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Multivariate morphometric analysis of *Petrorhagia* subsect. *Saxifragae* (*Caryophyllaceae*) in Greece, with a new species from SE Peloponnisos: *P. laconica*

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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 Greek endemic *P. graminea*, but clearly distinct from both species by a combination of morphological characters, including indumentum, inflorescence, calyx, capsule and seed characters.

Key words: *Caryophyllaceae*, Greece, morphology, multivariate morphometric analysis, new species, Peloponnisos, *Petrorhagia*, *Petrorhagia* subsect. *Saxifragae*, taxonomy

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Introduction

Caryophyllaceae include approximately 3000 species, distributed in about 100 genera (Hernández-Ledesma & al. 2015). The family has a primarily N-temperate distribution with a diversity centre in the E Mediterranean and Irano-Turanian regions, while presence in the tropics and the S hemisphere is limited and mostly confined to higher elevations (Bittrich 1993; Greenberg & Donoghue 2011; Rabaler & Hartman 2005; Hernández-Ledesma & al. 2015). The phylogenetic relationships within *Caryophyllaceae* is a field of active research (Fior & al. 2006; Harbaugh & al. 2010; Greenberg & Donoghue 2011;

Hernández-Ledesma & 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 (Ser.) Link (*Caryophylloideae*, *Caryophylleae*) is a small genus of c. 33 species, distributed in the Mediterranean area, Europe and W Asia (from the Canary Islands east to Kashmir), with a diversity centre in Greece and Turkey (Ball & Heywood 1964; Georgiou 1997). In the past, it was placed in different genera such as *Dianthus* L., *Gypsophila* L., *Kohlrauschia* Kunth and *Tunica* Scop. Evidence from both traditional and phylogenetic systematics support its recognition as a distinct

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taxon at the genus rank, which clusters as sister to a clade including *Dianthus* and *Velezia* L. (Ball & Heywood 1964; Harbaugh & al. 2010; Greenberg & Donoghue 2011; Pirani & al. 2014; Hernández-Ledesma & al. 2015; Hilooğlu & al. 2016).

Recent data on the infrageneric classification of *Petrorhagia* is still scarce, as the genus is rather poorly sampled. The available data (Greenberg & Donoghue 2011; Hilooğlu & 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, *P. sect. Petrorhagia* includes ten species (seven of them occurring in Greece and five endemic to the country) distributed in two subsections: *P. subsect. Saxifragae* Ball & Heywood and *P. subsect. Thessalae* Ball & Heywood. The two subsections are distinguished by stem branching (much branched vs simple or with few branches), inflorescence (flowers solitary or fasciculate vs capitate) 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 SE 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 *P. sect. Petrorhagia*, and they resemble *P. saxifraga* (L.) Link, a widely distributed species in C and S Europe and SW 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 Peloponnisos, described here as *Petrorhagia laconica*, support its classification in *P. subsect. Saxifragae*. Species delimitation within *P. subsect. Saxifragae* has never been studied in detail. All species of *P. subsect. Saxifragae*, except *P. riphaea* (Pau & Font Quer) Ball & Heywood 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 *P. subsect. Saxifragae* and to evaluate the taxonomic validity of the two recently discovered deviating *Petrorhagia* populations. More specifically, we aim: (1) to clarify the patterns of morphological variation within *P. subsect. Saxifragae* in Greece, (2) to disclose the level of morphological differentiation for recognized species, (3) to indicate the most informative characters for the iden-

tification of the species, and (4) 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 2017+) belonging to *Petrorhagia fasciculata* (Margot & Reut.) P. W. Ball & Heywood, *P. graminea* (Sm.) P. W. Ball & Heywood, *P. phthiotica* (Boiss. & Heldr.) P. W. Ball & Heywood and *P. saxifraga*. We collected several specimens of *P. laconica* in the field. All specimens included in the analyses were selected so as to represent the entire distribution range of the taxa 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 (see Supplementary Material online). 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 & 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 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). Capsule and seed morphological characters, although with a diagnostic value in the genus *Petrorhagia* (Ball & Heywood 1964), were excluded from the morphometric analyses as most studied specimens were in flower, lacking capsules and seeds. These characters, however, were used for the documentation of morphological differences among *P. laconica* and its closest relatives. 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, 5th and 95th 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 deviated from a normal distribution using the Shapiro-Wilk statistic

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 unbranched part of flowering stem (cm) |
| a4 | Length of the inflorescence (cm) |
| a5 | Glandular hairs present at the lower 1/3 of the stem |
| a6 | Glandular hairs present at the middle 1/3 of the stem |
| a7 | Glandular hairs present at the upper 1/3 of the stem |
| a8 | Eglandular hairs present at the lower 1/3 of the stem |
| a9 | Eglandular hairs present at the middle 1/3 of the stem |
| a10 | Eglandular hairs present at the upper 1/3 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 |

and we \log_{10} -transformed any characters that deviated from normality. Then, we computed the correlation coefficients between each character pair in order to reveal highly correlated characters and to ensure that no high correlations (> 0.95 – Španiel & al. 2017) were present that could potentially distort the discriminant analysis. As a second step, we performed several stepwise canonical and classificatory Discriminant Analyses (DAs) in order to: (1) identify the variables having the highest potential as diagnostic characters and (2) test the effectiveness of the discrimination rules by using the Leave-One-Out cross-validation (LOO). The value of the inclusion and the exclusion criterion in the stepwise DAs was set at $F = 0.05$ and $F = 0.1$, respectively. Wilk's lambda was used to assess the significance of each variable and Discriminant Function (DF). 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 analysing the ranges of the characters using core functions in the R computing environment (R Core Team 2015). All the analyses were carried out using IBM SPSS 24. The correlation coefficients were calculated with the “usdm” (Naimi & al. 2014) package, while the stepwise discriminant analysis plots 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 only character that had a correlation coefficient above the threshold was a10 (eglandular hairs present at the upper 1/3 of the stem) and was thus excluded from further analyses.

Multivariate analyses

The DA clearly discriminated 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 S3). 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 explained 86.6 %, 11.5 %, 1.3 % and 0.6 % of the total variation, respectively (Table 2, Fig. S1). Among the morphological characters used, the presence and the type of the indumentums are the most informative characters for species distinction in *P. subsect. Saxifragae*. The highest 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). The five *Petrorhagia* species included in the analyses are clearly distinct from one another (Table S3, Fig. 1, S1, S2).

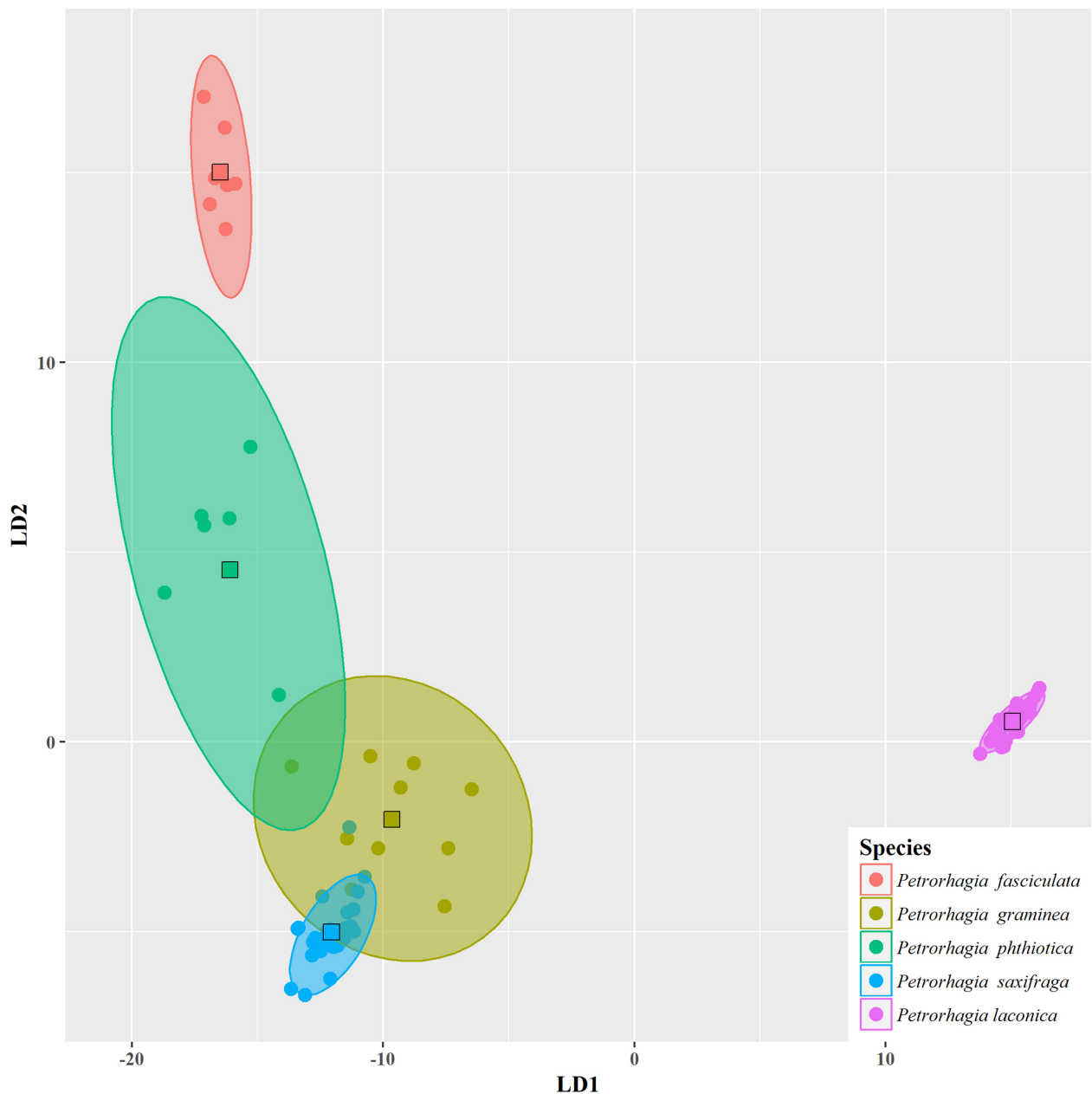


Fig. 1. Stepwise discriminant analysis plot for the five *Petrorhagia* species. The squares indicate the function group centroid of each species.

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 closely related and/or poorly differentiated taxa (e.g., Kougioumoutzis & 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 *P. laconica*.

Petrorhagia subsect. *Saxifragae* is a taxonomically complicated group with several overlapping characters. Our morphometric analyses, however, showed that all species are clearly distinguished from each other. Their distinction can be achieved even without using morphological seed 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 pres-

Table 2. Stepwise discriminant analysis results for the five *Petrorhagia* species included in the present study based on 14 morphological characters, as well as the eigenvalues of the discriminant functions 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 correlation of the characters 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 |

ence of eglandular hairy stems throughout their length is a unique feature of *Petrorhagia laconica*. *Petrorhagia fasciculata* and *P. phthiotica* are the only species with an indumentum of 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 a 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. phthiotica* and *P. saxifraga* 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 *Petrorhagia laconica* from all other species of *P. sect. Saxifragae* (Table S2, Fig. 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 no 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. *Petrorhagia 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 *P. 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. *Petrorhagia laconica* and *P. saxifraga* have narrow basal leaves compared to the other species. Furthermore, cauline leaves are diminishing in size toward the apex in all species, but diminishing in leaf length only, while leaf width remains stable, has been observed only in *P. laconica* (Table S2, Fig. S1).

Taxonomy

Petrorhagia laconica Trigas, Kalpoutz. & Kougioum., **sp. nov.** – Fig. 2.

Holotype: Greece, Peloponnisos, sand dunes c. 3.5 km NW of Neapoli village, 36°31.721'N, 23°01.273'E, 2 m, 22 May 2017, *Trigas 6311* (ACA; isotypes: B, UPA).

Diagnosis — Related to *Petrorhagia saxifraga*, from which it differs in the following morphological characters: underground stock rooting at nodes; stems procumbent to ascending or erect, glaucous-green, simple or rarely with few (1–3[–6]) lateral branches at upper 1/3, minutely papillose-scabridulous except glabrous at 1–4 upper internodes; leaves closely appressed to stem, rigid; flowers solitary or rarely paired, 1(or 2) per flowering stem, rarely up to 6; calyx minutely glandular pubescent; ribs purplish brown, obscurely 1-veined; capsule 2.5–3 mm long, sparsely tuberculate at proximal 1/2; seeds with flat, non-thickened margin.

Description — Plants perennial, with branched, slender, woody *underground stock* rooting at nodes, producing few to several flowering stems. *Flowering stems* procumbent to ascending or erect, glaucous-green to purplish brown, 7–40 cm long, simple or rarely with few (1–3[–6]) lateral branches at upper 1/3, minutely papillose-scabridulous ex-



Fig. 2. *Petrorhagia laconica* – A: sand-dune habitat NW of Neapoli village in SE Peloponnissos with evergreen shrubs (locus classicus), 22 May 2017; B: individual with simple, procumbent-ascending stems at the locus classicus, 16 May 2014; C: middle part of stem with leaves; D: upper part of stem with flower; E: flower, lateral view; F: flower, apical view; G: immature capsule; H: seed, dorsal face; I: seed, ventral face. – Scale bars: C–F: 3 mm; G: 1 mm; H, I: 500 μ m. – All photographs by Panayiotis Trigas except G by Eleftherios Kalpoutzakis.

cept glabrous at 1–4 upper internodes; *internodes* markedly unequal, larger at middle of stem, diminishing in size toward base and apex. *Leaves* linear-subulate, $\frac{1}{3}$ – $1\frac{1}{4}$ as long as internodes, rigid, 1-veined, united at base into a sheath 1–1.5 mm long, with a purplish brown ring at base, apex acuminate to caudate; *lower leaves* 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, 1 (or 2) per flowering stem, rarely up to 6, subtended by 4 straw-coloured, lanceolate, hyaline, glabrous *epicalyx bracts*, sometimes purplish-tinged at midrib. *Calyx* cylindrical, 3–6 mm long, minutely glandular pubescent; *ribs* purplish brown, obscurely 1-veined; *teeth* oblong-triangular, 0.5–1 mm long, apex obtuse. *Petals* white, 4–8 × 1.5–3 mm, abaxial surface purple-veined, adaxial surface with three longitudinal purplish stripes at base, with glabrous claw not distinctly delimited from emarginate lamina. *Anthers* white. *Ovary* oblong-ovoid, c. 1 mm long; *anthophore* green, c. 0.5 mm long, glabrous. *Capsule* pale brown, ovoid, 2.5–3 mm long, smooth or sparsely tuberculate at proximal $\frac{1}{2}$. *Seeds* blackish brown, ovate-oblong, 1–1.2 × 0.7–0.8 mm, both surfaces reticulate, margin flat, not thickened, apex long mucronate.

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, c. 25 km N-NW of the first population. It is the southernmost distributed species of *P.* subsect. *Saxifragae* in Europe.

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 sclerophyllous shrubs, such as *Anthyllis hermanniae* L., *Ceratonia siliqua* L., *Pistacia lentiscus* L., *Pyrus spinosa* Forssk. and *Smilax aspera* L. *Petrorhagia laconica* grows in stabilized sand dunes among the shrubs, together with other sand dune adapted taxa, such as *Anthemis tomentosa* L., *Asphodelus ramosus* L., *Centaurea sonchifolia* L., *Elytrigia juncea* (L.) Nevski, *Linaria tenuis* (Viv.) Spreng. and *Scirpoides holoschoenus* (L.) Soják.

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 *Lavandula stoechas* L., *Olea europaea* L., *Phlomis fruticosa* L., *Pistacia lentiscus* and *Pyrus spinosa* the predominant species. *Petrorhagia laconica* usually grows in small patches among the shrubs, together with *Ballota acetabulosa* (L.) Benth., *Cistus salviifolius* L., *Globular-*

ia alypum L., *Hypericum triquetrifolium* Turra, *Sarcopoterium spinosum* (L.) Spach, *Silene nocturna* L., etc.

Conservation status — In Neapoli Lakonias, *Petrorhagia laconica* grows on a large sandy beach c. 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 to 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 300–400 individuals, mainly concentrated at the E part of the beach. The Sykea population counts c. 500 individuals distributed in an area of c. 0.1 km².

Habitat loss and touristic development are the main threats that *Petrorhagia laconica* faces at its locus classicus. Sand dunes have been partly turned into cultivated fields at the E half of the beach, almost certainly leading to a significant population decline. The 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 *Petrorhagia 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 & al. 2012). The recent discovery of *Petrorhagia laconica* indicates that the species should not be common in this area. The habitat types in which 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 extent of occurrence (EOO) does not exceed 25 km² and since *P. laconica* is a very local species, its area of occupancy (AOO) is much smaller, apparently less than 1 km². Ergo, due to: (1) the restricted EOO and AOO, (2) the current existence of max. 900 mature individuals distributed in two populations and (3) the high possibility that the extant localities and populations could be eradicated as a result of human interference, *P. laconica* is assigned to the Endangered (EN) IUCN (2001) category, following criteria B1ab(i,ii,iii,iv)+2ab(i,ii,iii,iv). *Petrorhagia laconica* should be carefully monitored, cultivated ex situ in botanical gardens and special conservation measures be taken to safeguard its populations.

Table 3. Main morphological differences among *Petrorhagia laconica*, *P. saxifraga* and *P. graminea*.

| Character | <i>P. laconica</i> | <i>P. saxifraga</i> | <i>P. graminea</i> |
|-------------------|--|---|---|
| Underground stock | rooting at nodes | not rooting at nodes | not rooting at nodes |
| Stems | procumbent to ascending or erect, glaucous-green, simple or rarely with few (1–3[–6]) lateral branches at upper 1/3, minutely papillose-scabridulous except glabrous at 1–4 upper internodes | arcuately ascending to erect, green, usually branched to much-branched, glabrous to shortly papillose or scabrid-pubescent at least in lower part | ascending to erect, green, sparingly branched, glabrous to densely pubescent |
| Leaves | closely appressed to stem, linear-subulate, rigid | ± spreading, linear to linear-lanceolate, herbaceous | ± spreading, linear, herbaceous |
| Inflorescence | flowers solitary or rarely paired, 1 (or 2) per flowering stem, rarely up to 6 | flowers solitary or in fascicles, 2–59 per flowering stem | flowers solitary or in fascicles of 2 or 3, 2–37 per flowering stem |
| Epicalyx bracts | present | present | absent |
| Calyx | minutely glandular pubescent; ribs purplish brown, obscurely 1-veined | glabrous or occasionally sparsely eglandular pubescent; ribs green, 3-veined | densely eglandular pubescent to glabrous; ribs green, 3-veined |
| Capsule | 2.5–3 mm long, smooth or sparsely tuberculate at proximal 1/2 | 3.5–5 mm long, smooth | 3–5 mm long, smooth |
| Seeds | ovate-oblong, 1–1.2 × 0.7–0.8 mm, margin flat, not thickened | ovate, 0.9–1.6 × 0.6–1.1 mm, margin curved, thickened | oblong to suborbicular, 0.8–1.5 × 0.7–1 mm, margin ± curved, slightly thickened |
| Flowering period | May–August | May–August | September–January |

Etymology — The specific epithet refers to Laconia (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.

Taxonomic relationships — *Petrorhagia laconica* undoubtedly belongs to *P. 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). *Petrorhagia dianthoides* (Sm.) P. W. Ball & Heywood, *P. fasciculata*, *P. grandiflora* Iatrou and *P. thessala* (Boiss.) P. W. Ball & Heywood have a flat, thin seed margin, similar to that of *P. laconica*.

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

Petrorhagia laconica appears to be one of the most distinct species in *P. sect. Petrorhagia*. The results of multivariate morphometric analyses (Tables 2, S3, Fig. 1, S1, S2) support its distinct taxonomic position with-

in *P. subsect. Saxifragae*. The closest related species seem to be *P. saxifraga* and *P. graminea*, which, however, show significant morphological differences from *P. laconica* (see also Table 3). The possibility of vegetative reproduction (underground stolons connecting different individuals have been observed in the field) 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 & 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 centre for *Petrorhagia*, because more than 30 % of the species diversity of the whole genus is hosted in this small region.

Additional specimens examined — GREECE: Peloponnisos, c. 3.5 km S-SE of Sykea village, 36°43.970'N, 22°57.079'E, 130 m, macchie and phrygana among olive groves, on flysch, 6 May 2013, *Kalpoutzakis 4294* (ACA); *ibid.*, 29 Jul 2017, *Kalpoutzakis 4959* (ACA); c. 2.5 km S-SE of Sykea village, 36°44.552'N, 22°57.209'E, 120 m, macchie and phrygana among olive groves, on flysch, 22 May 2017, *Trigas 6312* (ACA).

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References

- Ball P. W. & Heywood V. H. 1964: A revision of the genus *Petrorhagia*. – Bull. Brit. Mus. (Nat. Hist.), Bot. **3**: 119–172.
- Bittrich V. 1993: *Caryophyllaceae*. – Pp. 206–236 in: Kubitzki K., Rohwer J. G. & Bittrich V. (ed.), Families and genera of vascular plants **2**. – Berlin, Heidelberg & New York: Springer Verlag.
- Coode M. J. E. & Cullen J. 1967: *Petrorhagia* (Ser.) Link. – Pp. 131–135 in: Davis P. H. (ed.), Flora of Turkey and the East Aegean Islands **2**. – Edinburgh: University Press.
- Dillenberger M. S. & Kadereit J. W. 2014: Maximum polyphyly: multiple origins and delimitation with plesiomorphic characters require a new circumscription of *Minuartia* (*Caryophyllaceae*). – Taxon **63**: 64–88.
- Dimopoulos P., Raus Th., Bergmeier E., Constantinidis Th., Iatrou G., Kokkini S., Strid A. & Tzanoudakis D. 2013: Vascular plants of Greece: an annotated checklist. – Berlin: Botanic Garden and Botanical Museum Berlin-Dahlem; Athens: Hellenic Botanical Society. [Englera **31**].
- Fior S., Karis P. O., Casazza G., Minuto L. & Sala F. 2006: Molecular phylogeny of the *Caryophyllaceae* (*Caryophyllales*) inferred from chloroplast *matK* and nuclear rDNA ITS sequences. – Am. J. Bot. **93**: 399–411.
- Georgiou O. (1997) *Petrorhagia* (Ser.) Link. – Pp. 333–343 in: Strid A. & Tan K. (ed.) Flora hellenica **1**. – Königstein: Koeltz Scientific Books.
- Greenberg A. K. & Donoghue M. J. 2011: Molecular systematics and character evolution in *Caryophyllaceae*. – Taxon **60**: 1637–1652.
- Greuter W. 2012: Results of the Seventh “Iter Mediterraneum” in the Peloponnese, Greece, May to June 1995. (Occasional Papers from the Herbarium Greuter – N° 1). – Bocconea **25**: 5–127.
- Harbaugh D. T., Nepokroeff M., Rabeler R. K., McNeill J., Zimmer E. A. & Wagner W. L. 2010: A new lineage-based tribal classification of the family *Caryophyllaceae*. – Int. J. Plant Sci. **171**: 185–198.
- Hernández-Ledesma P., Berendsohn W. G., Borsch Th., Mering S. von, Akhani H., Arias S., Castañeda-Noa I., Eggli U., Eriksson R., Flores-Olvera H., Fuentes-Bazán S., Kadereit G., Klak C., Korotkova N., Nyffeler R., Ocampo G., Ochoterena H., Oxelman B., Rabeler R. K., Sanchez A., Schlumpberger B. O. & Uotila P. 2015: A taxonomic backbone for the global synthesis of species diversity in the angiosperm order *Caryophyllales*. – Willdenowia **45**: 281–383.
- Hilooğlu M., Poyraz I. E., Poyraz I., Ataşlar E. & Sözen E. 2016: Genetic relationships among some Turkish *Petrorhagia* (Ser.) Link (*Caryophyllaceae*) taxa using ISSR markers. – Phytotaxa **272**: 165–172.
- Huberty C. J. & Olejnik S. 2006: Applied MANOVA and Discriminant Analysis, 2nd ed. – Hoboken: Wiley and Sons.
- Iatrou G. 1985: *Petrorhagia grandiflora* sp. nov. (*Caryophyllaceae*) from Greece. – Nord. J. Bot. **5**: 441–445.
- Kalpoutzakis E. & Constantinidis T. 2005: New data on the distribution of endemic and rare taxa in the flora of east Peloponnisos, Greece. – Bot. Chron. **18(2)**: 115–136.
- Kalpoutzakis E. & Constantinidis T. 2006: Additions and annotations to the flora of Peloponnisos (Greece). – Willdenowia **36 (Special Issue)**: 271–284.
- Kalpoutzakis E., Trigas P. & Constantinidis T. 2012: *Allium orestis* sp. nov. (*Amaryllidaceae*) from Paros and Taigetos mountains, south Peloponnisos, Greece. – Nord. J. Bot. **30**: 195–200.
- Kougioumoutzis K., Kalpoutzakis E. & Constantinidis T. 2017: Multivariate morphometric analysis and taxa delimitation in two narrow Greek endemics: *Astragalus maniacus* and *Aethionema saxatile* subsp. *corinthiacum*. – Pl. Biosyst. **151**: 108–116.
- Marhold K. 2011: Multivariate morphometrics and its application to monography at specific and infraspecific levels. – In: Stuessy T. F. & Lack H. W. (ed.), Monographic Plant Systematics: fundamental assessment of plant biodiversity. – Ruggell: Gantner.
- Naimi B., Hamm N. A., Groen T. A., Skidmore A. K. & Toxopeus A. G. 2014: Where is positional uncertainty a problem for species distribution modelling? – Ecology **37**: 191–203.
- Pirani A., Zarre S., Pfeil B. E., Bertrand Y., Assadi M. & Oxelman B. 2014: Molecular phylogeny of *Acanthophyllum* (*Caryophyllaceae*, *Caryophylleae*), with emphasis on subgeneric classification. – Taxon **63**: 592–607.
- R Core Team 2015: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. – Published at <https://www.R-project.org/> [accessed 3 Jul 2017].
- Rabeler R. K. & Hartman R. L. 2005: *Caryophyllaceae*. Pp. 3–8 in: Flora of North America Editorial Committee (ed.), Flora of North America north of Mexico **5**. – Oxford: University Press.
- Španiel S., Zozomová-Lihová J. & Marhold K. 2017: Revised taxonomic treatment of the *Alyssum montanum* – *A. repens* complex in the Balkans: a multivariate morphometric analysis. – Plant Syst. Evol. **303**: 1413–1442.
- Thiers B. 2017+ [continuously updated]: Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden’s Virtual Herbarium. Published at <http://sweetgum.nybg.org/science/ih/> [accessed 3 Aug 2017].

- Trigas P., Iatrou G. & Karetos G. 2007: Species diversity, endemism and conservation of the family *Caryophyllaceae* in Greece. – *Biodivers. Conserv.* **16**: 357–376.
- Trigas P., Tsiftsis S., Tsiripidis I. & Iatrou G. 2012: Distribution patterns and conservation perspectives of the endemic flora of Peloponnese (Greece). – *Folia Geobot.* **47**: 421–439.
- Wickham H. 2009: *ggplot2: Elegant Graphics for Data Analysis*. – New York: Springer.

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