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Multivariate morphometric analysis and taxa delimitation in two narrow Greek endemics: *Astragalus maniaticus* and *Aethionema saxatile* subsp. *corinthiacum*

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Abstract
Astragalus maniaticus and *Aethionema saxatile* subsp. *corinthiacum* have vague taxonomic relationships. *Astragalus maniaticus* was placed in sect. *Hypoglottis* but shows affinities to *A. suberosus* subsp. *haarbachii* of sect. *Platyglottis*. The *Aethionema saxatile* complex is an intriguing group due to the wide distribution and morphological variability of its taxa. In order to elucidate the variation patterns of these two taxa and to test their morphological identity, we carried out several multivariate morphometric (stepwise canonical and classificatory) analyses. They revealed that *Astragalus maniaticus* cannot be distinguished from *A. suberosus* subsp. *haarbachii* on morphological grounds. Therefore, it is transferred to sect. *Platyglottis* and regarded a heterotypic synonym of *A. suberosus* subsp. *haarbachii*. The taxonomic interpretation of *Aethionema saxatile* subsp. *corinthiacum* necessitated a broad revision of the *Ae. saxatile* group in Greece and detection of its relations with *Ae. rhodopaeum*, a Bulgarian endemic. The analyses showed that *Ae. saxatile* subsp. *corinthiacum* represents a distinct taxon; likewise, three additional subspecies receive support of similar strength: subsp. *creticum*, subsp. *graecum* and subsp. *oreophilum*. Intermediate forms between subsp. *oreophilum* and subsp. *saxatile* were detected in NW Greece. *Ae. rhodopaeum* is more variable than its original circumscription and is considered as another subspecies of *Ae. saxatile*.

Keywords: Aethionema, Astragalus, *conservation, discriminant analysis, endemics, Greece, morphometry, taxonomic revision*

Introduction

The southern Greek mainland consists of two neighbouring phytogeographical regions: Peloponnisos and Sterea Ellas (Pe and StE, hereafter). Many taxa new to science have been described from Pe and StE during the last decade (e.g. Strid & Tan 2005; Greuter 2012; Kalpoutzakis et al. 2012; Tan et al. 2013). These taxa have contributed to the significant number of Greek endemics found in these two regions: Pe and StE harbour 468 and 368 endemic taxa, respectively, (14.6 and 11.0% of their total flora, respectively – Dimopoulos et al. 2013).

Greek endemic taxa constitute a heterogeneous group in terms of evolutionary history and current distribution range. Not all of them are known from the same number of historical or recent collections that enable their unequivocal taxonomic interpretation. Rare or very local taxa, represented by very few vouchers, may need recollection and re-evaluation to achieve a good morphological definition and avoid taxonomic fluctuation with an impact on both diversity estimates and conservation planning (Isaac et al. 2004; Knapp et al. 2005; Mallet et al. 2005; IUCN 2014). If doubt is casted on morphology and species boundaries, supplemental experimental approaches may be useful or even decisive in drawing taxonomic conclusions (Jiménez et al. 2007).

This work focuses on two rare and little-known Greek taxa: *Astragalus maniaticus* Kit Tan & Strid (Fabaceae) from Peloponnisos (Tan & Strid 1997) and *Aethionema saxatile* (L.) R. Br. subsp. *corinthiacum* Kit Tan, G. Vold, Zarkos & Christodoulou (Brassicaceae) from Sterea Ellas (Zarkos et al. 2011).

Astragalus maniaticus (sect. *Hypoglottis* Bunge) has obscure affinities; it appears to be similar (albeit not related) to A. *austroaegaeus* Rech. f. from the Aegean region (Tan & Strid 1997; Tan & Iatrou 2001; Kougioumoutzis et al. 2012) and occurs in the southern and eastern parts of Peloponnisos. The species has already been studied in the field, evaluated and assigned to the Near-Threatened

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IUCN conservation category, yet doubt was casted regarding its taxonomic placement in sect. *Hypoglottis* as it appears morphologically closer to *A. suberosus* Banks & Sol. subsp. *haarbachii* (Boiss.) V. A. Matthews, belonging to sect. *Platyglottis* Bunge (Kalpoutzakis & Constantinidis 2009). Podlech (2008, 2011) on the other hand, accepted the two taxa as independent members of two different sections.

Aethionema saxatile subsp. *corinthiacum* is currently known from the lower, southern parts of Mt. Gerania (Zarkos et al. 2011), representing a local compact form with broadly oblong-elliptic to suborbicular fleshy leaves. This form was already known to Andersson et al. (1983), who preferred not to attribute to it a formal taxonomic recognition. Tan and Suda (2002) placed it under subsp. *graecum* (Boiss. & Spruner) Hayek; the latter being widely circumscribed to incorporate subsp. *oreophilum* I. A. Andersson et al. as well. Morphologically, *Ae. saxatile* subsp. *corinthiacum* shares similarities with *Ae. rhodopaeum* D. Pavlova, a Bulgarian serpentine endemic of the lower parts of eastern Rhodope Mountains (Pavlova 2007). If *Ae. saxatile* subsp. *corinthiacum* is indeed a distinct taxonomic entity, as rare and local as reported (only three collections are known so far, one being recent, see Zarkos et al. 2011), then conservation measures should probably be considered. Still, the elucidation of subspecies' morphological identity requires additional field work to discover new populations, coupled with detailed comparisons against the whole morphological variability encountered within Greek *Ae. saxatile* as a whole on one hand and the Bulgarian *Ae. rhodopaeum* on the other.

Given the uncertainty or sharp differences in opinion for *Astragalus maniaticus* and *Aethionema saxatile* subsp. *corinthiacum*, we performed several multivariate analyses (stepwise canonical and classificatory discriminant analyses), commonly applied to resolve species complexes (e.g. Henderson 2006; Brullo et al. 2011; Kaplan & Marhold 2012; Pinna et al. 2012; Agulló et al. 2013), in order to address the manifold aims of the present study, which were (i) to elucidate the morphological variation patterns between *Astragalus maniaticus* and *A. suberosus* subsp. *haarbachii*, as well as in the *Aethionema saxatile* complex in Greece, (ii) to test whether the taxa recognised in the literature can be reliably identified and by which morphological characters and (iii) to examine the taxonomic boundaries between the *Aethionema saxatile* complex and *Ae. rhodopaeum*.

Materials and methods

Data-sets

We constructed several data-sets to assist with multivariate analyses, as follows:

Data-set-1. The type specimen of *Astragalus maniaticus* (*Andersson* et al. 76, LD), specimens identified as *A. maniaticus* collected from south and east Pe and specimens from Pe and StE identified as *A. suberosus* subsp. *haarbachii* comprise data-set-1.

Data-set-2. Herbarium specimens deposited in ATH, ATHU, B and UPA (acronyms follow Thiers 2015) belonging to the representatives of the *Aethionema saxatile* complex in Greece comprise data-set-2. The type specimen of *Ae. saxatile* subsp. *corinthiacum* in ATH was also included.

Data-set-3. Herbarium specimens deposited in ATH, ATHU, B, SO and UPA belonging to the *Aethionema saxatile* complex and to *Ae. rhodopaeum* comprise data-set-3. The type specimens of *Ae. rhodopaeum* (D. Pavlova, SO 103994 & 103995) are included in data-set-3.

Data-set-4. Herbarium specimens deposited in ATH, ATHU and B belonging to *Aethionema saxatile* s.l., *Ae. saxatile* subsp. *oreophilum* and subsp. *saxatile,* comprise data-set-4.

Plant material

Data for the multivariate analyses were recorded from herbarium specimens as listed above. The specimens included in the analyses of data-set-1 and data-set-2 were selected so as to represent the taxa's entire distribution range in southern Greece (data-set-1) and Greece (data-set-2), as well as the morphological variation in each taxon. Only well-preserved and intact specimens were considered in the analyses. The total number of herbarium sheets included in data-sets-1 to 4 was 42, 110, 141 and 31, respectively (Table SI). Each specimen was preliminarily identified for data-set-1 as either *Astragalus maniaticus* or *A. suberosus* subsp. *haarbachii* and for data-set-2 as one of the four subspecies comprising the *Aethionema saxatile* complex in Greece according to Andersson et al. (1983) and Zarkos et al. (2011), i.e. subspecies *corinthiacum*, *creticum*, *graecum* and *oreophilum*. Morphologically intermediate specimens from contact zone areas were included in the analyses and considered under the morphologically predominant subspecies name.

Species identification and nomenclature are according to Andersson et al. (1983) and Dimopoulos et al. (2013), with Podlech (2008) also taken into consideration. A specimens' list of *Astragalus maniaticus* and *Aethionema saxatile* subsp. *corinthiacum*, together with basic data on their origin, is provided in Appendix I (see supplementary material).

Table I. Characters used in the morphometric analyses of data-sets-1–4.

Character abbreviation	Data-set	Detailed character definition
FtL	1	Legume length (mm)
FtW		Legume width (mm)
PL		Pedicel length (mm)
CL		Calyx length (mm)
CTL		Calyx teeth length (mm)
SdL		Standard length (mm)
LfN		Number of leaflet pairs
FL	2	Bilocular siliculae length (mm)
FW		Bilocular siliculae width (mm)
LfL		Leaf length at the upper half of flowering stem (mm)
LfW		Leaf width at the upper half of flowering stem (mm)
LfR		Upper flowering stem leaf length to width ratio
PeL		Petal length (mm)
SpL		Sepal length (mm)
SeL		Style length (mm)
FL	3 & 4	Bilocular siliculae length (mm)
FW		Bilocular siliculae width (mm)
LfL		Leaf length at the upper half of flowering stem (mm)
LfW		Leaf width at the upper half of flowering stem (mm)
LfR		Upper flowering stem leaf length to width ratio
SeL		Style length (mm)

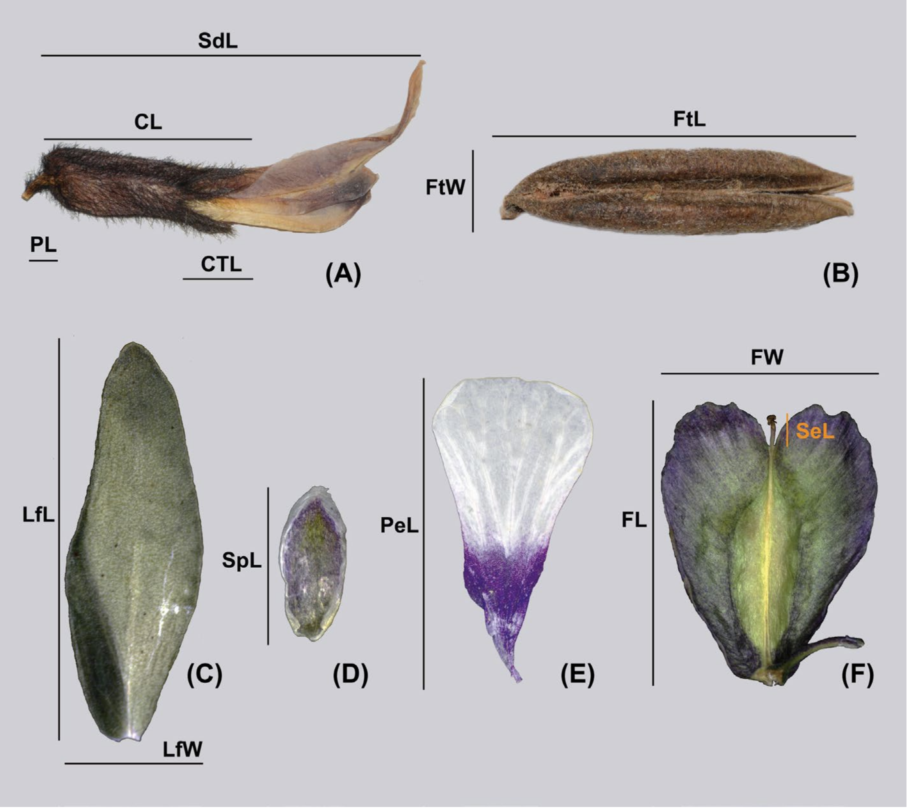

Figure 1. Measurement of morphological characters in: (A–B): *Astragalus suberosus* subsp. *haarbachii* or *A. maniaticus* and (C–F): the *Aethionema saxatile* complex in Greece. (A): Calyx and corolla (length of outstretched standard was measured), (B): ripe legume, (C): leaf from the upper half of stem, (D): sepal, (E): petal, (F): ripe silicula. Abbreviations as in Table I.

Characters recorded

Seven, eight and six quantitative morphological characters were scored for data-sets-1 & 4, data-set-2 and data-set-3, respectively (Table I & Figure 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 (Chater 1968; Andersson et al. 1983; Chater & Akeroyd 1993; Tan & Strid 1997, Tan & Suda 2002; Pavlova 2007; Podlech 2008). Additional characters were preliminarily evaluated but appeared non-significant and were not included in the study. Within this context, we measured for

Table II. Stepwise discriminant analysis results for data-set-1 based on seven morphological characters, as well as the discriminant function's eigenvalues and the proportion of variance (PV) explained by each discriminant function.

Character	*T*	*F*	WD	DF1
Eigenvalue	–	–	–	0.16
PV	–	–	–	100.0
WD	–	–	–	0.86
				SML
CL	0.92	0.00	0.97	**0.71**
FtW	0.92	0.02	0.93	−0.48
FtL	0.77	n.s.	n.s.	–
LfN	0.96	n.s.	n.s.	–
CTL	0.55	n.s.	n.s.	–
PL	0.78	n.s.	n.s.	–
SdL	0.67	n.s.	n.s.	–

Note: DF1 indicates the first discriminant function. *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 I. n.s.: statistically non-significant. Higher values are shown in bold.

data-sets-2 & -3, four additional characters (filament length and width, as well the presence/absence of a tooth and/or wing in the filament – the latter two as binary characters), yet none did emerge as significant (data not shown). Therefore, we excluded them from further analyses. All ATHU and UPA specimens were digitised using a flatbed scanner; the characters in ATHU, B, SO and UPA specimens were measured using the software program ImageJ (Schneider et al. 2012), while the characters in ATH 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 taxon 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 for each data-set and (ii) test the discrimination rules' effectiveness 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 both data-sets in relation to their taxon boundaries by analysing the characters' ranges with the box plot or histogram function of IBM SPSS 21. All the analyses were carried out using IBM SPSS 21. The maximum and proportional chance criteria were run under the Zclass algorithm kindly provided by John D. Morris (Florida Atlantic University).

Results

Characters scored

The basic statistical parameters for all data-sets are given in Table SII. The characters' morphological variation for data-sets-1 to 4 is graphically depicted in Figures S1–S4, respectively. The scored characters' distribution departed from normality in all data-sets; therefore, all the characters were $\log_{10}$-transformed prior to the analyses. After the logarithmic transformation, all the characters were normally distributed.

Multivariate analyses

I. Discriminant analysis. 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.

Data-set-1. There is no clear separation between *Astragalus maniaticus* and *A. suberosus* subsp. haarbachii (Figure S5). Only CL and FtW emerged as statistically significant (Table II), though with very high Wilk's lambda values, thus indicating that: (i) a very high proportion of variance is not explained by these two factors (i.e. there is not actually an effect from the grouping variable and the groups do not have different mean values) and (ii) there is tremendous ranges overlap across *A. maniaticus* and

Table III. Correct LOO cross-validation classification from the stepwise discriminant analysis of data-set-1.

Taxon	*Astragalus suberosus* subsp. *haarbachii*	*Astragalus maniaticus*	% Correct classification
Astragalus suberosus subsp. *haarbachii*	90.7	9.3	90.7
Astragalus maniaticus	75.0	25.0	25.0
PCC	<0.001	>0.5	<0.05
MCC	–	–	>0.5
Total	–	–	68.3

Note: PCC and MCC indicate the proportional chance criterion and the maximum chance criterion, respectively.

Table IV. Stepwise discriminant analysis results for data-set-2 based on eight morphological characters, as well as the discriminant functions' eigenvalues and the proportion of variance (PV) explained by each discriminant function.

Character	*T*	*F*	WD	DF1	DF2	DF3
Eigenvalue	–	–	–	9.32	0.95	0.18
PV	–	–	–	89.2	9.1	1.7
WD	–	–	–	0.04	0.43	0.85
					SML	
PeL	0.99	0.00	0.09	**0.72**	−0.36	−0.22
SeL	0.97	0.00	0.06	0.46	0.07	−0.12
SpL	0.97	0.00	0.05	0.40	0.11	0.32
LfW	0.52	0.00	0.07	0.18	**0.85**	0.20
LfL	0.51	0.00	0.05	0.17	0.32	**0.74**
LfR	0.95	n.s.	n.s.	–	–	–
FW	0.00	n.s.	n.s.	–	–	–
FL	0.44	n.s.	n.s.	–	–	–

Note: 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 I. n.s.: statistically non-significant. Higher values are shown in bold.

Table V. Correct LOO cross-validation classification from the stepwise discriminant analysis of data-set-2.

Taxon	A	B	C	D	% Correct classification
A	88.0	0.0	6.0	6.0	88.0
B	0.0	87.0	13.0	0.0	87.0
C	1.0	1.5	97.5	0.0	97.5
D	8.3	0.0	0.0	91.7	91.7
PCC	<0.001	<0.001	<0.001	<0.001	<0.001
MCC	–	–	–	–	<0.001
Total	–	–	–	–	92.4

Note: A, B, C and D indicate *Aethionema saxatile* subsp. *graecum*, *Ae. saxatile* subsp. *creticum*, *Ae. saxatile* subsp. *oreophilum* and *Ae. saxatile* subsp. *corinthiacum*, respectively. PCC and MCC indicate the proportional chance criterion and the maximum chance criterion, respectively.

A. suberosus subsp. *haarbachii*. Interestingly, PL, the most significant differentiating character between the sections *Hypoglottis* and *Platyglottis* to which *A. maniaticus* and *A. suberosus* subsp. *haarbachii*, respectively, belong (Chamberlain & Matthews 1970; Tan & Strid 1997), did not have statistically discriminating significance. Cross-validated classification (LOO) results also show that CL and FtW poorly distinguish between the two taxa, since overall classification success of *A. maniaticus* and *A. suberosus* subsp. *haarbachii* based on this character was 68.3%, being significantly better than random chance for the PCC ($p < 0.05$), but not for the MCC ($p > 0.05$; Table III); MCC is particularly appropriate in a two-group situation with different prior probabilities (Huberty & Lowman 2000). Moreover, 75.0% of the specimens identified as *A. maniaticus* were classified as *A. suberosus* subsp. *haarbachii* in the DA (Table III).

Data-set-2. The DA clearly discriminates between the taxa comprising the *Aethionema saxatile* complex. All but three of the characters (LfR, LfW & FL) emerged as statistically significant (Table IV), with LOO showing a 92.4% correct classification, being significantly better than random chance for both the PCC and MCC ($p < 0.001$, Table V). The remaining characters (PeL, SeL, SpL, LfL and FW) showed little overlap across the four taxa (Figure S6). The first (DF1), the second (DF2) and the third (DF3) discriminant function explain 89.2, 9.1 and 1.7% of the total variation, respectively. DF1 is influenced primarily by PeL, SeL and SpL. Specifically,

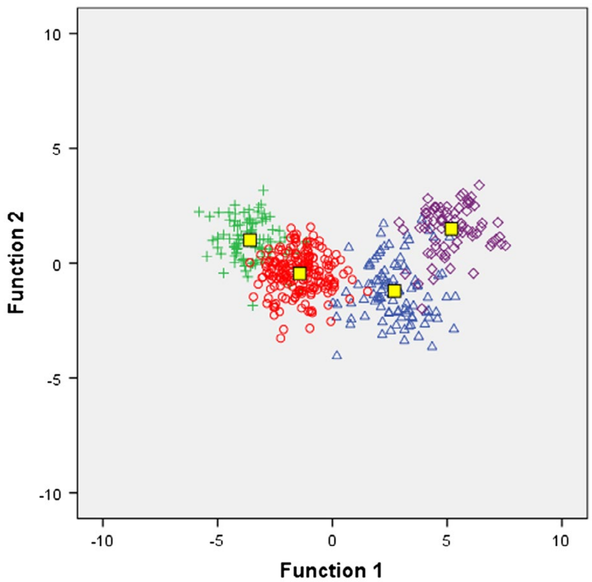

Figure 2. Stepwise discriminant analysis plot for data-set-2. Purple diamonds, green crosses, red circles and blue triangles indicate *Aethionema saxatile* subsp. *corinthiacum*, *Ae. saxatile* subsp. *creticum*, *Ae. saxatile* subsp. *oreophilum* and *Ae. saxatile* subsp. *graecum*, respectively. Yellow squares indicate the function group centroid of each taxon.

Aethionema saxatile subsp. *corinthiacum* has the longer petals, sepals and styles among the *Ae. saxatile* complex in Greece. LfW and LfL are the characters loading most heavily on DF2 and DF3, respectively, with *Ae. saxatile* subsp. *corinthiacum* having the wider and longer leaves than any other taxon of data-set-2. Undoubtedly, the taxa comprising the *Aethionema saxatile* complex in Greece are clearly distinct from one another (Figure 2). Nevertheless, there seems to be some slight overlap between *Ae. saxatile* subsp. *oreophilum* and *Ae. saxatile* subsp. *creticum*, as well as between *Ae. saxatile* subsp. *graecum* and *Ae. saxatile* subsp. *corinthiacum*; however, this phenomenon can be attributed to the close phylogenetic relationships between these taxa, since they are evolutionary young variants of the same species.

Data-set-3. *Aethionema saxatile* subsp. *corinthiacum* and *Ae. rhodopaeum* are fairly well separated from the rest of the taxa. All but one of the characters (LfW) emerged as statistically significant (Table SIII), with LOO showing a 70.6% correct classification, which was significantly better than random chance for both the PCC and MCC ($p < 0.001$, Table SIV). The remaining characters (PeL, SeL, LfL, LfR and FW) did not show much overlap across the four taxa (Figure S7). The first (DF1), the second (DF2), the third (DF3), the fourth (DF4) and the fifth discriminant function (DF5) explain 69.2, 20.1, 7.1, 3.1 and 0.5% of the total variation, respectively. DF1 is influenced primarily by SeL. Specifically, *Aethionema saxatile* subsp. *saxatile* has the smaller styles among the taxa included in data-set-3. LfR is the character loading most heavily on DF2, with *Ae. saxatile* subsp. *saxatile* having the highest leaf length:width ratio than any other taxon of data-set-3. DF3 on the other hand, is driven by LfL, with *Ae. rhodopaeum* having much shorter leaves than the other taxa. FL has a high loading on DF4, with *Ae. rhodopaeum* having the shortest fruits among data-set-3. Finally, DF5 is driven primarily by FW, with *Ae. saxatile* subsp. *saxatile* having the widest fruits. There seems to be some slight overlap between *Ae. saxatile* subsp. *oreophilum*, *Ae. saxatile* subsp. *creticum* and *Ae. rhodopaeum* (Figure S8), indicating that the latter would better merit a subspecific rank in the *Aethionema saxatile* complex. The lower classification success of data-set-3 compared to that of data-set-2 is attributable to the exclusion of PeL and SpL from the analysis, since the type specimens of *Ae. rhodopaeum* did not bear flowering shoots.

Data-set-4. The DA (Figure S9) indicates that certain specimens from SW Greece resembling *Aethionema saxatile* subsp. *saxatile* cannot be placed with certainty under either subsp. *oreophilum* or subsp. *saxatile*. Only PeL, SeL, LfR and FW emerged as statistically significant (Table SV). These factors, as well as the two discriminant functions have quite high Wilk's lambda values, indicating the vague identity of these unspecified specimens (Table SVI).

Discussion

Multivariate morphometrics is a powerful tool for the assessment of the variation patterns at the specific and infraspecific level and is particularly useful for drawing lines between complex taxa and discovering the most important characters that differentiate among them (Marhold 2011). In the present study, the application of multivariate morphometrics resulted in important conclusions with taxonomic consequences. Briefly, all plant specimens identified as either *Astragalus maniaticus* or *A. suberosus* subsp. *haarbachii* cannot be separated from one another by any morphological means; they represent a single taxon. Furthermore, *Aethionema saxatile* subsp. *corinthiacum* emerges as a distinct subspecies, independent from any of the other three subspecific taxa or presumed hybrids occurring in Greece and different from the Bulgarian *Ae. rhodopaeum*.

The multivariate morphometric analysis (Figure S5) demonstrated that, despite their placement in different sections, *Astragalus maniaticus* shares all important morphological features with *A. suberosus* subsp. *haarbachii*, an entity distributed in the

Balkans and Turkey (Podlech 1990, 2008; Taeb et al. 2012). The initial placement of *A. maniaticus* in sect. *Hypoglottis* (Tan & Strid 1997, Podlech 2008, 2011) following the sectional key provided by Chamberlain and Matthews (1970) was apparently induced by the short pedicels in the vouchers' pedunculate inflorescence, reported as 0–1 mm (Tan & Strid 1997), 0–2 mm (Tan & Iatrou 2001) or 2–3 mm (Podlech 2008) long. Flower pedicels are present in all our specimens; their length ranges between 1 and 4 mm (Table SIIa), thus supporting the placement of *A. maniaticus* in sect. *Platyglottis.* There are no other morphological features present in any of our specimens (see Appendix I in supplementary material for specimens examined) or even in the *A. maniaticus* holotype (LD) that contradict this sectional transfer. Within sect. *Platyglottis,* the taxonomic relationships of *A. maniaticus* point straightforward to *A. suberosus* subsp. *haarbachii* and in the absence of any distinguishing characters between these two taxa, their merging is inevitable, with the former considered a heterotypic synonym of the latter. On the other hand, *A. austroaegaeus*, a species presumably similar but not closely allied to *A. maniaticus* being a member of sect. *Malacothrix* Bunge (Podlech 2008), exhibits noticeable differences from *A. suberosus* (Tan & Strid 1997).

The only variable morphological character detected in the *A. suberosus* subsp. *haarbachii* specimens from southern Greece concerns legume size (Figure S10). All the Peloponnesian populations seen by us and included in data-set-1 exhibit long and narrow (4–7 mm wide) fruits, thus belonging to var. *argolicus* (Hausskn.) Podlech. We presume that the type of *A. maniaticus* belongs to the same variety too, although the type specimen itself lacks fruits. Typical var. *haarbachii* with wider (6–12 mm) and often shorter fruits, sometimes covered by a denser and somewhat longer indumentum compared to var. *argolicus*, was seen in specimens from Sterea Ellas. The two varieties do not exhibit independent distribution ranges (Podlech 1990) and populations with variable fruit size have also been observed by us, for example, on Mt. Gerania.

The elucidation of *Astragalus maniaticus* identity and its merging with *A. suberosus* subsp. *haarbachii* has a direct impact on its conservation priorities. *A. suberosus* subsp. *haarbachii* is a widespread taxon, particularly common in south & east Greek mainland, often found in human-affected habitats (e.g. open fields, olive groves, roadsides and margins of cultivated land). There are many populations known so far and their favourable habitats at low and middle altitudes (0–1000 m) are abundant, thus supporting the placement of the subspecies under the LC (Least Concern) IUCN (2001) category.

Aethionema saxatile complex is a taxonomically difficult group in Greece and different treatments for its subspecific taxa have been proposed (Andersson et al. 1983; Chater & Akeroyd 1993; Tan & Suda 2002). In the Brassicaceae in general, fruit morphology is primarily used in taxa delimitation, while floral characters are usually treated as less significant (Appel & Al-Shehbaz 2003). *Aethionema* particularly, shows significant variation in habitat, floral structure and colour, fruit morphology and heterocarpy (Al-Shehbaz et al. 2006). The morphological characters that emerged as significant in our analyses partly correspond to those previously reported as diagnostic (Andersson et al. 1983) and include floral parts, i.e. petal length (PeL), sepal length (SpL) and style length (SeL) and leaf dimensions. None of the fruiting characters alone could distinguish between the taxa in Greek *Ae. saxatile*, the only exception being the percentage of unilocular to bilocular fruits that separates subsp. *creticum* from the rest of the complex (Andersson et al. 1983). Our results indicate that subsp. *corinthiacum* is an independent subspecies rather than a local ecotype, receiving the same strength of support as the other three Greek subspecies: *creticum*, *graecum* and *oreophilum*. The latter, not accepted by Chater and Akeroyd (1993) and Tan and Suda (2002), should be re-established. It differs from subsp. *graecum* in statistically important characters including petal, sepal and style length: subsp. *oreophilum* has distinctively smaller petals, sepals and styles and flowering specimens are easily distinguished in the vast majority of cases. The presence of a fifth subspecies, subsp. *saxatile*, in NW Greece could not be confirmed beyond any doubt: those specimens with narrow leaves, short petals and sepals and very short styles seem to partition between typical subsp. *saxatile* represented by specimens from countries like France, Germany, Austria, Italy and former Yugoslavia and Greek subsp. *oreophilum*. The existence of "pure" subsp. *saxatile* in Greece cannot be ruled out, however, particularly since Andersson et al. (1983) map this subspecies very close to the Greek–Albanian borders, at the Albanian side. Further collections from NW Greece are advisable. A diagnostic key for the Greek *Aethionema saxatile* group is provided in the Appendix I (see supplementary material).

Aethionema saxatile subsp. *corinthiacum* has fleshy, broadly oblong-elliptic to suborbicular leaves and in these respects resembles *Ae. rhodopaeum*, a member of the *Ae. saxatile* group. According to Pavlova (2007), *Ae. rhodopaeum* differs from the *Ae. saxatile* complex in the following characters: inner filament length always greater than half the petal length, lack of teeth and wings on inner filaments, longer (2–3.5 mm) style and dark purple petals. In the Greek *Ae. saxatile* specimens examined, filament teeth may be absent in quite a large proportion (ca.

20%) of the flowers examined, particularly those with long and narrow filaments. The occurrence of wings along the inner filaments is related to their width: well-formed wings generally result in wider filaments. However, filament width and presence vs. absence of teeth did not emerge as significant in a preliminary morphometric analysis of the *Ae. saxatile* complex and consequently excluded from further analyses. Regarding style length, the examination of the *Ae. rhodopaeum* specimens kept in SO revealed some important evidence: the intact style measured in the fruiting specimens ranged from 0.3 to 1.0 mm and was substantially shorter than the 2–3.5 mm of length originally reported. These style values fall within the *Ae. saxatile* range of variation. Nevertheless, *Ae. rhodopaeum* is still fairly well separated from the five *Ae. saxatile* subspecies included in our study (Figure S8). Taking into consideration, (i) style length in *Ae. rhodopaeum* may be more variable than previously thought and that it emerged as an important discriminating feature in our analyses, (ii) inner toothless filaments can also be found in the *Ae. saxatile* subspecies and (iii) the lack of distinct morphological discontinuities between the examined taxa, we propose that *Ae. rhodopaeum* is better included in the *Ae. saxatile* complex rather than maintained as an independent species. The following nomenclatural combination seems necessary:

Aethionema saxatile subsp. *rhodopaeum* (Pavlova) Constantinidis, Kougioumoutzis & Kalpoutzakis, **comb. & stat. nov.**

Basionym: *Aethionema rhodopaeum* D. Pavlova in Bot. J. Linn. Soc. 155: 535, 2007.

Aethionema saxatile subsp. *corinthiacum* was described from the lower, southern parts of Mt. Gerania, at c. 130 m. Recently, we discovered new populations from the southern, western and northern parts of the same mountain, at an altitude of c. 10–950 m. These new populations grow on serpentine, sandstone or marl substrate. The subspecies predominately occurs on serpentine but is not an obligate serpentinophyte. Two specimens from the Kamena Vourla area (Willing 22633, B; Stamatiadou 10940, ATH) may also belong in this taxon, though having slightly larger styles. The inclusion of all the recently collected specimens in our analyses revealed that the range of seven morphological characters (FL, FW, LfL, LfW, PeL, SeL and SpL – Table SIIb) is expanded: the minimum bilocular fruit length and width and the maximum leaf length and width on one hand, together with petal and style length on the other are substantially lower and higher, respectively, than that reported by Zarkos et al. (2011); regarding sepal length, it is substantially smaller. A revised description is provided in Appendix I (see supplementary material).

Aethionema saxatile subsp. *corinthiacum* is not common on Mt. Gerania and has the narrowest distribution of all Greek *Ae. saxatile* subspecies. Each of the subpopulations found comprises between 20 and 150 mature individuals, meaning that the total population may be less than 2000 individuals. The plants colonise rocky slopes and cliffs, road embankments, horizontal or inclined areas with gravel, scree or bare soil, openings in *Pinus halepensis* forest and dry streambeds. Plants with almost white to entirely pink petals may exist at the same locality. Given its local distribution and rarity, subsp. *corinthiacum* is a candidate for a more detailed evaluation of its conservation priorities in the future.

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Supplemental data

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