Multivariate morphometric analysis of *Petrorhagia* sect. *Petrorhagia* subsect. *Saxifragae* in Greece, with the description of a new species from SE Peloponnisos

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Abstract

The intra-generic relationships within the intriguing genus *Petrorhagia* are currently debated, as the genus is underrepresented in recent taxonomic studies. In this study, we investigate the relationships among the species of *Petrorhagia* sect. *Petrorhagia* subsect. *Saxifragae* occurring in Greece. Based on a combination of ordination methods and discriminant analyses of 36 macromorphological characters, five species are recognized in Greece. The analyses showed that all currently known species from Greece (*P. fasciculata*, *P. graminea*, *P. phthiotica* and *P. saxifraga*) represent distinct taxonomic entities. According to our results, two recently collected populations from SE Peloponnisos represent a fifth, new *Petrorhagia* species, which is first described and illustrated here as *P. laconica*. It is related to the widespread *P. saxifraga* and the Peloponnesian endemic *P. graminea*, but clearly distinct from both species by a combination of morphological characters, including indumentums, inflorescence, calyx, capsule and seed characters.

Key words: Caryophyllaceae, Greek flora, Morphology, *Petrorhagia laconica*

Introduction

Caryophyllaceae include approximately 3000 species, distributed in about 100 genera (Hernández-Ledesma et al. 2015). It has a primarily north-temperate distribution with a diversity center in the eastern Mediterranean and Irano-Turanian regions, while presence in the tropics and the southern hemisphere is limited and mostly at higher elevations (Bittrich 1993, Greenberg & Donoghue 2011,

Rabeler & Hartman 2005, Hernández-Ledesma et al. 2015). The phylogenetic relationships within Caryophyllaceae is a field of active research (Fior et al. 2006, Harbaugh et al. 2010, Greenberg & Donoghue 2011, Hernández-Ledesma et al. 2015, etc.) and genera delimitation has been altered in some cases, reflecting the results of recent molecular studies (e.g. Dillenberger & Kadereit 2014).

Petrorhagia (Seringe in Candolle 1824: 354) Link (1831: 235) (Caryophylloideae, Caryophylleae) is a small genus of ca. 33 species, distributed in the Mediterranean area, Europe and western Asia (from the Canary Islands east to Kashmir), with a diversity center in Greece and Turkey (Ball & Heywood 1964, Georgiou 1997). In the past, it was placed in different genera like *Dianthus* Linnaeus (1753: 409), *Tunica* Scopoli (1772: 298), *Kohlrauschia* Kunth (1838: 108) and *Gypsophila* Linnaeus (1753: 406). Evidence from both traditional and phylogenetic systematics, support its recognition as a distinct taxon at the genus rank, which clusters as sister to a clade including *Dianthus* Linnaeus (1753: 409) and *Velezia* Linnaeus (1753: 332) (Ball & Heywood 1964, Harbaugh et al. 2010, Greenberg & Donoghue 2011, Pirani et al. 2014, Hernández-Ledesma et al. 2015, Hilooğlu et al. 2016).

Recent data on the infrageneric classification of *Petrorhagia* is still scarce, as the genus is rather under-sampled. The available data (Greenberg & Donoghue 2011, Hilooğlu et al. 2016) are fragmentary and do not allow a reliable infrageneric classification. As a result, most recent treatments of the genus follow the monograph of Ball & Heywood (1964) that recognizes five sections within *Petrorhagia*, distinguished by petal and seed characters, life-cycle, leaf venation and the presence/absence of epicalyx bracts. Out of the five sections, sect. *Petrorhagia* includes ten species (seven of them present in Greece and five endemic to the country) distributed into two subsections: subsect. *Saxifragae* Ball & Heywood (1964: 148) and subsect. *Thessalae* Ball & Heywood (1964: 156). The two subsections are distinguished by stem branching (much branched vs. simple or with few branches), inflorescence (flowers solitary or fasciculate vs. capitates) and bract (lanceolate or ovate, membranous, 1-veined vs. broadly ovate or suborbicular, membranous and 1-veined or brown-scarious and many-veined) morphological characters.

In May 2014, during field work in south-eastern Peloponnisos, we detected a population of an interesting *Petrorhagia* species, growing on coastal sand-dunes, an unusual habitat for the members of the genus. Careful examination of the collected specimens revealed that plants from SE Peloponnisos belong to *Petrorhagia* sect. *Petrorhagia*, and they resemble *P. saxifraga* (Linnaeus 1753: 413) Link (1831: 235), a widely distributed species in central and southern Europe and south-western Asia. After a detailed morphological study, it became apparent that plants from SE Peloponnisos are clearly distinct from *P. saxifraga*, as well as from all known *Petrorhagia* species

and they belong to a new, undescribed species. Dr. E. Kalpoutzakis had also found the same species in May 2013 in another locality of SE Peloponnisos.

The morphological characters of the new species from SE Peloponissos, described here as *Petrorhagia laconica*, support its classification in subsect. *Saxifragae*. The taxonomy of the subsect. *Thessalae* is rather clear compared to that of the subsection *Saxifragae*. The species belonging to the latter are notorious for their taxonomic complexity; their confusing taxonomy seems to be due to the rather few species-delimitating characters.

Species delimitation within *Petrorhagia* sect. *Petrorhagia* subsect. *Saxifragae* has never been studied in detail. All species of subsect. *Saxifragae*, except *P. riphaea* (Pau & Font Quer) Ball & Heywood (1964: 156) that is the only species of this group distributed in Africa (Morocco), are distributed in Greece. Thus, the scope of this study is manifold; it aims to resolve the apparent ambiguities within subsection *Saxifragae* and to evaluate the taxonomic validity of the two recently discovered deviating *Petrorhagia* populations. More specifically, we aim: (a) to clarify the patterns of morphological variation within *Petrorhagia* sect. *Petrorhagia* subsect. *Saxifragae* in Greece, (b) to disclose the level of morphological differentiation for recognized species, (c) to indicate the most informative characters for the identification of the species, and (d) to describe the newly discovered species.

Material and methods

Plant Material

Data for the multivariate analyses were recorded from herbarium specimens deposited in UPA and ATH (acronyms follow Thiers 2015) belonging to *Petrorhagia fasciculata*, *P. graminea*, *P. phthiotica* and *P. saxifraga*. We collected several specimens of *P. laconica* in the field. The specimens included in the analyses were selected so as to represent the taxa's entire distribution range in Greece, as well as the morphological variation in each species. Only well-preserved and intact specimens were considered in the analyses. The total number of herbarium specimens included in the analyses was 97. A list of the examined specimens is provided in Table S1. Each specimen was preliminary identified as one of the five aforementioned species. Species identification and nomenclature are according to Ball & Heywood (1964), Georgiou (1997) and Dimopoulos *et al*. (2013).

Characters recorded

Twenty-seven quantitative and 9 qualitative (binary) morphological characters were scored for the herbarium specimens included in our analyses (Table 1). The selected characters represent all those previously quoted as diagnostic and are the ones most commonly used in the latest comprehensive monographs, regional and local floras (Ball & Heywood 1964, Coode & Cullen 1967, Georgiou 1997). All the characters in the herbarium specimens were measured under a stereomicroscope using a ruler with the precision of 0.5 mm.

Morphometric Analyses

Basic statistical parameters (mean, minimum and maximum value, standard deviation, 5^{th} and 95^{th} percentiles) were calculated for each species included in the analyses. We used a combination of ordination methods and discriminant analyses in the morphometric analyses of the specimens (Marhold 2011).

We first tested whether any character did not have a normal distribution using the Shapiro-Wilk statistic and we $\log_{10}$-transformed any characters that deviated from normality. As a second step, we performed several stepwise canonical and classificatory Discriminant Analyses (DAs) in order to: i) identify the variables having the highest potential as diagnostic characters and ii) test the discrimination rules' effectiveness by using the Leave-One-Out cross-validation (LOO). The inclusion and the exclusion criterion's value in the stepwise DAs was set at F = 0.05 and F = 0.1, respectively. Wilk's lambda was used to assess each variable's and Discriminant Function's (DF) significance. The Maximum Chance Criterion (MCC) and the Proportional Chance Criterion (PCC) were used to determine whether the prediction equation was better than random chance (Huberty & Olejnik 2006). The DAs were checked for outliers. Finally, we examined the morphological variation in our dataset in relation to the species boundaries by analyzing the characters' ranges using core functions in the R computing environment (R Core Team 2015). All the analyses were carried out using IBM SPSS 24. The stepwise discriminant analysis plots, as well as the violin boxplots were visualized via the ggplot2 (Wickham, 2009) package in the R computing environment. The Maximum and Proportional Chance criteria were ran under the Zclass algorithm kindly provided by John D. Morris (Florida Atlantic University).

Results

Characters scored

The basic statistical parameters for all species are given in Table S2. The characters' morphological variation is graphically depicted in Figure S1, respectively. The scored characters' distribution

departed from normality; therefore, all the characters were $\log_{10}$-transformed prior to the analyses. After the logarithmic transformation, all the characters were normally distributed.

Multivariate analyses

We performed several canonical and discriminant analyses in order to find the most important characters separating the *a priori* defined groups, as well as to test the potential success of such separations. The DA clearly discriminates the five *Petrorhagia* species. Sixteen of the characters emerged as statistically significant (Table 2), with LOO showing a 100% correct classification, being significantly better than random chance for both the PCC and MCC ($P < 0.001$, Table 3). These characters showed no to little overlap across the five species (Fig. 1). The first (DF1), the second (DF2), the third (DF3) and the fourth (DF4) discriminant function explain 86.6%, 11.5%, 1.3% and 0.6% of the total variation, respectively (Table 2, Figs. 2, S2). Among the morphological characters used, the presence and the type of the indumentums are the most informative characters for species distinction in *Petrorhagia* sect. *Petrorhagia* subsect. *Saxifragae*. The most discriminative function (DF1) is primarily influenced by the presence of eglandular hairs at the middle part of the stem (a9). The presence of glandular hairs on calyx (a34) and eglandular hairs at the lower part of the stem (a8) have also high discriminant validity in DF1 (Table 2). The presence of eglandular hairs on calyx (a35) and glandular hairs at the middle/upper part of the stem (a6/a7) are the characters loading most heavily on DF2 and DF3, respectively (Table 2). Undoubtedly, the five *Petrorhagia* species included in the analyses are clearly distinct from one another (Table 3, Figs. 1-2).

Discussion

Multivariate morphometrics provide a powerful mean in order to assess the variation patterns at various taxonomic levels and is especially useful in disentangling the boundaries between complex taxa (e.g., Kougioumoutzis et al. 2015), as well as in unveiling the most informative and discriminating characters that differentiate among them (Marhold 2011). In the present study, the application of multivariate morphometrics resulted in important conclusions with taxonomic consequences. More specifically, the results of morphometric analyses enabled the delimitation of five well-separated species and the elucidation of the identity of the deviating *Petrorhagia* populations from SE Peloponnese: the latter constitute a new, distinct and rather rare taxon, herein described as *Petrorhagia laconica*.

Petrorhagia sect. *Petrorhagia* subsect. *Saxifragae* is a taxonomically complicated group with several overlapping characters. Our morphometric analyses, however, showed that all species are

clearly distinguished each other. Their distinction can be achieved even without using seed morphological characters, an important diagnostic feature in the genus *Petrorhagia*. The latter were not included in the morphometric analyses, as most specimens used were not in fruit.

The presence and type of hairs on stem and calyx have emerged as important diagnostic characters. The presence of uniformly eglandular hairy stems throughout their length is a unique feature of *P. laconica*. *P. fasciculata* and *P. phthiotica* are the only species with uniformly glandular hairs on stems, while the stems of *P. graminea* are covered by a mixture of glandular and eglandular hairs, at least at base. All examined specimens of *P. saxifraga* had glabrous stems. Furthermore, *P. laconica* is the only species with uniformly glandular hairy calyx. The calyx of *P. graminea* is predominantly covered by eglandular hairs, often mixed with few glandular hairs. All examined specimens of *P. fasciculata* had eglandular hairy calyces, while all calyces in *P. saxifraga* and *P. phthiotica* were glabrous.

Length of the inflorescence, number of lateral branches and flowers per flowering stem, width of lower leaves and the ratio pedicel/calyx length further differentiate *P. laconica* from all other species of sect. *Saxifragae* (Table S2, Figure S1). Moreover, *P. laconica*, together with *P. saxifraga*, are the only species with calyces enclosed by epicalyx bracts; in the specimens of all other species (including *P. fasciculata*) there are not bracts enclosing the calyx. The small petals and the large number of flowers per terminal inflorescence unit easily distinguish *P. fasciculata* from all other species. *P. graminea* and *P. saxifraga* are the only species with 3-veined sepals and they also have the largest petals. The 3-veined leaves and the short stems further differentiate *P. phthiotica* from all other members of sect. *Saxifragae*.

The results of our analyses have revealed a diagnostic character not previously used in the genus *Petrorhagia*. Although the length of leaves is similar in all species examined, leaf width varies among species. *P. saxifraga* and *P. laconica* have narrow basal leaves compared to the other species. Furthermore, cauline leaves are diminishing in size towards apex in all species, but diminishing in leaf length only, while leaf width remains stable, has been observed only in *P. laconica* (Table S2, Figure S1).

Taxonomy

Petrorhagia laconica Trigas, Kalpoutzakis & Kougioumoutzis, sp. nov. (Fig. 3)

Related to *P. saxifraga*, from which it differs in the following morphological characters: underground stock rooting at nodes; stems glaucous-green, procumbent to ascending or erect, simple

or rarely with few [1–3 (–6)] racemose lateral branches at the upper 1/3, minutely pappilose-scabridulous except the glabrous 1–4 upper internodes; leaves rigid, closely appressed to stem; flowers solitary or rarely paired, 1 (–2) per flowering stem, rarely up to 6 and then inflorescence racemose; calyx minutely glandular pubescent; ribs obscurely 1-veined, purplish-brown; capsule 2.5–3 mm long, sparsely tuberculate at the lower half; seeds with flat, not thickened margins.

Type:—GREECE. Peloponnisos: sand dunes ca. 3.5 km NW of Neapoli village, 2 m, 36°31.721' N, 23°01.273' E, 22 May 2017, *Trigas 6311* (holotype ACA, isotype UPA).

Perennial herb with branched, slender, underground woody stock rooting at nodes, producing few to several flowering stems. Flowering stems glaucous-green, procumbent to ascending or erect, 7–40 cm long, green to purplish-brown, simple or rarely with few [1–3 (–6)] paniculate lateral branches at the upper 1/3, minutely pappilose-scabridulous except the glabrous 1–4 upper internodes; internodes markedly unequal, larger at the middle of the stem, diminishing in size towards base and apex. Leaves linear-subulate, acuminate to caudate, rigid, 1-veined, 5/4–1/3 as long as internodes, united at the base into a sheath 1–1.5 mm long, with a purplish-brown ring at the base; lower ones 7–19 × 0.5–1 mm, minutely roughly papillose at margin, otherwise glabrous; cauline leaves closely appressed to stem, 3–16 × 0.5–1 mm, glabrous. Flowers solitary or rarely paired at apices of stems and branchlets, subtended by 4 hyaline, lanceolate, glabrous, straw-colored epicalyx bracts, sometimes purplish tinged at midrib. Calyx 3–6 mm long, cylindrical, minutely glandular pubescent; ribs obscurely 1-veined, purplish-brown; teeth 0.5–1 mm long, oblong-triangular, obtuse. Petals 4–8 × 1.5–3 mm, white, upper surface with three longitudinal purplish stripes at the base, lower surface purple veined, with glabrous claw not distinctly delimited from the emarginate lamina. Anthers white. Ovary ca. 1 mm long, oblong-ovoid; anthophore ca. 0.5 mm long, glabrous, green. Capsule 2.5–3 mm long, pale brown, ovoid, smooth or sparsely tuberculate at the lower half. Seeds 1.0–1.2 × 0.7–0.8 mm, ovate-oblong, with flat, not thickened margins, mucronate with a long, terminal mucro, reticulate on both surfaces, blackish-brown.

Etymology:—Species name refers to Laconia (also known as Lacedaemonia), a region that covers almost the entire SE part of the Peloponnisos, where the new species was collected. This name goes back to the historical times, when Laconia was the principal region of the Spartan state.

Distribution:—*Petrorhagia laconica* is distributed in the Malea Peninsula in SE Peloponnisos. Two populations have been discovered so far; the first one is located at the Gulf of Neapoli, right across Elafonisos Island, while the second one was found close to Sykea village, ca. 25 km N-NW of the

first population. It is the southernmost distributed species of *Petrorhagia* sect. *Petrorhagia* subsect. *Saxifragae* in Europe.

Habitat and ecology:—*Petrorhagia laconica* is known to grow in two different habitats. In Neapoli, all individuals were observed to grow in the inner sand dune zone, usually surrounded by sclerophyllus shrubs, like *Ceratonia siliqua* Linnaeus (1753: 1026), *Pistacia lentiscus* Linnaeus (1753: 1026), *Pyrus spinosa* Forsskål (1775: 211), *Smilax aspera* Linnaeus (1753: 1028) and *Anthyllis hermanniae* Linnaeus (1753: 720). *P. laconica* grows in stabilized sand dunes among the shrubs, together with other sand dune adapted taxa, like *Scirpoides holoschoenus* (L.) Soják (1972: 127), *Elytrigia juncea* (L.) Nevski (1933: 17), *Asphodelus ramosus* Linnaeus (1753: 310), *Centaurea sonchifolia* Linnaeus (1753: 915), *Linaria tenuis* (Viv.) Sprengel (1825: 795) and *Anthemis tomentosa* Linnaeus (1753: 893).

Close to Sykea village, *Petrorhagia laconica* grows in small remnant patches of macchie and phrygana formations that form a mosaic among the abundant olive groves. The population occupies a small area at 120–140 m a.s.l., on flysch. Shrub species composition of macchie is similar to that of the *locus classicus*, with *Pistacia lentiscus*, *Pyrus spinosa*, *Olea europaea* Linnaeus (1753: 8), *Lavandula stoechas* Linnaeus (1753: 573) and *Phlomis fruticosa* Linnaeus (1753: 584) being the predominant species. *P. laconica* usually grows in small patches among the shrubs, together with *Sarcopoterium spinosum* (L.) Spach (1846: 43), *Ballota acetabulosa* (L.) Bentham (1834: 595), *Cistus salviifolius* Linnaeus (1753: 524), *Globularia alypum* Linnaeus (1753: 95), *Hypericum tetrapterum* Fries (1823: 236), *Silene nocturna* Linnaeus (1753: 416), etc.

Additional specimens examined:—GREECE. Peloponnisos: ca. 3.5 km S-SE of Sykea village, macchie and phrygana among olive groves, on flysch, 130 m, 36°43.970’ N, 22°57.079’ E, 06 May 2013, *Kalpoutzakis 4294*. ibid.: 29 July 2017, *Kalpoutzakis 4959*. ca. 2.5 km S-SE of Sykea village, macchie and phrygana among olive groves, on flysch, 120 m, 36°44.552’ N, 22°57.209’ E, 22 May 2017, *Trigas 6312*.

Conservation:—In Neapoli Laconias, *Petrorhagia laconica* grows on a large sandy beach ca. 3.5 km long, between Neapoli and Viglafia village. The width of sand dunes along this beach ranges from 30 to 150 m, and the species is exclusively confined at the inner sand dune zone, where it is fairly rare. It usually forms small groups of 5–30 individuals, but single remote plants were also observed. The total population of Neapoli is estimated to include ca. 300–400 individuals, mainly concentrated at the eastern part of the beach. The Sykea population counts ca. 500 individuals distributed in an area of ca. 0.1 km^2.

Habitat loss and touristic development are the main threats that *P. laconica* faces at its lo*cus classicus*. Sand dunes have been partly turned into cultivated fields at the eastern half of the beach,

almost certainly leading to a significant population decline. Species' habitat also hosts thousands of swimmers during summer, being an unpredictable risk factor for its long term survival. The whole area, however, belongs to the Natura 2000 network of protected areas (Periochi Neapolis kai Nisos Elafonisos, GR2540002), and numerous human activities that could create additional risks for the population of *P. laconica* in Neapoli area are fortunately forbidden.

Habitat loss is also the main threat that *P. laconica* faces in Sykea area, as more than 50% of the area previously covered with natural vegetation have been turned into cultivated fields. As a result, it is irrefutable that the species has suffered a severe population decline in the near past, which can be estimated as probably approaching the percentage of habitat loss.

The flora of SE Peloponnisos has been intensively explored during the last decades (e.g. Kalpoutzakis & Constantinidis 2005, 2006, Greuter 2012, Kalpoutzakis et al. 2012). The recent discovery of *P. laconica* indicates that the species should not be common in this area. The habitat types that it grows (especially the mosaic with macchie and phrygana formations), however, are abundant in SE Peloponnisos. Consequently, the existence of additional populations of this tiny and easily overlooked species cannot be ruled out. The species' extent of occurrence (EOO) does not exceed 25 km^2 and since *P. laconica* is a very local species, its area of occupancy (AOO) is much narrower, apparently less than 1 km^2. Ergo, due to: i) the restricted EOO and AOO, ii) the current existence of max. 900 mature individuals distributed in 2 populations and iii) the high possibility that the extant species' localities and populations could be eradicated as a result of human interference, *Petrorhagia laconica* is assigned to the Endangered (EN) IUCN (2001) category, following criteria B1ab(i,ii,iii,iv)+2ab(i,ii,iii,iv). *P. laconica* should be carefully monitored, cultivated ex situ in botanical gardens and special conservation measures be taken to safeguard its populations.

Taxonomic relationships:—*Petrorhagia laconica* undoubtedly belongs to *Petrorhagia* sect. *Petrorhagia*, as it is indicated by its 1-veined leaves, the presence of epicalyx bracts, the not abruptly clawed petals, the reticulate, blackish-brown seeds and the perennial life-cycle. The thickened seed margin is not a common feature of all species in *P.* sect. *Petrorhagia*, as it is indicated by Ball & Heywood (1964). *P. fasciculata*, *P. thessala* (Boiss.) P.W. Ball & Heywood (1964: 156), *P. dianthoides* (Sm.) P.W. Ball & Heywood (1964: 158) and *P. grandiflora* Iatrou (1985: 441) have flat, thin seed margins, similar to that of *P. laconica*.

Within *Petrorhagia* sect. *Petrorhagia*, *P. laconica* shows close affinities to the members of subsect. *Saxifragae*. The solitary or paired flowers and the lanceolate, 1-veined epicalyx bracts advocate for the inclusion of *P. laconica* within subsect. *Saxifragae*. Flower and seed morphological characters further support this taxonomic view. The simple or sparingly branched stems, however, resemble the members of subsect. *Thessalae*, which otherwise have significant morphological

differences from *P. laconica* (e.g. capitate inflorescence, broadly ovate or sub-orbicular bracts, larger and different petals, etc.).

P. laconica appears to be one of the most distinct species in *P*. sect. *Petrorhagia*. The results of multivariate morphometric analyses (Tables 2, 3, Figures 1, 2) clearly support its distinct taxonomic position within subsect. *Saxifragae* The most related species seem to be *P. saxifraga* and *P. graminea*, which, however, show significant morphological differences from *P. laconica* (see also Table 4). The possibility of vegetative reproduction and the tuberculate capsule have not been recorded in other Greek *Petrorhagia* species, and they probably represent unique features within the whole genus. The discovery of *P. laconica* is in line with Trigas et al. (2007, 2012), which state that SE Peloponnese is one of the important regions in Greece in terms of endemic plant species richness and conservation, as well as a significant diversity center for *Petrorhagia,* since more than 30% of the whole genus' species diversity is hosted in this small region.

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TABLE 1. Characters used in the morphometric analyses.

Character abbreviation	Detailed character definition
a1	Number of flowering stems
a2	Length of flowering stems (cm)
a3	Length of the unbrached part of flowering stem (cm)
a4	Length of the inflorescence (cm)
a5	Glandular hairs present at the lower part of the stem
a6	Glandular hairs present at the middle part of the stem
a7	Glandular hairs present at the upper part of the stem
a8	Eglandular hairs present at the lower part of the stem
a9	Eglandular hairs present at the middle part of the stem
a10	Eglandular hairs present at the upper part of the stem
a11	Number of flowers per flowering stem
a12	Number of lateral branches per flowering stem
a13	Length of lateral branches per flowering stem (cm)
a14	Number of flowers per lateral branch
a15	Number of flowers per terminal unit of the inflorescence
a16	Length of lower leaves (mm)
a17	Width of lower leaves (mm)
a18	Length of middle leaves (mm)
a19	Width of middle leaves (mm)
a20	Length of internodes at the middle of the stem (mm)
a21	Number of leaves at the middle of the stem
a22	Ratio of leaves/internodes length at the middle of the stem
a23	Number of veins on leaves
a24	Length of petals (mm)
a25	Width of petals (mm)
a26	Length of calyx (mm)
a27	Length of calyx teeth (mm)
a28	Width of calyx teeth at their base (mm)
a29	Length of pedicels (mm)

a30	Bract length (mm)
a31	Ratio pedicel length/calyx length
a32	Ratio petal length/calyx length
a33	Number of veins on sepals
a34	Glandular hairs present on calyx
a35	Eglandular hairs present on calyx
a36	Epicalyx bracts present

TABLE 2. Stepwise discriminant analysis results for the five *Petrorhagia* species included in the present study based on fourteen morphological characters, as well as the discriminant functions' eigenvalues and the proportion of variance (PV) explained by each discriminant function. DF1, DF2, DF3 and DF4 indicate the first, the second, the third and the fourth discriminant function, respectively. F, T and WD indicate the F-test P values, the tolerance values, as well as the Wilk's lambda values of the discriminant functions and characters retained in the stepwise discriminant analysis, respectively. SML indicates the loadings from the stepwise discriminant analysis (i.e. the characters' correlation with the discriminant functions). Character abbreviations follow Table 1. Higher values are shown in bold.

Character	**T**	**F**	**WD**	**DF1**	**DF2**	**DF3**	**DF4**
Eigenvalue	-	-	-	205.23	27.22	3.12	1.38
PV	-	-	-	86.6	11.5	1.3	0.6
WD	-	-	-	0.00	0.00	0.10	0.42
					SML		
a9	0.85	0.01	0.00	**0.35**	0.09	-0.06	0.11
a34	0.42	0.00	0.00	0.26	0.06	0.07	0.16
a15	0.58	0.00	0.00	-0.06	0.22	0.03	0.09
a35	0.51	0.00	0.00	-0.06	0.23	**0.69**	-0.23
a31	0.09	0.00	0.00	0.06	-0.03	-0.36	0.02
a8	0.45	0.00	0.00	0.20	0.03	0.34	0.32
a29	0.08	0.00	0.00	-0.09	-0.13	-0.29	0.03
a28	0.67	0.01	0.00	-0.06	-0.16	0.25	-0.14
a25	0.60	0.03	0.00	-0.07	-0.15	0.24	-0.02
a7	0.28	0.00	0.00	-0.05	0.18	-0.19	**0.53**
a6	0.16	0.00	0.00	-0.05	0.18	-0.19	**0.53**
a5	0.24	0.00	0.00	-0.09	**0.38**	-0.21	0.44
a14	0.38	0.00	0.00	-0.13	0.00	-0.08	0.37
a4	0.69	0.03	0.00	-0.15	0.00	0.02	0.28
a27	0.23	0.00	0.00	-0.05	-0.04	0.11	-0.26
a30	0.60	0.03	0.00	0.03	0.03	-0.04	-0.11

TABLE 3. Correct Leave-One-Out (LOO) cross-validation classification from the stepwise discriminant analysis. A, B, C, D and E indicate *Petrorhagia fasciculata*, *P. graminea*, *P. phtiothica*, *P. saxifraga* and *P. laconica*, respectively. PCC and MCC indicate the Proportional Chance Criterion and the Maximum Chance Criterion, respectively.

Taxon	**A**	**B**	**C**	**D**	**E**	**% Correct Classification**
A	100.0	0.0	0.0	0.0	0.0	100.0
B	0.0	100.0	0.0	0.0	0.0	100.0
C	0.0	0.0	100.0	0.0	0.0	100.0
D	0.0	0.0	0.0	100.0	0.0	100.0
E	0.0	0.0	0.0	0.0	100.0	100.0
PCC	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
MCC	-	-	-	-		< 0.001
Total	-	-	-	-		100.0

TABLE 4. Main morphological differences among *Petrorhagia laconica*, *P. saxifraga* and *P. graminea*.

Character	***P. laconica***	***P. saxifraga***	***P. graminea***
Underground stock	Rooting at nodes	Not rooting at nodes	Not rooting at nodes
Stems	Glaucous-green, procumbent to ascending or erect, simple or rarely with few [1–3 (–6)] paniculate lateral branches at the upper 1/3, minutely pappilose-scabridulous except the glabrous 1–4 upper internodes	Green, arcuately ascending to erect, usually branched to much-branched, diffusely paniculate, glabrous to shortly pappilose or scabrid-pubescent at least in lower part	Green, sparingly branched, dichotomously divaricate, glabrous to densely pubescent
Leaves	Linear-subulate, rigid, closely appressed to stem	Linear to linear-lanceolate, herbaceous, ± spreading	Linear, herbaceous, ± spreading
Inflorescence	Flowers solitary or rarely paired, 1 (–2) per flowering stem, rarely up to 6 and then inflorescence paniculate	Paniculate, flowers solitary or in fascicles, 2–59 per flowering stem	Dichotomously divaricate, flowers solitary or in fascicles of 2–3, 2–37 per flowering stem
Epicalyx bracts	Present	Present	Absent
Calyx	Minutely glandular pubescent; ribs obscurely 1-veined, purplish-brown	Glabrous or occasionally sparsely eglandular pubescent; ribs 3-veined, green	Densely eglandular pubescent to glabrous; ribs 3-veined, green
Capsule	2.5–3 mm long, smooth or sparsely tuberculate at the lower half	3.5–5 mm long, smooth	3–5 mm long, smooth
Seed	1.0–1.2 × 0.7–0.8 mm, ovate-oblong, with flat, not thickened margins	0.9–1.6 × 0.6–1.1 mm, ovate, with thick curved margins	0.8–1.5 × 0.7–1.0 mm, oblong to sub-orbicular, with slightly thickened, ± curved margins
Flowering period	May-August	May-August	September-January

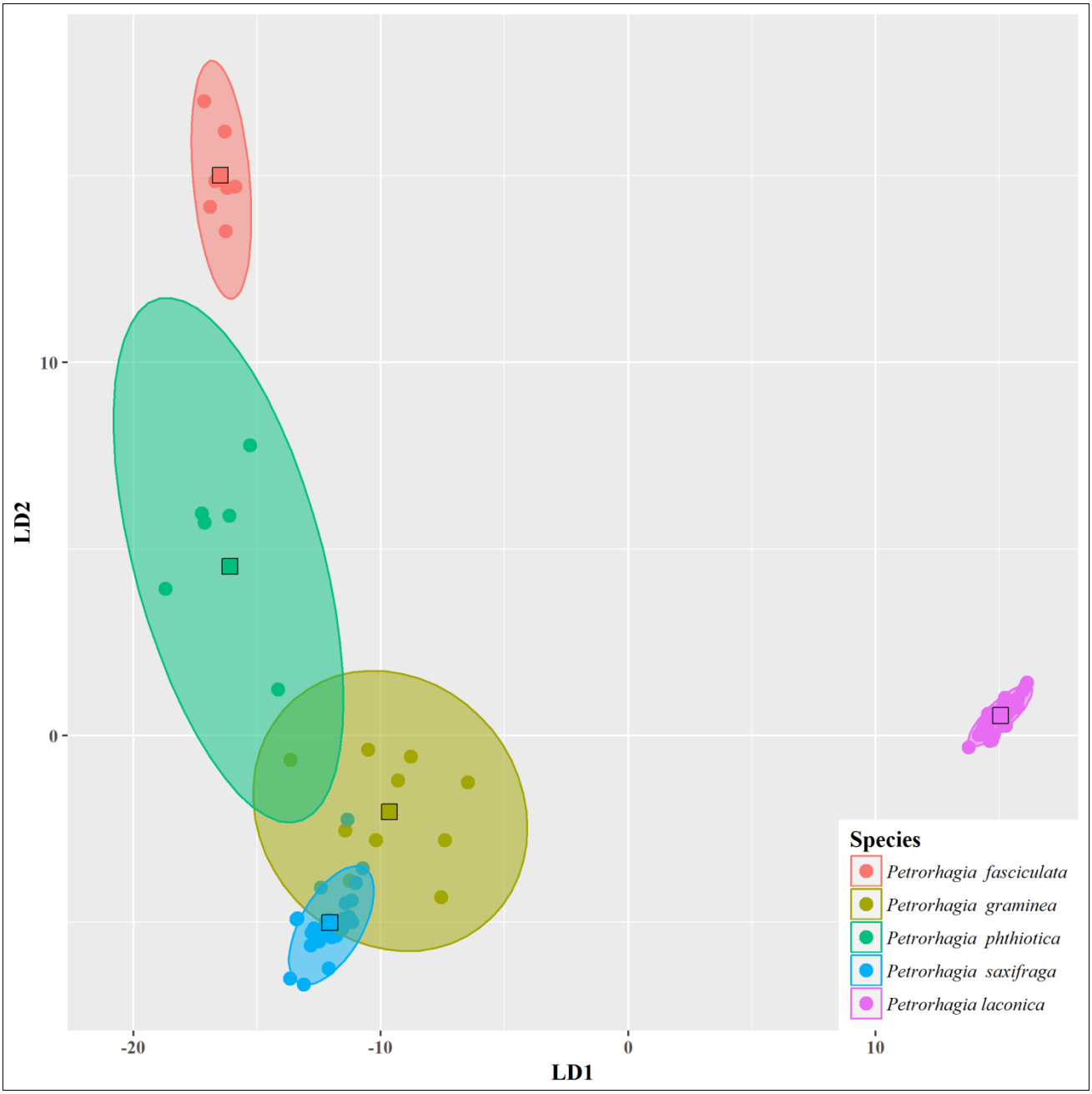

FIGURE 1. Stepwise discriminant analysis plot for the five *Petrorhagia* species. The squares indicate the function group centroid of each species.

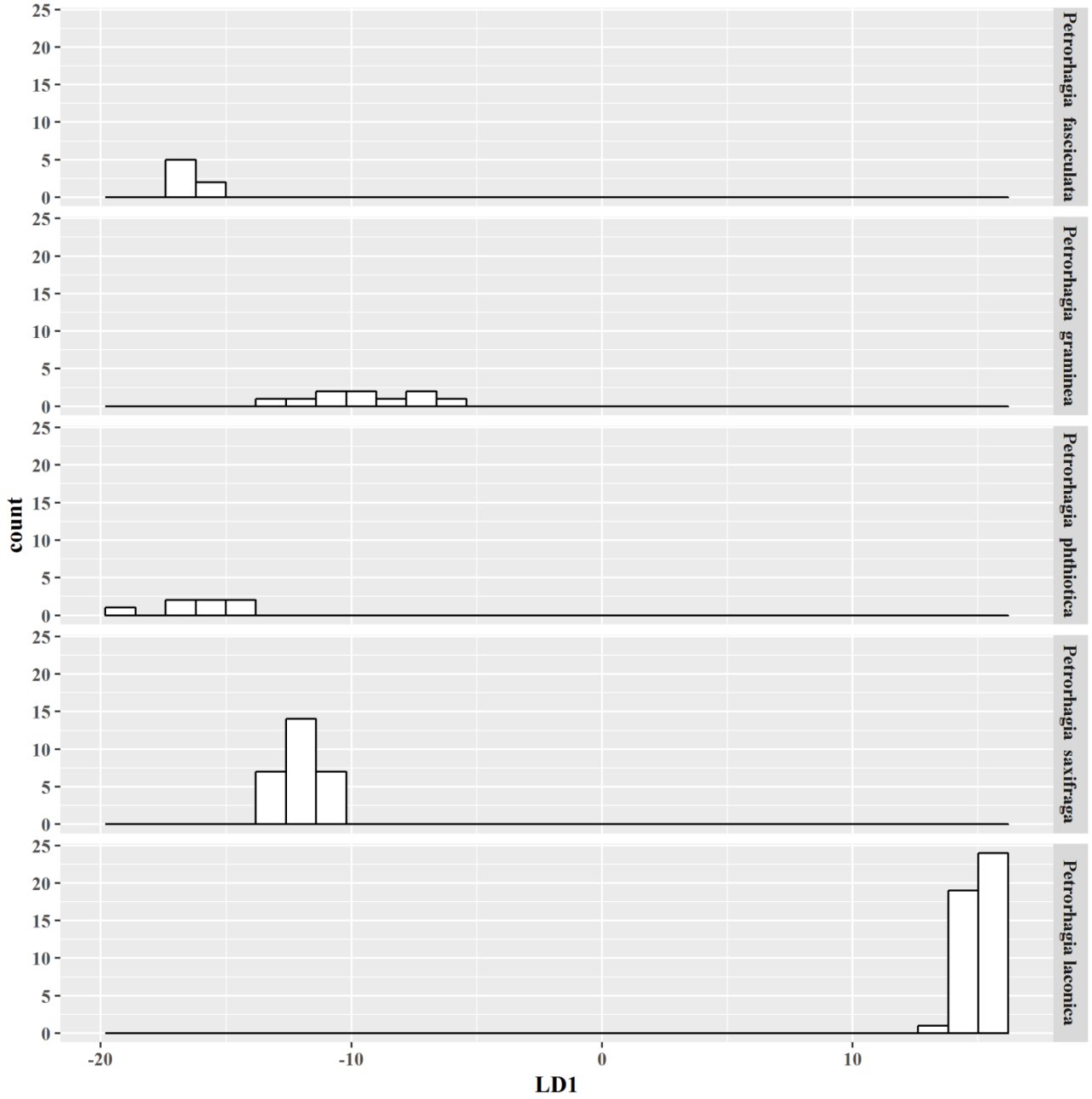

FIGURE 2. Frequency distribution of discriminant scores for the first discriminant function (LD1). The numbers on the x axes represent the discriminant function values.

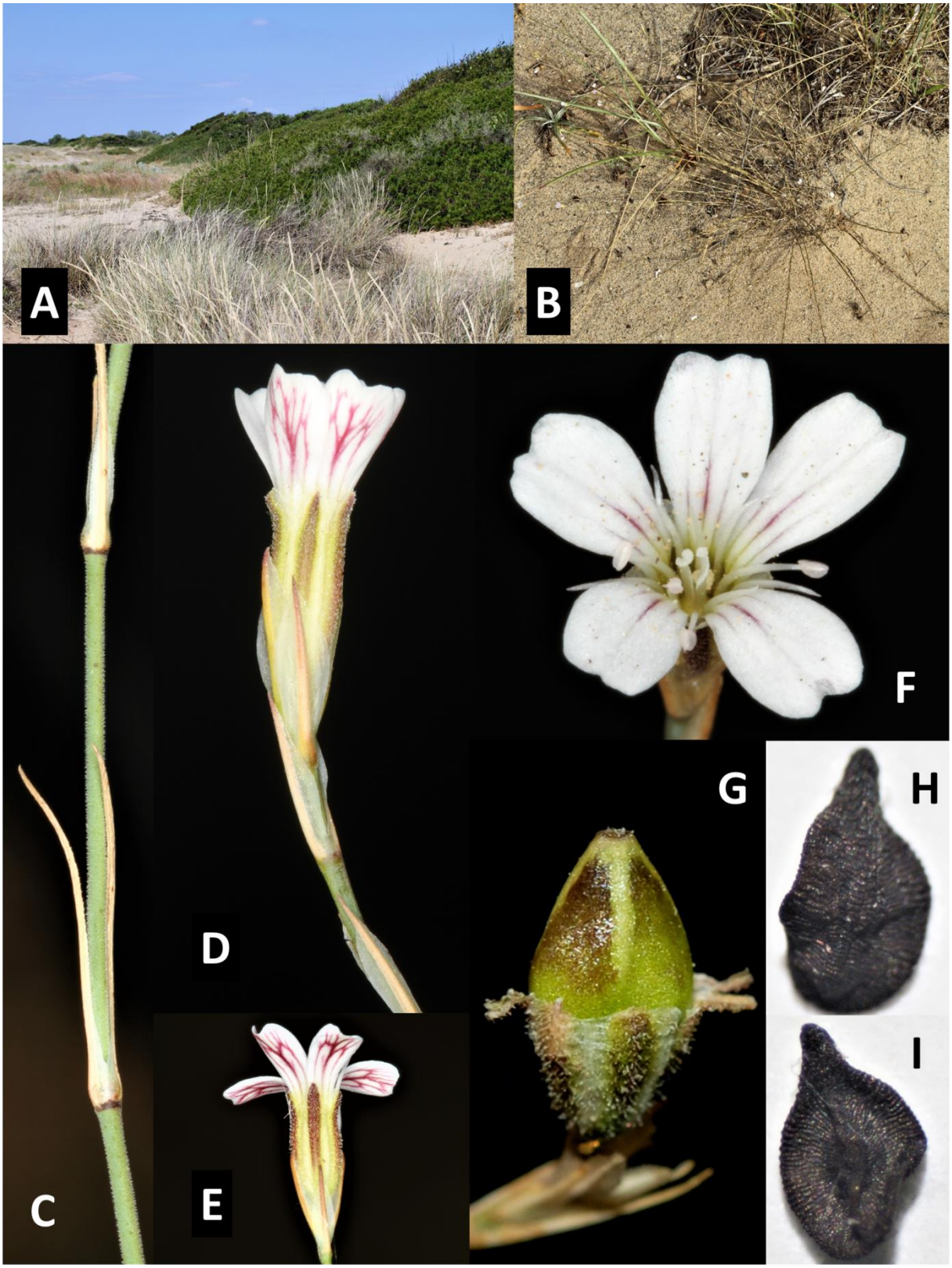

FIGURE 3. *Petrorhagia laconica*: (A) sand dunes northwest of Neapoli village in SE Peloponnisos, *locus classicus*, (B) habit, (C) middle part of stem with leaves, (D) upper part of stem with flower, (E) flower, side view, (F) flower, face view, (G) immature capsule, (H) seed, upper surface, (I) seed, lower surface.