tested with the following parameters: Hartigan–Wong algorithm, 50,000 iterations, 50,000 random sets and ten repetitions for each value of *k*.

Linear Discriminant Analyses (LDA), which are commonly used in morphological analysis to predict individuals' membership to a priori defined groups, are not allowed on a mix of qualitative and quantitative characters. However, Saporta and Cappe de Baillon (1977) developed an alternative method, named DISQUAL, which can take into account both types of characters in a single LDA. The DISQUAL method implies, first, to perform a Multiple Correspondence Analysis (MCA). Therefore, the set of data previously used (84 individuals × 12 characters) was transformed into a complete disjunctive table in which each quantitative variable was split into four classes in order to perform the MCA. Classes with too few individuals were grouped with their neighbouring class. Finally, a LDA was carried out on a selection of factors produced by the MCA, as reported in Saporta and Cappe de Baillon (1977).

Two different models of validation techniques were used with the LDA in order to test the clustering depending on the question addressed. The first model was the "leave-one-out" cross-validation, in which each individual is sequentially removed, then tested using the discriminant function computed on all remaining individuals. This validation was used for specimens located in the area analysed by Greuter (1995b, 1997). The second model was a specific case of the "2-fold" cross-validation method where two subsets, one training set used to determine the function and one validation set to test the function, were a priori determined in order to test specific groups of individuals. For each technique, ten replicates were performed.

In order to have a similar treatment for all quantitative characters and to take into account non-normal distributions, Kruskal–Wallis tests for non-parametric variables (with $\alpha = 0.05$) were carried out on LDA groups. For multiple tests, the significance level α was adjusted using a modified False Discovery Rate (FDR) method (Benjamini and Yekutieli 2001), which is less conservative than the Bonferroni's correction. The correction is calculated as follow: $\alpha / \sum_{i=1}^{k} (1/i)$, k being the number of tests performed. Boxplots, first and third quartiles, min and max values, medians and means were also computed. The results were further used for taxa description.

All statistical analyses were performed using the R software (R Development Core Team 2012). FAMD and MCA were performed using the ade4 library functions "dudi.mix" and "dudi.acm" (Thioulouse et al. 1997;

Table 1 List of the 84 characters examined with their coding states

Stump		
1	CESP	Caespitose form: (2) absent (3) poor (one stem) (4) medium (2–4 stems) (5) abundant (many stems)
2	P.VE	Vegetative part: (2) absent (4) rosette (8) vegetative stems (16) rosettes and vegetative stems
Stem		
3	T.BA[a]	Base: (2) absent (no rosette) (4) numerous scale of petiole (8) withered rosette (16) dense rosette
4	T.NB[a]	Internode number on the stem (below the inflorescence): integer
5	T.TY	Internodes type: (3) increasingly long (4) irregular (5) equal
6	T.RA	Ramification of the stem: (2) absent (4) small branches usually without flowers (8) 1–2 flowered branches (16) numerous flowered branches
7	T.HA	Plant height, in cm
8	T.LO	Plant height without inflorescence, in cm
9	T.H/	T.LO/T.HA ratio
10	T.DI	Stem diameter, in mm
11	T.D./	T.DI/T.HA ratio
12	T.DE	Indumentum density: (2) glabrous (4) sparsely (<2 hairs/mm^2) (8) densely (3–30 hairs/mm^2) (16) very densely (+30 hairs/mm^2)
13	T.L	Indumentum length: (2) absent (4) short (<0.2 mm) (8) medium (0.3–0.4 mm) (16) long (0.4–0.7 mm) (32) very long (>0.7 mm)
14	T.CO	Indumentum type: (2) absent (3) spreading (4) retrorse
Cauline leaves (second node from the base)		
15	F.FO	Shape of cauline leaves: (4) oblong (8) elliptic (16) oval (32) attenuated obovate
16	F.LG	Relative width: (4) narrow (8) medium (16) large
17	F.PE	Relative petiole length: (2) absent (4) short (8) long
18	F.EX	Apex (4) obtuse (8) acute (16) acuminate
19	F.LO	Lamina length including petiole, in mm
20	F.LA	Lamina width, in mm
21	F.DE	Indumentum density on the adaxial surface: (2) glabrous (3) sparsely (4) densely (5) very densely
22	F.L	Indumentum length: (2) absent (4) short (8) medium (16) long
23	F.MA	Indumentum on margin: (2) glabrous (4) the same as the upper surface (8) denser than the upper surface (16) longer than the upper surface
24	F.F	Indumentum on abaxial surface: (2) glabrous (3) the same as the upper surface (4) vein more pubescent (5) more pubescent than the adaxial
25	FB.F	Shape of basal leaves: (4) oblong (8) elliptic (16) oval (32) attenuated obovate
26	F/FB	Comparison between basal and cauline leaves: (1) identical (2) different
Inflorescence		
27	I.HA	Length, in cm
27bis	I.ET	Inflorescence state: (1) flower (2) fruit (3) flower and fruit (4) floral buds
28	I.LA	Width, cm
29	I.L/	I.HA/I.LA ratio
30	I.FO	Shape: (2) sub-umbellate (4) corymbiform (8) pyramid-shaped
31	I.SY	Branching type: (3) opposite branching (4) irregular (5) alternate branching
32	I.TY	Type: (3) erect (4) nutant
33	I.NB[a]	Number of internodes (within the inflorescence): integer
34	I.H	Length of the upper three internodes, in mm
34bis	DNPF[a]	Distance between the first node (the upper node) and the prophylls of terminal dichasium
35	I.PE	Peduncle length at the fourth node, in mm
36	I.BR	Length of the corresponding bract, in mm
37	I.B/	Ratio I.PE/I.BR
38	I.DE	Indumentum density (at the second internode from the extremity): (2) glabrous (4) sparsely (8) densely (16) very densely
39	I.L.	Indumentum length: (2) absent (3) short (4) medium (5) long
40	I.GL	Proportion of glandular hairs compared to the eglandular hairs (%): (2) 0 (3) 1–49 (4) 50–99 (5) 100
41	I.P/	I.PE/I.H ratio

Table 1 continued

Flower		
42	FLRE[a]	Distribution at branches tips (within the whole inflorescence): (3) homogeneous (4) floccosus
42bis	NBGL[a]	Condensed verticillaster number (except the terminal dichasium): integer
42ter	NGL/	NBGL/I.NB ratio
42 quarter	FLTH	Flowers distribution on thyrsoids (at the third node) (3) homogeneous (4) floccosus
43	FLNB	Number of flowers within the main inflorescence: integer
43bis	FLMR	Mean number of flowers per node: integer
44	FLMO[a]	Average number of flowers per dichasium: integer
45	FLMA	Maximum number of flowers per dichasium: integer
45bis	FLCT	Number of flowers in the terminal dichasium: integer
45ter	FLC1	Number of flowers at first node: integer
45 quarter	FLC2	Number of flowers at second node: integer
45 quint	FLC3	Number of flowers at third node: integer
46	FLTY	Type: (4) erect (8) nutant
47	FLPE	Length of pedicel, in mm
Calyx		
48	C.LO[a]	Length, in mm
49	C.LA[a]	Maximum width, in mm
50	C.LMA	Minimum width, in mm
51	C.L/	C.LO/C.LA ratio
52	C.FO	Shape: (4) cylinder-shaped (8) cylinder-clavate shaped (16) clavate-shape
53	C.BA	Base type: (3) attenuate (4) truncate (5) navel-like
54	C.DE	Indumentum density (2) glabrous (3) sparsely (4) densely (5) very densely
55	C.L.[a]	Indumentum length: (2) absent (4) very short (<0.08 mm) (8) short (0.08–0.45 mm) (16) long (+0.45 mm)
56	C.LOC	Indumentum localisation: (2) absent (3) only on the veins (4) mainly on the veins (5) everywhere
57	C.GL[a]	Proportion of glandular hairs compared to the eglandular hairs (%): (2) 0 (3) 1–49 (4) 50–99 (5) 100
58	C.DI	Relative length of teeth: (4) short (8) long
59	C.MA	Teeth margin: (2) absent (4) narrow (8) thick
Anthophore		
60	G.LO[a]	Length, in mm
61	G.L/	G.LO/C.LO ratio
62	G.PU	Indumentum: (2) glabrous (3) sparsely (4) densely
Fruit		
63	FRLO	Length, mm
64	FRLA	Width, in mm
65	FRFO	Shape: (3) oblong (4) ovate (5) conical
66	FRG/	Ratio G.LO/FRLO
67	FRDE	Fruit length out of calyx, in mm
Petal		
68	P.ON	Claw length, in mm
69	P.PU	Claw pubescence: (2) glabrous (8) ciliate on the margin
70	P.O/	Ratio P.ON/C.LO
71	P.AU	Auricle: (2) absent (3) narrow (4) thick
72	P.LI	Limb length, in mm
73	P.FE	Importance of the cleft: (3) below 1/3 (4) from 1/3 to 2/3 (5) more than 2/3
74	E.PU	Pubescence on stamen filaments: (2) glabrous (3) pubescent

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[a] Characters used for the multivariate analysis. Underlined characters are constant or non-informative

Chessel et al. 2004; Dray and Dufour 2007; Dray et al. 2007). For “dudi.mix”, classes coded by numbers were transformed into letters for all qualitative characters. LDA was run using the “lda” function of the MASS library (Venables and Ripley 2002), *k*-means clustering used the “*k* means” function included by default in the R software. Graphics illustrating FAMD results were obtained using “scat.mix.numeric” and “scat.mix.categorical” functions from RVAideMemoire library and the function “s.class” from ade4 library. For means comparisons, “kruskal.test” and “p.adjust” functions, included by default in R, were used.

Results

Specimens were classified a priori into five groups according either to Greuter’s taxonomic treatment (1995b, 1997), or to specific characters or to geographic features. As indicated by Greuter (1995b), specimens from Epirus (NPi, SPi) seem to present shared morphological features between *S. gigantea* subsp. *gigantea* and subsp. *rhodopea*. The Greek specimens from Aetolia-Acarnania (StE) and from the North of Euboea (WAe) were also difficult to assign using Greuter’s (1997) key. Therefore, all these specimens were a priori classified into a ‘hybrid’ group. Finally, specimens from Turkey, a country that Greuter (1995b, 1997) did not consider, were classified into a ‘Turkey’ group. This preliminary clustering into five groups (*gigantea, hellenica, rhodopea,* ‘hybrid’ and Turkey) played no role in the exploratory multivariate analyses.

Multivariate and clustering analyses

The FAMD, performed on 84 individuals and 12 characters, shows that the first and second factorial axes (F1 and F2) accounted for 39.75 % of the total variation (Fig. 3) and that 65.28 % was explained for by the first five axes (Table 2). The variables’ correlation coefficients with each factorial axes are given in Online Resource 3. On F1, four variables have correlations higher than 0.40 (FRLE, flowers distribution at peduncle tips: 0.67, NBGL, number of condensed verticillasters: 0.63, T.NB, number of internodes below the inflorescence: 0.55 and C.L., indumentum length: 0.44), whereas for F2 only one variable displays such a high correlation (C.LA, calyx width: 0.61). On F1 × F2 axes (Fig. 3), the Turkey barycentre is found near the *hellenica* one, whereas the ‘hybrid’ group is located in between the *gigantea* and the *rhodopea* groups. On the F1 × F3 axes (Online Resource 4), the respective barycentre of *hellenica*, Turkey and ‘hybrid’ are close to each other’s and placed between those of *gigantea* and *rhodopea*. The results of *k*-means clustering analyses with $k = 3$–5 (Table 3) always show three main clusters, which overall correspond to the three subspecies. Similar results are found when a Principal Coordinate Analysis (PCoA) based on the Gower’s distance is computed. Clustering with $k = 4$ defines a cluster of five insular individuals, a priori placed into the *gigantea* group, whereas $k = 5$ adds one cluster essentially formed by the ‘hybrid’ group (7 over 10 individuals) plus four specimens a priori classified as *gigantea*. The results with $k = 5$ are illustrated on the F1 × F2 scheme (Fig. 3) as well as on F1 × F3 and F1 × F4 schemes (Online Resources 4 and 5, respectively). The analysis shows that two of the five clusters (black and green) are clearly differentiated on the F1 axis, while the red and the blue clusters, which occur in an intermediate position on the F1 axis, are better differentiated on the F2 axis. The fifth cluster (sky blue) is clearly isolated on the F3 axis (see Online Resource 4). Considering F1 × F2 scheme (Fig. 3), the respective barycentre of *k*-means clusters (black, red and green) overlap with those of the *gigantea*, *hellenica*, and *rhodopea* groups, respectively, as a priori defined using Greuter’s key (Fig. 3; Table 3). The barycentre of the blue cluster is slightly off-centred with respect to that of the ‘hybrid’ group. The fifth cluster barycentre (sky blue) is clearly distinct from that of the black cluster on the F3 axis (see Online Resource 4). Consequently, each cluster can be characterised as follow, using the correlation circle for quantitative variables (Fig. 3), individuals’ character state for each qualitative variable (Online Resource 6) and the correlation coefficients (Online Resource 3):

- The green cluster consists of individuals with large calyces that are always covered by subsessile glands (<0.08 mm), a large anthophore, a loose inflorescence (without condensed verticillasters) with relatively few flowers, more nodes on the stem below the inflorescence (and fewer in the inflorescence) than those in other clusters, as well as an entirely or partially withered rosette at flowering. Table 3 and Fig. 3 show that all individuals belonging to this cluster were a priori classified into the *rhodopea* group, except one specimen (DP_120_1: ‘hybrid’ group from StE).
- Conversely, the black cluster is mainly characterised by small glandular or eglandular calyces always with hairs or glands >0.08 mm, a small anthophore, an inflorescence with numerous flowers, partly condensed in verticillasters, fewer internodes below the inflorescence (and more in the inflorescence) than those in other clusters, and a greening or few withered rosette leaves at flowering. Excepting three individuals (i.e. Heldreich_3067: *hellenica* group from StE; Balansa_s.n._2 and Huber-Morath_2157: Turkey group), all these

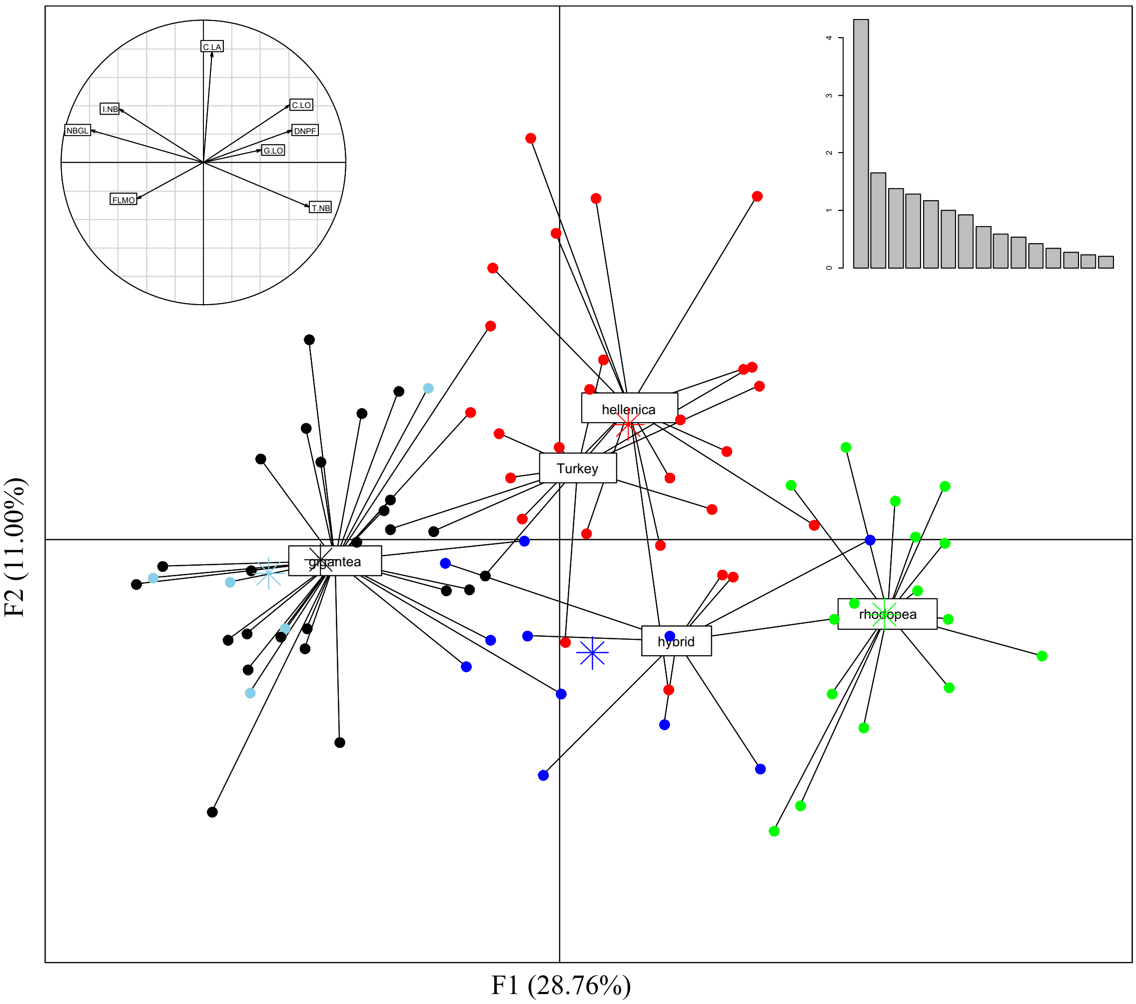

Fig. 3 Projection on FAMD F1 × F2 axes with eigenvalues *barplot* at the *upper right corner* and *correlation circle* at the *upper left corner*. *Colours* were given according to the results of the *k*-means analysis and stars correspond to the five *k*-means clusters' barycenter. *Links* and *labels* refer to a priori groups

individuals were a priori assigned to the *gigantea* group.

- The sky blue cluster, which comprises five individuals from the islands of Kriti, Samos and Cyprus differ from the previous cluster by calyces covered by a mix of glandular and eglandular hairs (>0.08 mm) and inflorescences with a high number of flowers. All these individuals were a priori assigned to the *gigantea* group.
- Individuals of the red cluster display calyces endowed with glandular hairs (>0.08 mm) that are larger than in the three former groups. The inflorescence is either partly condensed in verticillasters of flowers (as in the black cluster) or more lax (as in the green cluster). The rosette leaves are usually withered at flowering. This cluster comprises most individuals assigned to the *hellenica* group (15 from 16), eight individuals from the Turkey group (from 10) and two individuals of the *gigantea* group from Chios (DP_161_1 and Aucher-Eloy_493).
- Finally, the blue cluster differs from the previous one by a narrower eglandular or glandular calyx (always with hairs >0.08 mm) and rosette leaves that can be greening or withered at flowering. This cluster comprises seven individuals of the 'hybrid' group (from 10), originating from Pindus (SPi) and Euboea (WAe) as well as four individuals of the *gigantea* group, all from the Ionian Islands.

The overall congruence between the a priori classification and the *k*-means clustering reaches 69 %, whereas it is 67, 70, 94 and 100 % for the *gigantea,* 'hybrid', *hellenica* and *rhodopea* groups, respectively. Eighty percent of the Turkey group is merged with the *hellenica* cluster. The relatively low congruence value found for the *gigantea* group (67 %) is due to specimens displaying a large

Table 2 FAMD eigenvalues

Axes	Inertia	Cumulated inertia	Ratio	Cumulated ratio
1	4.314	4.314	0.288	0.28757247
2	1.649	5.963	0.398	0.39752393
3	1.381	7.343	0.490	0.48956533
4	1.285	8.628	0.575	0.57521827
5	1.164	9.792	0.653	0.65278907
6	0.997	10.789	0.719	0.71928607
7	0.921	11.710	0.781	0.78068713
8	0.715	12.425	0.828	0.82832553
9	0.587	13.012	0.867	0.8674898
10	0.534	13.546	0.903	0.90305867
11	0.416	13.962	0.931	0.9308232
12	0.337	14.299	0.953	0.95329753
13	0.272	14.572	0.971	0.97145253
14	0.225	14.797	0.986	0.986482
15	0.203	15.000	1.000	1

number of flowers and particular indumentum on calyces (mix of glandular and eglandular hairs), which form a specific cluster (sky blue) and to the four other specimens from Ionian Islands being classified as blue. A similar low congruence is found for the 'hybrid' group, which was built from heterogeneous origins, and which merely correspond to the blue cluster.

Linear discriminant analyses (LDA)

The Greek, Macedonian and Bulgarian specimens a priori assigned to subspecies *gigantea*, *hellenica* and *rhodopea* following Greuter's key were used in a first LDA in order to test Greuter's subspecies delimitation (Greuter 1995b, 1997). The results of this LDA (Table 4) show that 90.6 % of all individuals (58/64) were 'correctly' assigned using the leave-one-out validation method. Table 4(b) shows that subspecies *rhodopea* is clearly delimited, whereas two individuals of the *hellenica* subspecies are classified into the *gigantea* cluster (specimens Heldreich_3067 and DP_107_1 from the same locality near Arachova in Beotia, StE). Conversely, four individuals move from the *gigantea* subspecies to the *hellenica* cluster, i.e. specimen DP_121_3 from Lefkas (IoI) and three specimens from Chios (EAe) out of six: Rechinger_5408, DP_161_1 and Aucher-Eloy_493.

An additional LDA was computed and showed that the Turkey and 'hybrid' groups cannot be considered as distinct (results not shown). Therefore, all individuals from Turkey were then tested against the three subspecies in order to identify the group to which they belong. The results of this LDA (Table 3) show that nine specimens from Turkey (90 %) were assigned to the *hellenica* cluster. The remaining specimen, Bourgeau_s.n., was placed into the *gigantea* cluster. Although this latter individual presented almost the same features as all the other specimens from Turkey, it differs by the number of internodes below the inflorescence (5 against 7–10 for other Turkey specimens) and by a short distance between the first node and the prophylls of the terminal cyme, two characters that are preferably found in the *gigantea* group. However, this specimen, as well as the five other duplicates of Bourgeau's gathering, displays a retrorse stem indumentum, which is specific to individuals from Turkey. The same observation holds for specimen Balansa_s.n._2 for which LDA probabilities equivalently support an assignment to the *gigantea* and to the *hellenica* clusters (Table 3).

For the 'hybrid' group, the results of an additional LDA (Table 3) show that the three individuals of population DP_120 (StE) are unambiguously placed into the *hellenica* group. Individuals of population DP_105 (Euboea) are shared between the *gigantea* (2 individuals) and the *hellenica* group (1 individual), whereas specimens from Epirus are shared between the *gigantea* (1 individual) and the *hellenica* group (3 individuals).

Comparison of *k*-means and LDA for final assignment

The LDA results for the three subspecies in Greece are largely congruent with that of *k*-means clustering (Table 3). Furthermore, the LDA and *k*-means consistently cluster the Turkish specimens in subspecies *hellenica* (80 % overlap). Both analyses, however, point out some problematic populations, i.e. Chios (EAe), Epirus (SPi), Euboea (WAe), the Ionian Islands (IoI) and Beotia (StE). In addition, the sky blue cluster determined by the *k*-means analysis is clearly assigned to the *gigantea* subspecies according to LDA. Because the five concerned individuals are morphological extremes, the LDA classification is favoured against the *k*-means one for further analyses.

According to our analyses, specimens from Chios are shared between subspecies *gigantea* and *hellenica* (Table 3), although Greuter (1995b, 1997) attributed them to subspecies *gigantea*. Due to this ambiguous classification and to a geographically intermediate location between subspecies *gigantea* and *hellenica*, this population might be of true hybrid origin, and therefore requires further investigation. For that reason, this population is not taken into account in subsequent analyses in the present study.

Individuals from Epirus (SPi) and Euboea (WAe) were grouped together with the Ionian Islands specimens (*gigantea* subspecies) in a specific cluster by *k*-means but seem arbitrarily assigned to either the *gigantea* or the

Table 3 A priori classification of individuals together with *k*-means and LDA results

Specimens	Locality	A priori classification	*k*-means clustering			LDA a posteriori probabilities		
			$k = 3$	$k = 4$	$k = 5$	*gigantea*	*hellenica*	*rhodopea*
Baldacci_6_1	KK: Kriti	*gigantea*	2	4	Sky blue	1.00	0.00	0.00
Baldacci_6_2	KK: Kriti	*gigantea*	2	4	Sky blue	1.00	0.00	0.00
Baldacci_6_4	KK: Kriti	*gigantea*	2	4	Sky blue	1.00	0.00	0.00
DP_154_1	EAe: Rhodos	*gigantea*	2	1	Black	1.00	0.00	0.00
DP_154_3	EAe: Rhodos	*gigantea*	2	1	Black	1.00	0.00	0.00
DP_155_1	EAe: Samos	*gigantea*	2	1	Black	1.00	0.00	0.00
DP_155_2	EAe: Samos	*gigantea*	2	4	Sky blue	1.00	0.00	0.00
DP_155_3	EAe: Samos	*gigantea*	2	1	Black	1.00	0.00	0.00
DP_157_1	EAe: Samos	*gigantea*	2	1	Black	1.00	0.00	0.00
DP_157_2	EAe: Samos	*gigantea*	2	1	Black	1.00	0.00	0.00
DP_157_3	EAe: Samos	*gigantea*	2	1	Black	1.00	0.00	0.00
forsyth-Major_245_1	KK: Karpathos	*gigantea*	2	1	Black	0.99	0.01	0.00
forsyth-Major_245_2	KK: Karpathos	*gigantea*	2	1	Black	1.00	0.00	0.00
forsyth-Major_668	EAe: Ikaria	*gigantea*	2	3	Black	1.00	0.00	0.00
Greuter_S4098	KK: Kriti	*gigantea*	2	1	Black	1.00	0.00	0.00
Rechinger_ 8162	KK: Karpathos	*gigantea*	2	1	Black	1.00	0.00	0.00
Rechinger_7160	EAe: Rhodos	*gigantea*	2	1	Black	1.00	0.00	0.00
Rechinger_8286	KK: Karpathos	*gigantea*	2	1	Black	0.88	0.12	0.00
Rechinger_8517	EAe: Rhodos	*gigantea*	2	1	Black	1.00	0.00	0.00
Rigo_s.n.	Cyprus	*gigantea*	2	1	Black	1.00	0.00	0.00
Sintenis_664_1	Cyprus	*gigantea*	2	1	Black	1.00	0.00	0.00
Sintenis_664_2	Cyprus	*gigantea*	3	4	Sky blue	1.00	0.00	0.00
Margot_621	IoI: Zakinthos	*gigantea*	2	1	Black	0.99	0.01	0.00
Margot_s.n.	IoI: Zakinthos	*gigantea*	2	1	Blue	1.00	0.00	0.00
DP_121_1	IoI: Lefkas	*gigantea*	2	1	Blue	1.00	0.00	0.00
DP_121_3	IoI: Lefkas	*gigantea*	2	1	Blue	0.00	1.00	0.00
Hofmann_s.n.	IoI: Lefkas	*gigantea*	3	1	Blue	1.00	0.00	0.00
Rechinger_5408	EAe: Chios	*gigantea*	2	1	Black	0.05	0.95	0.00
DP_159_2	EAe: Chios	*gigantea*	2	1	Black	1.00	0.00	0.00
DP_161_1	EAe: Chios	*gigantea*	3	3	Red	0.00	1.00	0.00
DP_161_2	EAe: Chios	*gigantea*	3	3	Black	0.98	0.02	0.00
DP_161_3	EAe: Chios	*gigantea*	2	3	Black	1.00	0.00	0.00
Aucher-Eloy_493	EAe: Chios	*gigantea*	3	3	Red	0.21	0.79	0.00
DP_107_1	StE	*hellenica*	3	3	Red	0.76	0.24	0.00
DP_107_2	StE	*hellenica*	3	3	Red	0.00	1.00	0.00
DP_107_3	StE	*hellenica*	3	3	Red	0.00	1.00	0.00
DP_107_4	StE	*hellenica*	3	3	Red	0.00	1.00	0.00
DP_110_1	StE	*hellenica*	3	2	Red	0.00	1.00	0.00
DP_110_2	StE	*hellenica*	3	3	Red	0.00	1.00	0.00
DP_110_3	StE	*hellenica*	3	2	Red	0.00	1.00	0.00
Heldreich_3067	StE	*hellenica*	2	3	Black	1.00	0.00	0.00
DP_170	Pe	*hellenica*	3	3	Red	0.00	1.00	0.00
DP_66	Pe	*hellenica*	1	2	Red	0.00	1.00	0.00
DP_78_1	Pe	*hellenica*	3	2	Red	0.00	1.00	0.00
DP_78_2	Pe	*hellenica*	1	2	Red	0.00	1.00	0.00
DP_79	Pe	*hellenica*	3	3	Red	0.00	1.00	0.00
DP_80	Pe	*hellenica*	3	2	Red	0.01	0.99	0.00

Table 3 continued

Specimens	Locality	A priori classification	*k*-means clustering			LDA a posteriori probabilities		
			$k = 3$	$k = 4$	$k = 5$	*gigantea*	*hellenica*	*rhodopea*
DP_95_1	Pe	*hellenica*	3	2	Red	0.00	1.00	0.00
DP_95_2	Pe	*hellenica*	3	3	Red	0.00	1.00	0.00
Abd-ur-Rahman_s.n.	NC	*rhodopea*	1	2	Green	0.00	0.00	1.00
Behr_s.n.	Macedonia	*rhodopea*	1	2	Green	0.00	0.00	1.00
Burdet_10203	NE	*rhodopea*	1	2	Green	0.00	0.00	1.00
DP_137	NC	*rhodopea*	1	2	Green	0.00	0.00	1.00
DP_140	NC	*rhodopea*	1	2	Green	0.00	0.00	1.00
DP_185	StE	*rhodopea*	1	2	Green	0.00	0.00	1.00
DP_197	NC	*rhodopea*	1	2	Green	0.00	0.00	1.00
DP_206	NE	*rhodopea*	1	2	Green	0.00	0.00	1.00
DP_207	NE	*rhodopea*	1	2	Green	0.00	0.00	1.00
Hartvig_8500	NC	*rhodopea*	1	2	Green	0.00	0.00	1.00
Podlech_37588	StE	*rhodopea*	1	2	Green	0.00	0.00	1.00
Rechinger_3283b	NC	*rhodopea*	1	2	Green	0.00	0.00	1.00
Rechinger_6034	NE	*rhodopea*	1	2	Green	0.00	0.00	1.00
Rechinger_8935	NC	*rhodopea*	1	2	Green	0.00	0.00	1.00
Stříbrny_s.n.	Bulgaria	*rhodopea*	1	2	Green	0.00	0.00	1.00
Balansa_s.n._1	Turkey: B1	Turkey	2	3	Red	0.02	0.98	0.00
Balansa_s.n._2	Turkey: B1	Turkey	2	3	Black	0.43	0.57	0.00
Boissier_s.n._1	Turkey: B1	Turkey	3	2	Red	0.00	1.00	0.00
Boissier_s.n._2	Turkey: B1	Turkey	3	3	Red	0.15	0.85	0.00
Boissier_s.n._3	Turkey: C2	Turkey	3	3	Red	0.05	0.95	0.00
Boissier_s.n._4	Turkey: B1	Turkey	1	2	Red	0.00	1.00	0.00
Boissier_s.n._5	Turkey: B1	Turkey	3	2	Red	0.00	1.00	0.00
Bourgeau_s.n.	Turkey: C2	Turkey	3	3	Red	1.00	0.00	0.00
Charpin_26251	Turkey: C2	Turkey	3	3	Red	0.01	0.99	0.00
Huber-Morah_2157	Turkey: C2	Turkey	2	3	Black	0.00	1.00	0.00
DP_120_1	StE	hybrid	1	2	Green	0.00	1.00	0.00
DP_120_2	StE	hybrid	1	2	Red	0.00	1.00	0.00
DP_120_3	StE	hybrid	1	2	Red	0.00	1.00	0.00
DP_123_1	SPi	hybrid	1	2	Blue	0.06	0.94	0.00
DP_147	SPi	hybrid	1	2	Blue	0.00	1.00	0.00
DP_148	SPi	hybrid	1	2	Blue	0.11	0.89	0.00
DP_151	SPi	hybrid	2	1	Blue	1.00	0.00	0.00
DP_105_1	WAe	hybrid	2	1	Blue	0.01	0.99	0.00
DP_105_2	WAe	hybrid	1	2	Blue	1.00	0.00	0.00
DP_105_3	WAe	hybrid	2	1	Blue	1.00	0.00	0.00

The column $k = 5$ refers to colours used in Fig. 3 to identify the five *k*-means clusters, whereas 1–4 are used for *k*-means analyses with $k = 3$–4. Codes of phytogeographic areas in Greece: KK, Kriti-Karpathos area; EAe, Eastern Aegean; IoI, Ionian Islands; StE, Sterea Ellas; Pe, Peloponnese; NC, North Central; NE, North East; SPi, South Pindus; WAe, West Aegean area. Codes of the phytogeographic areas in Turkey: Turkey 2.1, Aegean coastline area; Turkey 2.2, West mainland area; Turkey 3.1, West Mediterranean area. Codes of the phytogeographic areas on Cyprus: 7, Kyrenia range (limestone). Within a given group, individual whose assignment disagrees with that of the majority is underlined

hellenica cluster using LDA. Based on a wider sampling, specimens from Epirus (NPi, SPi) always display similar indumentum of eglandular hairs on the calyx, whereas glandular or eglandular calyces are found in the Euboea population DP_105 (3 individuals). If the Epirote specimens are considered as belonging to a single

Table 4 LDA contingency tables

	gigantea	*hellenica*	*rhodopea*
(a) Without CV			
gigantea	33	0	0
hellenica	0	16	0
rhodopea	0	0	15
		0 % misclassification	
(b) With CV			
gigantea	29	4	0
hellenica	2	14	0
rhodopea	0	0	15
		9.4 % misclassification	

Rows are the a priori classifications. Columns are the a posteriori classifications

CV cross-validation using the leave-one-out technique

geographically distinct group from the Euboea populations, it becomes easier to differentiate them from the three subspecies by several morphological characters although they remain close to the Ionian Islands specimens (Table 5 and Online Resource 7). The Epirote specimens are therefore considered as a separate cluster named Epirus.

The Euboea populations, which were assigned to the *hellenica* subspecies by Greuter (1995b, 1997), mainly differ from this subspecies by the variability of the calyx indumentum. For this reason, the specimens from Euboea were removed from further analyses since they would require more investigations.

Specimens from Lefkas and Zakynthos (Ionian Islands) are mainly classified as *gigantea* (4/5) using LDA or included in the blue cluster (4/5). These specimens display specific calyx indumentum of eglandular hairs also found in specimens belonging to the blue cluster but, as before, we decided to follow the LDA classification and attributed them to subspecies *gigantea*, as did Greuter (1995b, 1997).

The assignation of one to two individuals from Beotia (StE) to the *gigantea* group by *k*-means and LDA analyses seems, after checking, to be due to local morphological variations. Specimens from this locality were kept in subspecies *hellenica* since the identification of all other specimens from the same populations was straightforward, even though their general morphology was less characteristic of *hellenica* than other populations belonging to this subspecies.

Thus, our morphological analyses as well as the associated decisions permitted the redrawing of the geographic distribution of the *S. gigantea* complex and highlighted some ambiguous populations showing variable mixed characters (i.e. populations from Chios and Euboea). The resulting distribution, illustrated in Fig. 4, is slightly different from Greuter's one (1995b, fig. 3 p. 113). Further morphological investigations within subspecies *gigantea* and *hellenica* revealed, respectively, two and three morphodemes (Table 5 and Online Resources 8 and 9), whose distribution correspond to the main phytogeographic regions. However, no morphologic variation was recorded within *S. gigantea* subsp. *rhodopea*.

Discussion

Distribution of *Silene gigantea*

The analysis of specimens from numerous herbaria and from our own observations (Online Resource 2 and Fig. 4) show that the overall distribution of *S. gigantea* corresponds to what is mentioned in the Med-Checklist (Greuter et al. 1984), in disagreement with several authors (Boissier 1867; Post 1896, 1932; Bouloumoy 1930; Thiébaut 1936; Coode and Cullen 1967; Yıldız and Çırpıcı 2013). Indeed, all specimens previously reported as *S. gigantea* from southern Turkey (Hatay region), NW Syria and Lebanon were mistaken for *S. confertiflora*. Therefore, *S. gigantea* seems absent from these areas.

Structure of *Silene gigantea*

Four main clusters are shown to meaningfully describe the structure revealed by both *k*-means and LDA analyses. Three of them, the *gigantea, hellenica* and *rhodopea* groups, roughly correspond to the three subspecies described by Greuter (1995b, 1997). The fourth one corresponds to a new morphogroup, named Epirus in this study. The analyses also show that the Turkey group actually belongs to subspecies *hellenica*. In addition to the former clusters, two populations, i.e. from Chios and Euboea, proved to be morphologically ambiguous and were discarded from further analyses. These two populations would need additional analyses (genetic ones for instance) to conclude on their assignment.

All specimens of *S. gigantea* (178) observed in this study, but not included in the FAMD analysis, corroborate the former results, obtained on a wide geographic sampling of 84 individuals. This study therefore fully supports the taxonomic delimitation of Greuter (1995b, 1997) and shows that three taxa (and possibly a fourth one) can be recognised within the *S. gigantea* complex throughout its distribution range. The *rhodopea* subspecies is the most distinctive one compared to the others, especially due to the indumentum of small glands on calyces and absence of condensed verticillasters. Subspecies *gigantea* and *hellenica* are more similar and characters slightly overlap, due to the fact that they are both chasmophytic. The Epirus group is less well-delimited than the three subspecies and seems to be related to the Ionian Islands populations of

Table 5 Morphologic comparison of morphodemes within *Silene gigantea* subsp. *gigantea* and *S. gigantea* subsp. *hellenica* and the Epirus group, which comprises specimens from the Epirus area (NPi, SPi) as defined in this study

	S. gigantea subsp. *gigantea*		*S. gigantea*. subsp. *hellenica*			Epirus group
	deme 1	deme 2	deme 3	deme 4	deme 5	
Area	KK/EAe-Cyprus	IoI	Pe	StE	Turkey	NPi-SPi
Calyx indumentum (C.GL)	Hairs, glands or both mixed	Hairs	Glands	Glands	Glands	Hairs
Calyx length (C.LO), mm	5.5–7(–10)	8–10	7–10	(8–)9–10	6.5–9.5	8–10
Petal limb length (P.LI), mm	(2–)3–4(–5)	2.5–4.5	3–6	(3–)6–8	2–4	(2.5–)3–4.5
Petal claw length (P.ON), mm	(4–)5–7	6–8	6–7(–8)	6–8.5	5–7	5.5–7
Number of verticillasters (NBGL)	(0–)1–4	1–3	1–3	1–3	0–3	1(–3)
Distance between the first nodes and the prophylls of terminal dichasium (DNPF)	0.1–9(–23)	(1–)7–25(–55)	(3–)17–27(–55)	16–40(–80)	8–30	(3–)10–40
Number of flowers in the terminal dichasium (FLCT)	2–15(–22)	2–5	(1–)3–5(–6)	3–7	1–3(–6)	1–5
Number of flowers at the first node (FLC1)	2–24(–33)	3–7(–16)	3–8(–12)	2–7(–17)	2–6(–14)	1–8
Number of flowers at the second node (FLC2)	6–16(–28)	(0–)6–14	(0–)6–14	6–13(–19)	3–6(–13)	0–8(–16)
Number of stem internodes below inflorescence (T.NB)	(3–)4–8	8–11	(7–)9–11	6–8	(5–)7–9(–10)	11–13
Plant height below inflorescence (T.LO), cm	(13–)25–50–(75)	55–110	(40–)65–110	45–110	50–80(–110)	100–140
Inflorescence length (I.HA), cm	6–22(–38)	(55–)85–110	(7–)10–22(–25)	7–25	7–15(–25)	4.5–7(–11)
Length of stem indumentum (T.L), mm	0.4–0.7	Variable	0.4–0.7	0.4–0.7	<2	0.3–0.4
Type of stem indumentum (T.CO)	Spreading	Spreading	Spreading	Spreading	Retrorse	Hirsute

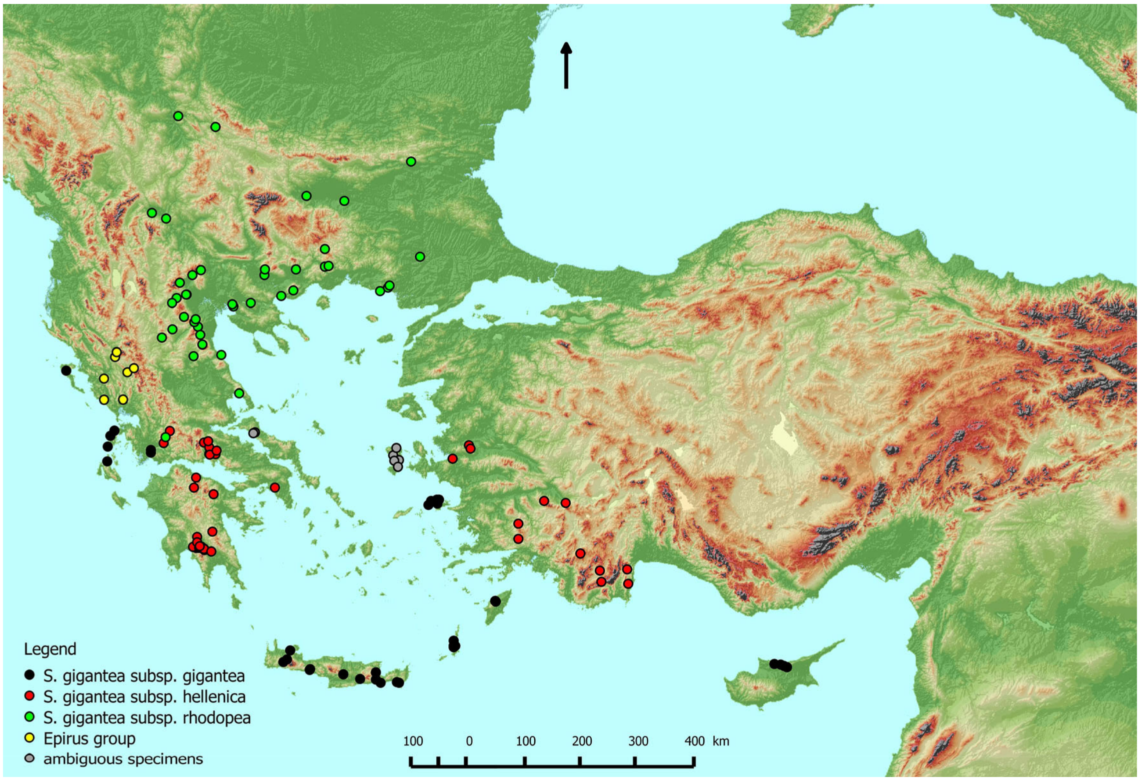

Fig. 4 Distribution map based on 178 observed individuals belonging to the three *Silene gigantea* subspecies as well as to the Epirus group (*yellow points*). Ambiguous specimens are coloured in *grey*. *Colours* on the map represent altitudinal ranges

subspecies *gigantea*. However, Online Resource 7 shows that most quantitative characters are significantly different among the four groups. Previous results and boxplot analysis (Online Resources 7, 8 and 9) allow the characterization of the three subspecies as well as of the Epirus group. Their morphological variations and taxonomic rank are discussed below.

Character significance

FAMD, combined with *k*-means analysis, proves to be an efficient tool to investigate structuring at the morphological level within *Silene gigantea*. In this study, the 12 selected morphological characters, among which six (T.BA, FLRE, C.L, C.GL, FLMO, NBGL) are those used by Greuter (1995b, 1997), correspond to the combination that reveals the most informative structure in the dataset. As already reported by Greuter (1995b), the calyx indumentum is a character of high taxonomic value, which appears to be constant in the *rhodopea* and *hellenica* subspecies, but that can vary in the *gigantea* subspecies from one island to another. However, as already mentioned, the Euboea specimens, with their variable calyx indumentum within the same population, represent an unsolved problem.

Distribution and morphological variation within *Silene gigantea* subsp. *gigantea*

Silene gigantea subsp. *gigantea* mainly occurs in the Greek Islands: Ionian Islands (IoI), Kriti-Karpathos (KK), East Aegean (EAe), except Lesbos from where it is absent, and Cyprus (Fig. 4). This subspecies is also found on the Greek mainland in a single locality, south of the Trichonis Lake in Etolia-Acarnania (StE). *Silene gigantea* subsp. *gigantea* probably exists on the Turkish coastlines between the city of Izmir and Marmaris (sectors B1 and C1 to C3), as reported by Coode and Cullen (1967). The occurrence of this taxon in the northern part of the Balkan Peninsula could be a misidentification of *S. gigantea* subsp. *rhodopea*.

Univariate statistics (Online Resource 8 and Table 5) computed on all individuals identified as *S. gigantea* subsp. *gigantea* by previous analyses allow defining two morphogroups.

Deme 1: Kriti-Karpathos/East Aegean-Cyprus

Meikle (1977) did not distinguish any intraspecific taxonomic rank within populations of *S. gigantea* on Cyprus. Yıldız (2006), who compared Turkish and Cypriot populations using morphological and palynological approaches, suggested recognising two different subspecies. Indeed, Cyprus populations are different from those from Turkey, since the former correspond to *S. gigantea* subsp. *gigantea* and the latter to *S. gigantea* subsp. *hellenica.* It should be noted, however, that the criterion of two scales on each petal suggested by Yıldız (2006) from the Cyprus specimens was not observed in the material used for this study. Therefore, *Silene gigantea* subsp. *gigantea* occurs on the islands of Kriti, Karpathos, Rhodos, Samos, Ikaria, Anafi, Nissiros, Kos, Agathonissios and Cyprus. It usually grows in crevices on calcareous cliffs between 25 and 700 m altitude. No collection has been reported from Kassos (E-Kriti), Kythera, Antikythera (W-Kriti) or from Lesbos (NE Aegean area). On Kriti, Karpathos and Rhodos, *S. gigantea* subsp. *gigantea* is more restricted, mainly being found on vertical cliffs in gorges, and forms small populations of chasmophytic plants. We noticed that, some years, individuals of these populations are almost all found as vegetative rosettes. On Samos, the subspecies is slightly more widespread from the coastline to mount Kerki (1433 m altitude). The single collection from Ikaria does not provide more information. On Cyprus, the species occurs only on the north face of the Pentadaktylos mountain range, which is the single calcareous substrate located in the North of the island. We did not observe the collection from Anafi (Kik), which is reported in the *Flora Hellenica* (Greuter 1997) and therefore we cannot confirm the presence of the subspecies in the Kiklad area. *Silene gigantea* subsp. *gigantea* does not seem to be present on other islands, but it probably occurs on the cliffs of the Aegean coastline of Turkey and in the Dilek Peninsula as reported by Coode and Cullen (1967).

Deme 2: Ionian Islands/Sterea Ellas

In the Ionian area, *Silene gigantea* subsp. *gigantea* occurs in Kerkira, Lefkass, Kefallinia and Zakynthos Islands between 180 and 360 m altitude. On the mainland, the subspecies is reported from a single locality in Etolia-Acarnania (StE) in the gorges located south of the Trichonis Lake between 100 and 500 m altitude. In contrast with populations from Kriti or Rhodos, *S. gigantea* subsp. *gigantea* from Lefkas is found growing in large populations with less restricting ecological requirements, as it is found on small calcareous cliffs at the edge of the maquis.

Silene gigantea subsp. *gigantea* is usually chasmophytic. This specificity is also found within some populations of *S. gigantea* subsp. *hellenica* from south Peloponnese (mount Taygetus). The specific adaptation to cliff habitats leads to a particular morphology, explaining why it is sometimes difficult to separate these two subspecies. However, the chasmophytic morphology with very condensed verticillasters of flowers is much more pronounced within subspecies *gigantea*, particularly in the Cretan and the East Aegean area (Samos). Compared with populations from deme 2, those from deme 1 are the most characteristic, usually with subsessile peduncles at each upper inflorescence node (except for the lower one), and an inflorescence which has a developed peduncle that generally opens at 90°. Small stems with few internodes, tall inflorescence with more flowers at the terminal and first verticillaster and small calices also characterise this morphotype. In addition, populations from deme 1 present a variable indumentum on the calyx (glandular on Ikaria, glandular or eglandular, or both, on Rhodos and Karpathos, mixed on Kriti, Samos and Cyprus), whereas only eglandular indumentum is found in deme 2. Noticeably, some individuals of deme 1 growing in crevices on cliffs develop several rosettes. This perennial feature has not been observed elsewhere.

Distribution and morphological variation within *Silene gigantea* subsp. *hellenica*

Silene gigantea subsp. *hellenica* occurs in Greece, the Peloponnese (Pe), as well as in the Attica, Boeotia and Phocis regions (StE). In Turkey, this subspecies is distributed in the following regions: Izmir, Manisa, Aydin, Denizli, Muğla (Aegean area), Antalya, Mersin (Mediterranean area) and Karaman (Central Anatolia area). Considering the weak sampling from Turkey (18 observed specimens from which 10 were measured) little is known about the ecology of the Turkey populations but they are reported between 500 and 1200 m altitude and in Greece they are widespread from 200 to 1100 m altitude.

In the Peloponnese, *S. gigantea* subsp. *hellenica* occurs in the South in the Taygetos and Parnonas mountains ranges where it grows mainly in gorges. It seems absent from Messenia (south-west Peloponnese) and Elis region (north-west Peloponnese) where the altitude is lower. In the north-west Peloponnese, it can be found in Achaea, in the surroundings of mounts Chelmos and Kyllini, and in the Corinthe region on mount Gerania. Here, the ecology is different from populations from the South Peloponnese, specimens are not specifically chasmophyte, but usually grow at the edges of maquis on rocky or earthy slopes. The presence of subspecies *hellenica* is not confirmed in the Attica region (despite an old record by Candolle, see Online Resource 2), but it is widespread in the Parnassus range. The Giona range seems to constitute the western limit of subspecies *hellenica* distribution. Thus, it is tempting to recognise three different demes within *S. gigantea* subsp. *hellenica* according to the following floristic regions: Peloponnese (Pe), Sterea Ellas (StE) and Turkey. However, this delimitation would be based on geographic and ecological arguments and not morphological features. It should be noted, however, that specimens from Turkey are characterised by particular indumentum (retrorse) at stem base which is not reported in the Greek populations. The highest number of internodes below the inflorescence, as found within populations from Peloponnese, is not expected for a chasmophytic morphotype. Differences found in calyx length (C.LO) and petal length (P.ON and P.LI) need more Turkish material to be confirmed (Online Resource 9; Table 5).

Distribution and morphological variation within *Silene gigantea* subsp. *rhodopea*

This subspecies occurs at the North of the Greek mainland (i.e. East Central (EC), North Central (NC) and North Eastern (NE) regions), Albania, Macedonia and Bulgaria from 20 to 1200 m altitude both on siliceous or calcareous bedrock. It grows in open rocky places or in crevices of small cliffs, usually at the edge of forests and never in chasmophytic conditions. In Greece the subspecies is widespread from the Olympus, Ossa and Pilion mountains to the Rhodope massif. No specimen from Albania was observed although the species has been reported in several Albanian Floras (Baldacci 1900; Demitri 1979, 1983; Paparisto et al. 1961, 1988; Vangjeli 2003). However, these citations most probably refer to subspecies *rhodopea*. In Macedonia, *S. gigantea* subsp. *rhodopea* seems to occur in the centre and the North (Jakupica mountain range). In Bulgaria and Serbia, it is widespread in the Stara Planina mountain range from the East to the West. It is absent in the range of Pindus and in occidental Turkey. Despite a disjunct distribution, the two specimens DP_118 (not measured) and DP_185 from the surrounding of the Trichonis Lake (Etolia-Acarnania, StE) seem to belong to *S. gigantea* subsp. *rhodopea* (confirmed by *k*-means and LDA analyses for DP_185), whereas a third occurrence (DP_120), close to the previous ones, better corresponds to subspecies *hellenica*. *Silene gigantea* subsp. *gigantea* is also reported in the same area, a little further south in a gorge. Therefore, the three subspecies seem to meet in this area, but additional collections would be necessary to confirm this.

No further morphological variation was detected within *S. gigantea* subsp. *rhodopea*, but no or very little material was available from Albania, Macedonia, Serbia and Bulgaria. Thus, further investigations in the northern Balkan Peninsula would be necessary to explore the morphological variation within this taxon.

The Epirus group

As already reported by Greuter (1995b), the specimens of *S. gigantea* from Epirus (NPi, SPi) present various mixed features. At first glance, such a mixed pattern seems to be due to hybridization and/or introgression between *S. gigantea* subsp. *gigantea* and *S. gigantea* subsp. *rhodopea*. LDA results assigned the four measured specimens from this region (DP_123_1, DP_147, DP_148 and DP_151) to *gigantea* or *hellenica* subspecies, indifferently. However, the morphological analysis of these specimens reveals several homogeneous features, among them the eglandular indumentum on the calyx which is of high taxonomic value. This character is shared with the Ionian Islands specimens of *S. gigantea* subsp. *gigantea* (Table 5; Online Resource 7). The present geographic distribution, as defined in this work (Fig. 4), does not support the existence of a recent hybrid zone between subspecies *gigantea* and *rhodopea*. Indeed, subspecies *gigantea*, which occurs in the Ionian Islands and in one mainland locality, is separated from subspecies *rhodopea* by the mountain range of Pindus, which crosses the Greek mainland from southern Albania to northern Peloponnesus. Our assumption is that contact between these two subspecies has not been possible in this region for a long time. Populations occurring in Epirus would have a narrow relationship with populations from Ionian Islands (as suggested by the specific indumentum feature of calyx) and would represent a morphological adaptation to mainland conditions after a colonisation from the Ionian Islands. Noticeable are the results of cultivation tests at the Botanical Garden of Geneva showing that the specific morphological features of population from Epirus (DP_123) are stable in the next generation, something that is not expected under the hybrid hypothesis.

Conclusion

Our study fully supports the taxonomic treatment proposed by Greuter (1995b, 1997). The diagnostic features he used to distinguish the three subspecies proved to be the most reliable ones amongst a high number of measured characters. *Silene gigantea* displays a large morphological plasticity related to ecological conditions, with specific adaptations to gorge cliffs or to insular conditions. Furthermore, our study shows that populations from Turkey and those from the southwestern part of mainland Greece are morphologically similar. Therefore, we suggest extending the distribution of *S. gigantea* subsp. *hellenica* to the Turkish mainland. Consequently, all specimens from Turkey presently identified as *S. gigantea* subsp. *rhodopea* actually refer to *S. gigantea* subsp. *hellenica*. The occurrence of *S. gigantea* subsp. *gigantea* along the Aegean coast of Turkey is, however, possible but not confirmed herein.

Our results also indicate that the analysed characters proved to be insufficient to unambiguously assign the specimens from Euboea and Chios.

Finally, we showed that the Cyprus specimens belong to *S. gigantea* subsp. *gigantea* and that populations from Epirus (NW Greece) are slightly differentiated but seem still to be related to the Ionian Islands populations of *S. gigantea* subsp. *gigantea*. Further investigations will be necessary on populations from Etolia-Acarnania where the two subspecies *gigantea* and *hellenica* seem to meet and on populations from the northern Balkan Peninsula to assess more precisely the morphological variation within *S. gigantea* subsp. *rhodopea*.

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