Commagene Journal of Biology

Toprak, (2023) *Comm. J. Biol.* 7(2), 164-170.
DOI: 10.31594/commagene.1404268

e-ISSN 2602-456X

Research Article/Araştırma Makalesi

Investigation of the Occurrence of *Silene fuscata* in Anatolia Based on Molecular Analysis

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Received: 13.12.2023 **Accepted:** 30.12.2023 **Published online:** 31.12.2023 **Issue published:** 31.12.2023

Abstract: *Silene fuscata* is an annual species distributed to a wide geography including Eastern Mediterranean, Northern Africa, and Southern Europe. The native range of this species is known as the Mediterranean Region. Presence of *S. fuscata* in Anatolia is reported by several comprehensive botanical literatures; however, the actual situation has never been investigated thoroughly. This study focuses on the occurrence of *S. fuscata* in Anatolia. In order to determine the existence of this species in the aforementioned area, nuclear and chloroplast-DNA sequence data from a wide range of *Silene* samples were analyzed. DNA sequence analyses and other available evidence display no support for the presence of *S. fuscata* in Anatolia.

Keywords: Amanos, est04, haradj, Mediterranean, pcr, phylogenetics, rps16, *Silene*.

Silene fuscata'nın Anadolu'daki Varlığının Moleküler Açıdan Araştırılması

Öz: *Silene fuscata*, Doğu Akdeniz, Kuzey Afrika ve Güney Avrupa'yı içeren geniş bir coğrafyaya yayılmış tek yıllık bir bitki türüdür. Akdeniz Bölgesi bu türün doğal yayılış alanı olarak bilinir. *S. fuscata*'nın Anadolu'da da yetiştiği birçok kapsamlı botanik çalışmada bildirilmiş olmakla birlikte türün mevcut durumu hiçbir zaman ayrıntılı olarak araştırılmamıştır. Bu makalede *S. fuscata*'nın Anadolu'daki varlığı incelenmiştir. Anadolu ve Anadolu dışından toplanan farklı *Silene* türlerinden nükleer ve kloroplast-DNA dizileri üretilerek bu bitki örneklerinin filogenetik ilişkileri irdelenmiştir. DNA dizi analizleri ve mevcut diğer kanıtlar *S. fuscata*'nın Anadolu'da yayılış göstermediğine işaret etmektedir.

Anahtar kelimeler: Amanos, est04, haradj, Akdeniz, pcr, filogenetik, rps16, *Silene*.

1. Introduction

The genus *Silene* L (Caryophyllaceae) exhibits a wide geographical distribution, primarily concentrated in the temperate regions of the Northern Hemisphere (Oxelman & Lidén, 1995; Oxelman et al., 2001). Mediterranean Region is one of the most *Silene* rich areas (Greuter, 1997; Naciri et al., 2022). In the most recent revision of the genus by Jafari et al. (2020), approximately 870 species of *Silene* from all over the world were assigned to 33 sections. These sections were further classified under three subgenera: *S.* subg. *Silene*, *S.* subg. *Behenantha* (Otth) Endl., and *S.* subg. *Lychnis* (L.) Greuter. The study by Jafari et al. (2020) has been one of the most inclusive studies of *Silene* to date and it provides a backbone classification based on morphological and molecular data. Nevertheless, taxonomy within *Silene* is still somewhat difficult at lower taxonomic levels such as section, subsection, group, and series. The main ground for this complexity is the high homoplasy associated with morphological characters like floral structures (number of styles, limb color, coronal scales, etc.), inflorescence, life habit, and type of indumentum among others. Such complexity may occasionally results in erroneous identification of species or inferences of "species complex", referring to a number of taxa with unclear boundaries (Scherz et al., 2019). The misidentification of the morphologically very similar taxa amplifies the issue by leading to incorrect understanding of their distribution patterns.

Silene fuscata Link ex Brot. is an annual Mediterranean species outspread to a large geography including North Africa, South Europe, and Eastern Mediterranean areas such as Cyprus, Lebanon, Syria, and Israel. It is a moderately common plant growing in open lands, rocky slopes, screes, and sandy fields (Danin & Fragman, 2016). The flowering time depends on ecological factors such as altitude, temperature, humidity and can range from early February to late June.

Anatolia is known as one of the native areas for *S. fuscata* (Güner et al., 2012). In the Flora of Turkiye (Coode & Cullen, 1967), *S. fuscata* is classified within Sect. *Atocion* Otth, a group of morphologically very similar annual Mediterranean species (Toprak et al., 2016) having an ambiguous phylogenetic position in the genus *Silene* (Aydın et al., 2014–a). Recent work by Jafari et al. (2020) shows that *S. fuscata* is nested within the Sect. *Silene*, belonging to S. subg. *Silene*, and thus distantly related to Sect. *Atocion*.

S. fuscata has been included in several recent molecular (Prieto–Benítez et al., 2016, Eggens et al., 2020; Mesbah, 2021; Toprak & Yıldız, 2022) and floristic studies (Greuter, 1995; Jeanmonod, 2015; Medina & Aedo, 2022). However, none of these studies employs any specimen of *S. fuscata* originated from Anatolia. Furthermore, there is no sample registry from Anatolia except for the two records in the Flora of Turkey (Coode & Cullen, 1967). Additionally, name of *S. fuscata* appears in a few highly localized floristic investigations (e.g., Orcan et al., 2004)

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and several regional herbaria [e.g., AIBU, VHLV, GUL, HUB (acronyms follow Thiers, 2023)] listing the name of the species in their online catalogue. Nevertheless, both of these resources fall short in providing further information on the species.

The aim of this study is to understand the occurrence of *S. fuscata* in Anatolia. By comparing the DNA sequence data of *S. fuscata* samples collected from outside of Anatolia to the sequences from putative Anatolian *S. fuscata,* their phylogenetic relationships are investigated. It is hypothesized that a possible Anatolian *S. fuscata* sample would display a closer relationship to any other *S. fuscata* species than it would to a distant *Silene* species.

2. Material and Methods

The plant material from Anatolia was collected via field trips performed between 2008 and 2019. The non-Anatolian plant material was provided by the herbarium of University of Gothenburg (GB). In order to sample Anatolian *S. fuscata,* the localities given by *Flora of Turkey and East Aegean Islands* (Davis,1965–1988) were visited several times. *Silene fuscata* material in the G, GB, HBG, HUB, K, UPS, WU (acronyms follow Thiers, 2023) herbaria were also examined. Considering *S. fuscata* localities given by Flora of Turkey, available specimen sheets, and local floristic studies; any *Silene* material growing in the reported places and nearby areas were sampled. However, only the material that shows morphological resemblance to *S. fuscata* was sequenced. Morphological evaluation of the samples was based on the considerations in *Flora of Turkey* (Coode & Cullen, 1967), *Flora Hellenica* (Greuter, 1997), and *Flora of Cyprus* (Meikle, 1977). The lectotype of *S. fuscata* [Fig. 1 (MA00031723!)] was also taken into account for morphological inference. The localities of the sequenced Anatolian samples are presented in Figure 2. The complete voucher list is provided as a Supplemental Table 1. In total, 46 Anatolian *Silene* samples showing a large overlapping morphology with *S. fuscata,* were analyzed. The remaining samples were *S. fuscata* from outside Anatolia and some other diverse *Silene* species used as references for phylogenetic estimation.

The chloroplast *rps16* (Oxelman et al., 1997) and nuclear *EST04* (Toprak et al., 2016) markers were used to compare phylogenetic position of putative Anatolian *S. fuscata* with *S. fuscata* from Algeria and Israel. The *EST04* data set was formed by newly generated (27%) and already published sequences while the *rps16* data set was made of sequences from Genbank and our published work elsewhere. New sequences were generated according to protocols available in Petri et al. (2013) and Aydin et al. (2014–b). The contigs were processed using Geneious Prime v2022.1.1 (Biomatters, http://www.geneious.com). Details of the applied PCR protocols, amplifying and sequencing primers are available in BoxTax Database (Oxelman *et al.,* 2013). Edited sequences were aligned with MUSCLE as implemented in the program MEGA v.11.0.13 (Tamura et al., 2021) and manually adjusted afterwards. Each data set was checked for parsimony informativeness using PAUP* v.4.0a 169 (Swofford 2002, 2021).

Figure 1. A- Image of a *S. fuscata* specimen (W. Schimper, 1932, Algeria (HBG-503531!)) B- Image of the Lectotype of *S. fuscata* (MA00031723!)

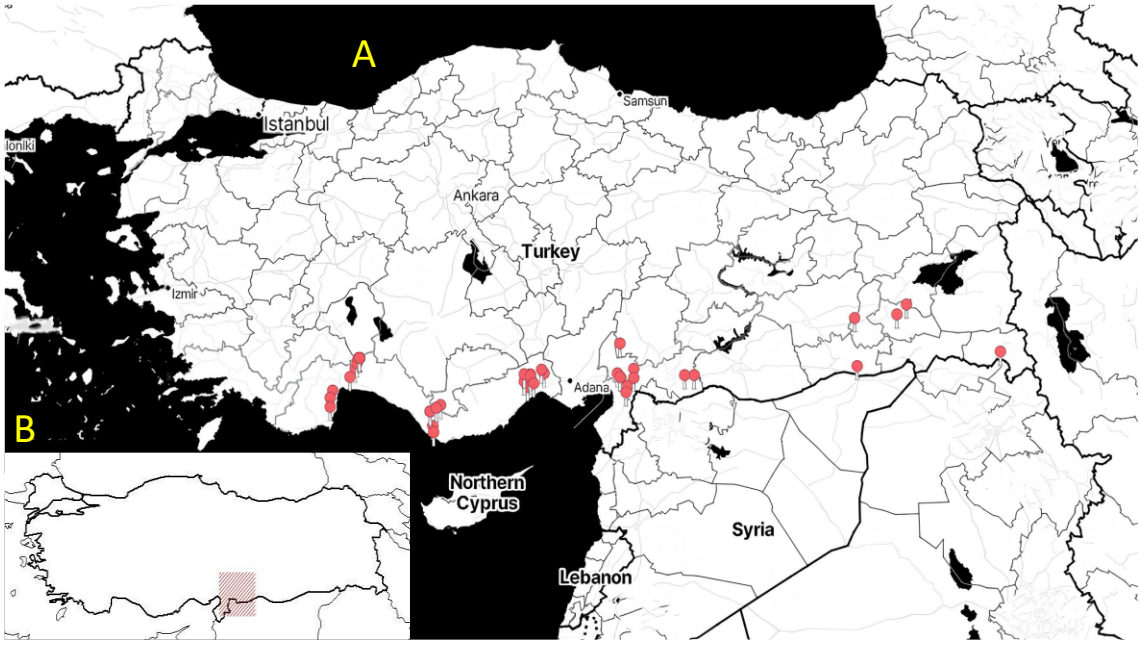

Figure 2. A- Map showing the geographical distribution of 46 putative Anatolian *S. fuscata* samples. The red icons refer to the geographic coordinates of the samples. B- Geographic distribution of *S. fuscata* based on the grids defined by Flora of Turkey and East Aegean Islands (Retrieved from TUBIVES (Bakis et al., 2011).

Maximum likelihood (ML) gene trees for 82 of *rps16*, and 78 of *EST04* sequences were estimated independently. ML gene trees were reconstructed via Randomised Accelerated Maximum Likelihood (RAXML) v.8.2.x (Stamatakis, 2014). A GTR+G model with 25 rate categories was set as substitution model for each locus. The analysis was run on a random starting tree with rapid bootstrapping of 1000 iterations. Each analysis was performed as three replicates. Because of its close phylogenetic position with the genus *Silene* (Oxelman et al. 1997), a sequence of *Atocion rupestre* was set as outgroup for both data sets. The remaining parameters were accepted as implemented by CIPRES portal (Miller, 2010). Resulting best ML tree of each locus was illustrated and inferred with FigTree v1.4.4 (Rambaut, 2018).

3. Results

The two *S. fuscata* samples reported by the Flora of Turkey and East Eagean Islands (Coode & Cullen, 1967) demonstrated highly similar morphology to the 46 analyzed samples.

The alignment of 82 *rps16* and 78 *EST04* sequences resulted in 991 and 783 nucleotide characters, respectively. Statistics of parsimony analysis for each alignment are given in Table 1. The ML gene trees of chloroplast (Fig. 3) and nuclear (Fig. 4) loci showed results that well congruent with each other and also with the phylogenetic relationships reported in the most recent revision of *Silene* (Jafari et al., 2020). *Atocion rupestre* and *Viscaria vulgaris* were located as the basal lineages and the remaining samples were placed in agreement with the content of the three subgenera. Both loci displayed non-Anatolian *S. fuscata* samples within the clade forming the *S.* subgenus *Silene*. None of the 46 samples from Anatolia was placed as closely related to *S. fuscata* from Algeria and Israel. These samples were shown as outside of genus *Silene* in the rps16 tree (Fig. 3), whereas they were distributed as sisters to the *S.* Subg. *Behenantha* and *S.* Subg. *Silene* by the *EST04* tree (Fig. 4), yet with weak support. The position of *S.* Subg. *Lychnis* was poorly understood due to the polytomy observed in both gene trees. *S. fuscata* was located in the group including the African samples classified under *S.* Subg. *Silene* by both loci.

Table 1. Statistics of parsimony analysis of *rps16* and *EST04* loci.

	rps16	*EST04*
Number of samples	82	78
Aligned matrix length [bp]	991	783
Constant characters [bp]	756	511
Variable characters [bp]	61	36
Parsimony-informative characters [bp]	174	236
Consistency index (CI)	0.800	0.758
Retention index (RI)	0.939	0.947
Homoplasy index (HI)	0.200	0.242
Rescaled consistency index (RC)	0.751	0.719

4. Discussion

In the last global revision of the genus (Jafari et al., 2020), *S. fuscata* is assigned to Sect. *Silene* belonging to *S.* Subg. *Silene*. In accordance with this classification, our analyses put *S. fuscata* in Subg. *Silene* together with allies as *S. italica*, S. *nutans*, *S. schafta, S.biafrae* among others. On the other hand, genetic data of 46 Anatolian *Silene* with a common morphology to *S. fuscata* show no affinity to non-Anatolian *S. fuscata*. Such a relationship signifies the lack of the species among the sequenced samples. Although our analyses were based on a limited number of non-Anatolian *S. fuscata* samples, the observed sister phylogenetic relationships among these geographically divergent samples yet their rather distant position to Anatolian *S. fuscata* represantives clearly remark the absence of the same lineage in Anatolia.

There are only two records of *S. fuscata* in the *Flora of Turkey and East Aegean Islands* (Coode & Cullen, 1967) and

both are based on the specimens collected by Haradjian (https://kiki.huh.harvard.edu/databases/botanist_index.html). These specimens (Fig. 5) are originated from "Kurd Dağı" area (see Özuslu and Tel, 2008 for the update on the regional names) within the Amanos Mountains in Southern Anatolia and preserved in the "G" herbarium. Modern *Silene* specialists verify the specimens of Haradjian as *S. aegyptiaca,* instead of *S. fuscata*.

Silene fuscata and *S. aegyptiaca* show a high degree of morphological similarity; however, their phylogenetic relatedness is comparatively distant (Jafari et al., 2020). The distribution range of both species is known to be overlapping largely in the Middle East but partly in Anatolia given the former species exist in the latter region. Our study covers a wide geographic area with samples from all over the South Anatolia including the whole Mediterranean range. Nevertheless, unlike the herbarium records (e.g., HUB 3704) and previous studies (Coode & Cullen, 1967; Orcan et al., 2004) our analyses strongly disagree with the occurrence of *S. fuscata* in Anatolia unless it is extremely rare to be sampled. In fact, *S. fuscata* is reported to be very rare in Greece (Strid, 2018).

Regarding the phylogenetic relationships of 46 putative *S. fuscata* samples, they form clades with unstable positions among the three subgenera of *Silene*. Indeed, these positions match to the reported positions for the members of Sect. *Atocion* (Erixon & Oxelman, 2008; Aydin et al., 2014–a; Toprak et al., 2016) and thus strongly indicate their relation with the members of Sect. *Atocion,* rather than *S. fuscata.* Besides, they show sister relationships with the *S. aegyptiaca* samples from Cyprus, Lebanon, and Israel. Furthermore, both of the gene trees place the 46 samples into two clades that well congruent with the geographic separation of the members of Sect. *Atocion* remarked by Toprak et al. (2016).

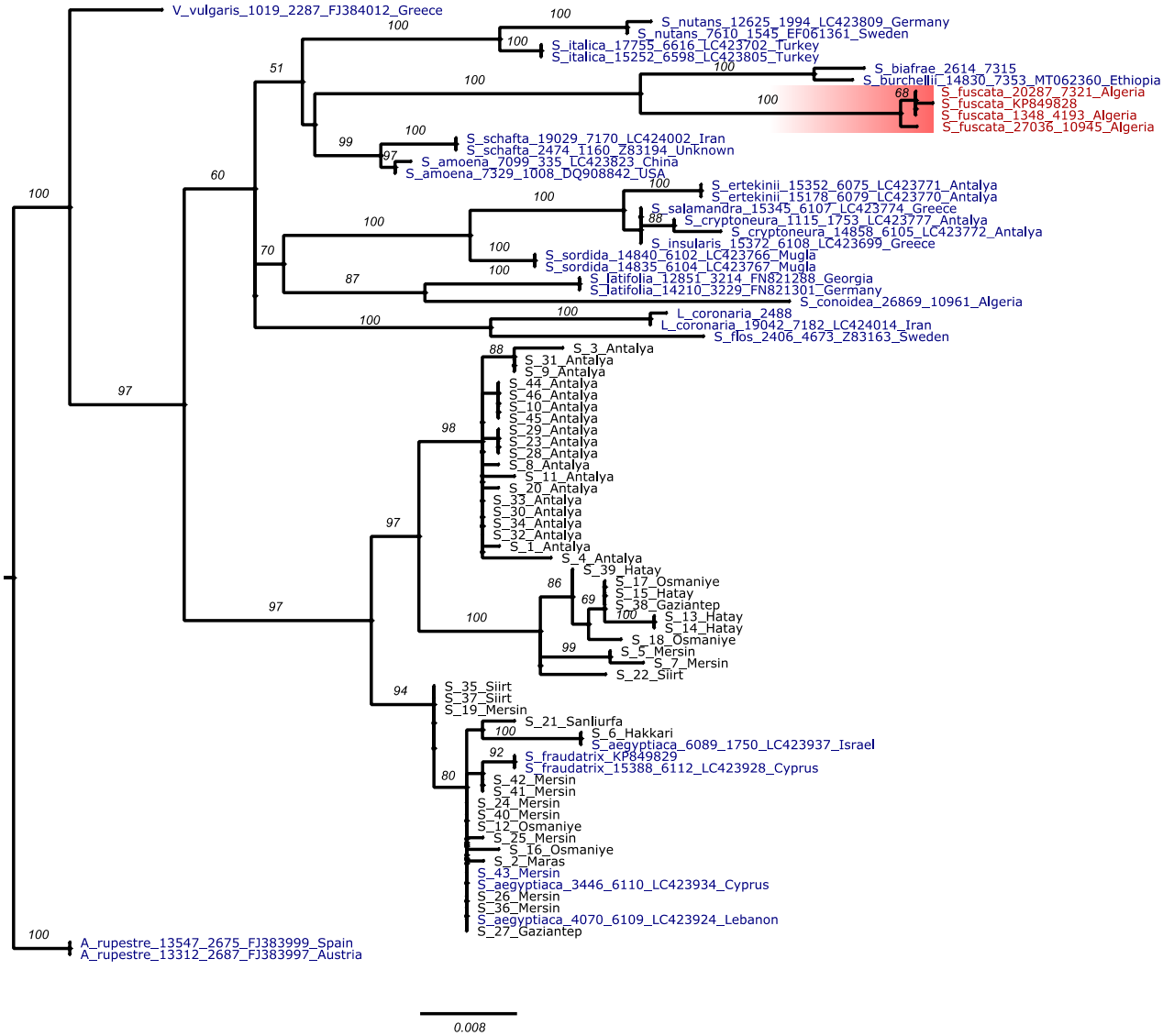

Figure 3. Maximum Likelihood gene tree of *rps16* locus, obtained from RAXML. The number above the branches indicates Bootstrap values (Bs>50 are shown). The numbers following the taxon name are specimen, sequence, and gene bank IDs, respectively. The 46 putative Anatolian *S. fuscata* samples are given as "S_1– 46". Non-Anatolian *S. fuscata* samples are highlighted by red color.

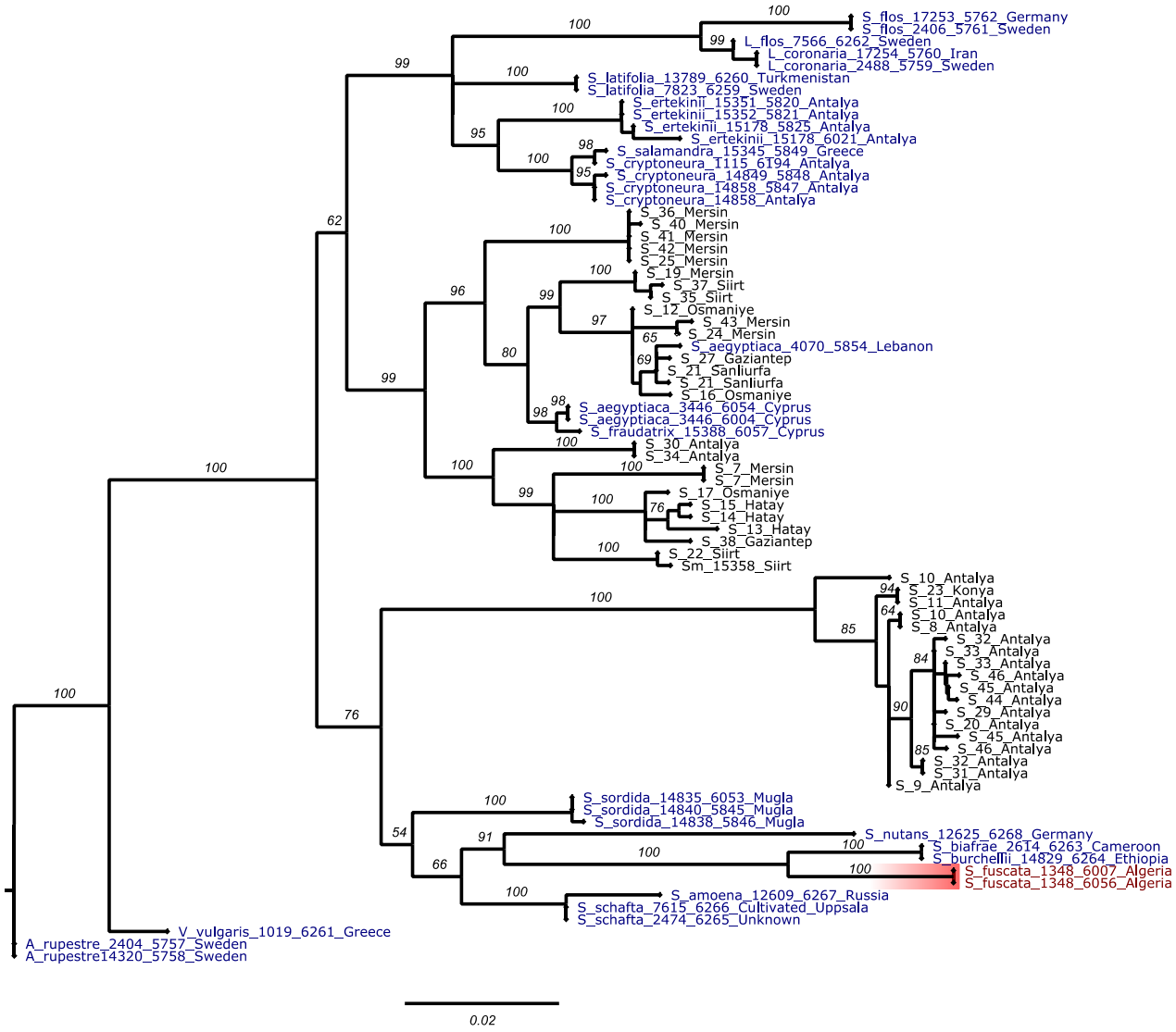

Figure 4. Maximum Likelihood gene tree of *EST04* locus, obtained from RAXML. The number above the branches indicates Bootstrap values (Bs>50 are shown). The numbers following the taxon name are specimen, sequence, and gene bank IDs, respectively. The 46 putative Anatolian *S. fuscata* samples are given as "S_1– 46". Non-Anatolian *S. fuscata* samples are highlighted by red color.

Despite the sampling effort, we were unable to get the material from the exact localities of Haradjian's specimens due to the insufficient descriptions of the exact points; however, considerable amount of materials were collected around the region reported as "Kurd Dağı" (Özuslu and Tel, 2008). We analyzed this material in addition to the material from precise location given by Orcan et al. (2004) and the localities given by the other records (e.g., HUB 3704; Gazipaşa, Macar köyü yaylası, H. Sümbül, AIBU; Hassa-Islahiye 3 km batı yamaç, K. Yıldız 0381-1, E. Minareci, M. Kuh). Nevertheless, none of them demonstrates any close relation to non-Anatolian *S. fuscata* and therefore increases the possibility of these specimens being confused with the allies of *S. aegyptiaca* complex.

The high homoplasy associated with the morphological evolution of *Silene* is well known (Petri & Oxelman, 2011; Aydin *et al.*, 2014b; Toprak *et al.*, 2016; Pfeil *et al.*, 2017; Naciri *et al.*, 2017; Jafari *et al.*, 2020). Convergent morphological evolution is another known issue for the genus (Naciri et al., 2022; Berardi et al., 2022). Consequently, the misidentification of the taxa and their distribution areas stand as a plausible explanation for the case of *S. fuscata* and the members of Sect. *Atocion*. Given the complex morphological evolution of the genus, the importance of using molecular data such as DNA sequences becomes critical for the accurate determination of many taxa of *Silene*.

Briefly, nuclear and chloroplast DNA sequences data provide no support for the occurrence of *S. fuscata* in Anatolia. The available evidence suggests an erroneous determination of the taxon, indicating a probable confusion with members of Sect. *Atocion*. Nevertheless, a better sampling strategy that covers samples from Greece, Italy, Portugal, and more of the Middle East as well as the sequences of type specimens would give a complete understanding of the case. Furthermore, a comprehensive morphological work covering the full variation is required for the proper identification keys to avoid confusions such as the one between *S. fuscata* and *S. aegyptiaca.*

Acknowledgments: The author is grateful to Bengt Oxelman (University of Gothenburg, Gothenburg); Kemal Yıldız (University of Celal Bayar, Manisa); Yamama Naciri & Daniel Jeanmonod (University of Geneva; Conservatoire et Jardin Botaniques, Geneva); A. Selçuk Ertekin (University of Dicle,

Diyarbakır) Staff of the Gothenburg Botanical Garden, and Mehmet Toprak (Antalya) for their valuable contributions. This work is partly supported by Scientific and Technological Research Council of Turkey (TUBITAK, BIDEB ID: 667895), and Dicle University Scientific Research Centre (DUBAP: FEN.22.015).

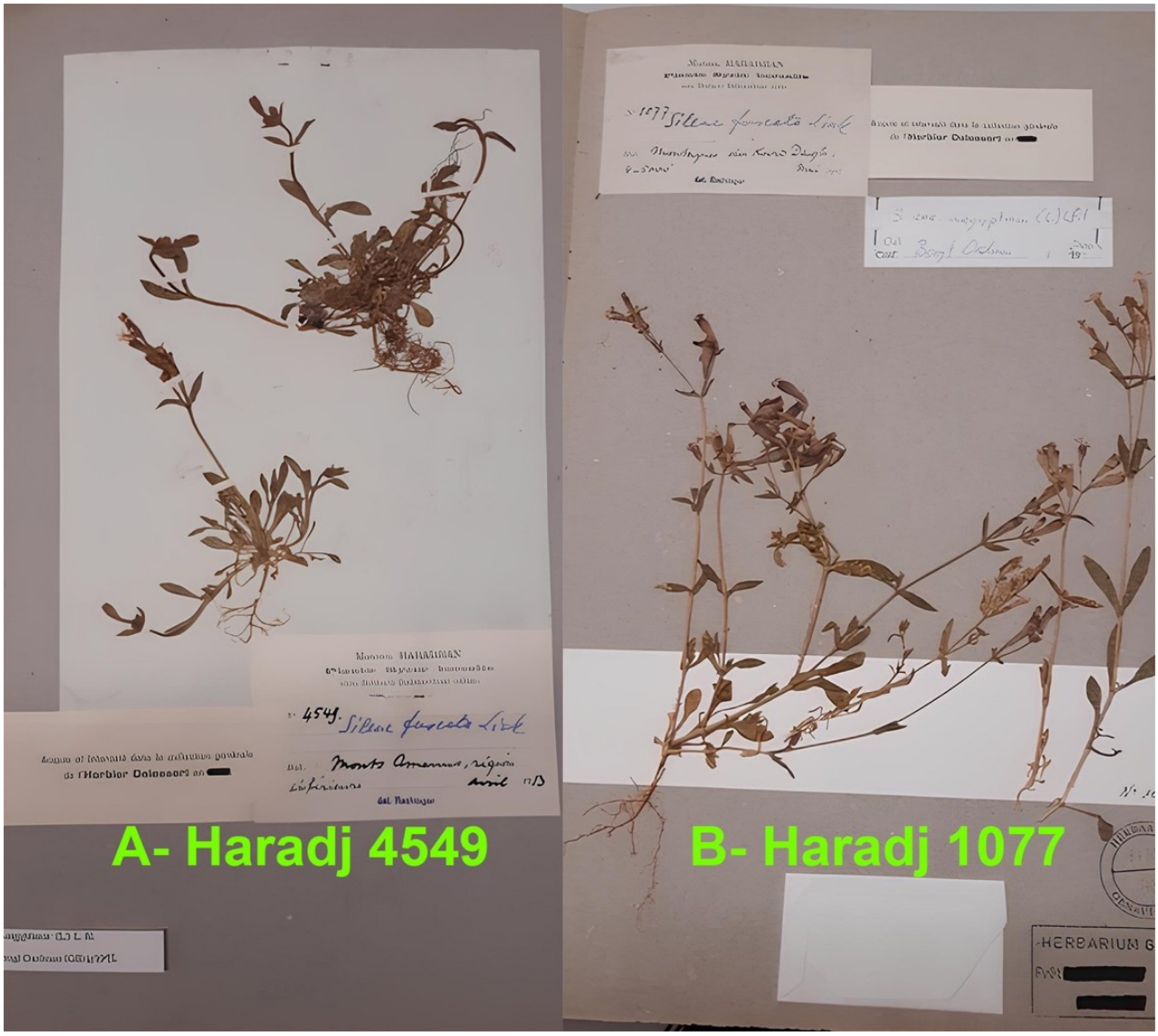

Figure 5. The two *S. fuscata* specimens of Haradjian (Haradj.) used for the description *S. fuscata* by Flora of Turkey and East Aegean Islands.

Ethics committee approval: Ethics committee approval is not required for this study

Conflict of interest: The author declares that there is no conflict of interest.

References

Aydin, Z., Ertekin, A.S., Långström, E., & Oxelman, B. (2014a). A new section of Silene (Caryophyllaceae) including a new species from South Anatolia, Turkey. *Phytotaxa*, 178(2), 098–112. http://doi.org/10.11646/phytotaxa.178.2.2

Aydin, Z., Marcussen, T., Ertekin, A.S., & Oxelman, B. (2014b). Marginal likelihood estimate comparisons to obtain optimal species delimitations in Silene sect. Cryptoneurae (Caryophyllaceae). *PLoS One*, 9e106990. https://doi.org/10.1371/journal.pone.0106990

Bakis Y, Babac MT, & Uslu E (2011). Updates and improvements of Turkish Plants Data Service (TÜBİVES). 6 th International Symposium on Health Informatics and Bioinformatics (HIBIT), (pp. 136-140). May 2-5, 2011, Izmir, Turkey.

Berardi, A.E., Betancourt Morejón, A.C., & Hopkins, R. (2022). Convergence without divergence in North American red-flowering Silene. *Frontiers in Plant Science*, 13, 945806. https://doi.org/10.3389/fpls.2022.945806

Coode, M.J.E., & Cullen, J. (1967). Silene L. In: Davis PH, editor. *Flora of Turkey and the East Aegean Islands*, Vol. 2. 1st ed. Edinburgh, UK: Edinburgh University Press, pp. 179–222.

Danin, A., & Fragman–Sapir, O. (2016) Flora of Israel and adjacent areas. Retrieved from: https://flora.org.il/en/plants/

Davis, P.H. (ed.). (1965–1985). Flora of Turkey and the East Aegean Islands, Vols.1–9. Edinburgh University Press, Edinburgh.

Davis, P.H., Mill, R.R., & Tan, K. (1988). *Silene* L. In: Davis, P.H., Mill, R.R., Tan, K. (Eds.). Flora of Turkey and the East Aegean Islands (Suppl. 1), Vol. 10. 1st ed. Edinburgh, UK: Edinburgh University Press, pp. 76–81.

Eggens, F., Jafari, F., Thollesson, M., Crameri, S., Zarre, S., & Oxelman, B. (2020). Phylogeny and species delimitation in Silene sect. Arenosae (Caryophyllaceae): a new section. *PhytoKeys* 159, 1–34. https://doi.org/10.3897/phytokeys.159.51500

Erixon, P., & Oxelman, B. (2008). Reticulate or tree–like chloroplast DNA evolution in Sileneae (Caryophyllaceae)?, *Molecular Phylogenetics and Evolution*, 48, 313–325. https://doi.org/10.1016/j.ympev.2008.04.015

Greuter, W. (1997). Silene L. In: Strid, A. & Tan, K. (eds.) *Flora Hellenica*, Vol. 1, Koenigstein: Koeltz Scientific Books, pp. 239–323.

Greuter, W. (1995). Silene (Caryophyllaceae) in Greece: a subgeneric and sectional classification. *Taxon*, 44(4), 543–581. https://doi.org/10.2307/1223499

Güner, A., Aslan, S., Ekim, T., Vural, M. & Babaç, M.T. (2012). Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını. İstanbul. Retrieved from: https://bizimbitkiler.org.tr/yeni/demos/technical/

Jafari, F., Zarre, S., Gholipour, A., Eggens, F., Rabeler, R.K, & Oxelman, B. (2020). A new taxonomic backbone for the infrageneric classification of the species-rich genus Silene (Caryophyllaceae). *Taxon,* 69, 337–368. https://doi.org/10.1002/tax.12230

Jeanmonod, D. (2015). Notes on Corsican flora, XXV. *Candollea* 70(1), 109–140. https://doi.org/10.15553/c2015v701a10

Medina, L., & Aedo, C. (2022). Vascular Plants from the Journey through Portugal (1797–1801) by Hoffmannsegg and Link at the Herbarium of the Real Jardín Botánico of Madrid. *Plants*, 11, 2438. https://doi.org/10.3390/plants11182438

Meikle, R.D. (1977). *Silene* L. in Meikle, R.D., (ed.), *Flora of Cyprus*, Vol: 1. Kew: Bentham– Moxon Trust and Royal Botanic Garden, 224–252.Mesbah, M. (2021). Etude taxonomique et biogéographique de quelques espèces du genre Silene L. (Caryophyllacées) en Algérie. Systématique, phylogénie et taxonomie. Université de Bejaia [Algérie], 2021. Français

Miller, M.A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES science gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop*, 14, 1–8. Retrieved from https://www.phylo.org/ (accessed: 10 Oct 2023).

Naciri, Y., Du Pasquier, P-E., Lundberg, M., Jeanmonod, D., & Oxelman, B. (2017). A phylogenetic circumscription of *Silene* sect. *Siphonomorpha* (Caryophyllaceae) in the Mediterranean basin. *Taxon,* 66, 91–108. https://doi.org/10.12705/661.5

Naciri, Y., Toprak, Z., Prentice, H.C., Hugot, L., Troia, A., Burgarella, C., & Jeanmonod, D. (2022). Convergent morphological evolution in the Italicae section of Silene in the Mediterranean Basin. *Frontiers in Plant Science*, 13, 695958. https://doi.org/10.3389/fpls.2022.695958

Orcan, N., Binzet, R., & Yaylalioglu, E. (2004). The flora of Findikpinari (Mersin–Turkey) Plataeu. *Flora Mediterrnea,* 14, 309–345. ISSN 1120–4052.

Oxelman, B., & Lidén, M. (1995). Generic boundaries in the tribe Sileneae (Caryophyllaceae) as inferred from nuclear rDNA sequences. *Taxon,* 44, 525–542. https://doi.org/10.2307/1223498

Oxelman, B., Lidén, M., & Berglund, D. (1997). Chloroplast rps16 intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Plant Systematics and Evolution*, 206, 393– 410. https://doi.org/10.1007/BF00987959

Oxelman, B., Lidén, M., Rabeler, R.K., & Popp, M. (2001). A revised generic classification of the tribe Sileneae (Caryophyllaceae). *Nordic Journal of Botany* 20, 515–518. http://doi.org/10.1111/j.1756-1051.2000.tb00760.x

Oxelman, B., Rautenberg, A., Thollesson, M., Larsson, A., Frajman, B., Eggens, F., Petri, A., Aydın, Z., Töpel, M., & Brandtberg–Falkman, A. (2013). *Sileneae taxonomy and systematics*. Retrived from http://www.sileneae.info

Ozuslu, E., & Tel, A.Z. (2008). Some Changes and Updating Processes of Localizations in Turkey's Flora (Flora of Turkey) Declared by Gaziantep/Turkey, *Biological Diversity and Conservation,* 1, 99–107. https://dergipark.org.tr/en/pub/biodicon

Petri, A., & Oxelman, B. (2011). Phylogenetic relationships within Silene (Caryophyllaceae) Sect. Physolychnis. *Taxon,* 60, 953–968. https://doi.org/10.1002/tax.604002

Petri, A., Pfeil, B.E., & Oxelman, B. (2013). Introgressive hybridization between anciently diverged lineages of Silene (Caryophyllaceae). *PLoS One*, 8(7), e67729. https://doi.org/10.1371/journal.pone.0067729

Pfeil, B.E., Toprak, Z., & Oxelman, B. (2017). Recombination provides evidence for ancient hybridisation in the Silene aegyptiaca (Caryophyllaceae) complex. *Organisms Diversity and Evolution,* 17, 717–726. https://doi.org/10.1007/s13127–017–0331–9

Prieto-Benítez, S., Millanes, A.M., Dötterl, S., & Giménez-Benavides, L. (2016). Comparative analyses of flower scent in Sileneae reveal a contrasting phylogenetic signal between night and day emissions. *Ecology and Evolution,* 6, 7869–7881. https://doi.org/10.1002/ece3.2377

Rambaut, A. (2018). FigTree v1.4.4. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. Retrieved from http://tree.bio.ed.ac.uk/software/figtree/

Scherz, M.D., Glaw, F., Hutter, C.R., Bletz, M.C., Rakotoarison, A., Köhler, J., et al. (2019). Species complexes and the importance of Data Deficient classification in Red List assessments: The case of Hylobatrachus frogs. *PLoS One,* 14(8), e0219437. https://doi.org/10.1371/journal.pone.0219437

Stamatakis, A. (2014). RAxML Version 8: A tool for Phylogenetic Analysis and Post–Analysis of Large Phylogenies. *Bioinformatics*, 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Strid, A. (2018). **In** Vascular plants of Greece: An annotated checklist (Dimopoulos & al. 2013). Retrieved from: https://portal.cybertaxonomy.org/flora-greece/cdm_dataportal/taxon/84468f6d-881e-4412-9175-ace4f5de4146#

Swofford, D.L. (2002). PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0. Sunderland, Massachusetts, *Sinauer Associates*.

Swofford, D.L. (2020). Phylogenetic Analysis Using Parsimony (PAUP) (Version 4.0a169). Retrieved from: http://phylosolutions.com/paup-test/ (accessed: 20 Sep 2023)

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution,* 38, 3022–3027. https://doi.org/10.1093/molbev/msab120

Thiers, B. (2023) [continuously updated]). Index Herbariorum: a global directory of public 22 herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Retrieved from http://sweetgum.nybg.org/science/ih/ (accessed 20 October 2023).

Toprak, Z., Pfeil, B.E., Jones, G., Marcussen, T., Ertekin, A.S., & Oxelman, B. (2016). Species delimitation with DISSECT: Evidence of high species diversity in the Silene aegyptiaca complex (Caryophyllaceae). *Molecular Phylogenetics and Evolution,* 102, 1–8. https://doi.org/10.1016/j.ympev.2016.05.024

Toprak, Z., & Yıldız, K. (2022). Conflicting signals in phylogenetic relatioships and taxonomy of Anatolian endemic Silene sordida (Caryophyllaceae). *Phytotaxa*, 548(2), 199–222. https://doi.org/10.11646/phytotaxa.548.2.5

Supplemental Table

Supplemental Table 1. Supplementary Material-Voucher list

Sample ID	Collector	Collector No	Locality	Herbarium
1146	Bengt Oxelman	1659	Mughla, 4 km southeast of Olu ̈Deniz. Pinus brutia- woodland.75 m.s.m.	GB
1151	Bengt Oxelman	1664	Antalya, Road Uz̈u ̈mlu ̈-Çameli Km 3.500 m.s.m.	GB
1155	Bengt Oxelman	1668	Mughla, Road Uz̈u ̈mlu ̈-Çameli Km 11. Open woodland. 1000 m.1000 m.s.m.	GB
1173	Bengt Oxelman	1686	Antalya, Road Korkuteli-Antalya Km 21. Crevices in steep W-facing limestone cliffs.1000 m.s.m.	GB
*1176	Bengt Oxelman	1690	Antalya, Road Antalya-Altınyaka, 21 km from turn-off point of main road Antalya- Finike. Woodland. 700 m.800 m.s.m.	GB
1184	Bengt Oxelman	1698	Antalya, Road Gu ̈zelbagh- Gu ̈ndoghmus, Km 4. Open woodland.800 m.s.m.	GB
1188	Bengt Oxelman	1702	Road Akseki- Beysehir, Km 9, 2 km before Imrasan Gecidi. Woodland.1400 m.s.m.	GB
3080	Balls	1089	Beiruth Dagh	E
*3245	Coode and Jones	1114	Maras, 5 miles south of Andirin. Carpinus/Quercus woodland scrub on crumbling metamorphic rock	E
3289	Davis	2732	Lefkara, cornfields and by roadside	E
3290	Davis	2346	Island of Cyprus. Yayla above Plalefka	E
3291	Davis	2846K	Island of Cyprus. Gaila (Kyrenia range)	E
*3299	Davis	15772	Antalya, Gebiz: Bozburun Dagh, between Tazli yayla & Kozludere	E
*3301	Davis and Polunin	25673	Antalya, Aksu. Calcareous maquis	E
3313	Davis and Hedge	26385	Mersin, Between Tarsus and Namrum near Samlar. Rocky limestone slopes 500 m.s.m.	E
*3315	Davis and Hedge	26462	Mersin, dist. Tarsus: gorge of Tarsus between Ulas and Samlar. Rocky limestone slope 150 m.s.m.	E
3330	Davis and Hedge	27506	Maras, Maras Go ̈ksun, near Yemis dagh. Shaley slopes 600 m.s.m.	E
3333	Davis and Hedge	28567	Mardin, Savur. Rocky limestone slopes 900 m.s.m.	E
3338	Davis	42571	Siirt, C9 Siirt. Gizre to Sirnak, 25 km below Sirnak. Bare shale hills	K
3340	Davis	42634	Siirt, C9 Siirt: Above Sirnak. Rocky limestone slopes. 1450 m.s.m.	K
3342	Davis	42822	Mardin, Cudi Dagh, above Hessana (d. Silopi). Earthy limestone screes facing W 1400-1500 m.s.m.	E
3446	Edmondson and McClintock	2933	Division 6, Island of Cyprus. Valley of r. Pedhieos NW of Pano Dheftera, 15 km SW of Nicosia. Alluvial terraces, field margin	E
3505	Feinbrun and Wendelbo	6180	Upper Galilee: Rama. Olive grove	GB
3926	Meyers and Dinsmore	5341	Palaestinae borealis: Mt Tabor. Fields	E
4070	Polunin	5251	Bzoumar near Jounie. Terrace cultivation, cornfield weed; 3000 ft.	E
*4290	Trelawny	1415	Hakkari, Village Piskasir on road to Oramar —28 miles from Yu ̈ksekova. Slope 50°. Fine brown sand, overlain with coarse pebbles (3.6“ diam.), 1830 m.s.m	E
4368	Wa ̈ngsjö	1493	Broummana (Lib. int. centr.) ad marginem viae 850 m.s.m.	LD
6089	Snogerup and Snogerup	3140	9 km NNW of Jericho on road to Ramallah. Suadetum semidesert.	LD
12114	Gunnar Samuelsson	3736	Near Aleppo. Prope Kefr Saghin, in agro stepposo, ca 375 m s. m., 375 m m.s.m	S

Sample ID	Collector	Collector No	Locality	Herbarium
*13999	Bengt Oxelman	2456	Road Tarsus- Çamliyayla, just S of Sarıkoyak. Virgin roadside gravel., 693 m.s.m	GB
*14980	Zeynep Aydin	2	Gazipasa, Antalya, Antalya, Gurcam village, 1000 m.s.m.	GB
*14999	Zeynep Aydin	8	Antalya, Gazipasa, Inal village, stony slope, 671 m.s.m	GB
*15004	Zeynep Aydin	9	Antalya, Gazipasa, Gazipasa to Belbasi, 1500 m.s.m	GB
*15018	Zeynep Aydin	9	Antalya, Gazipasa, Gazipasa to Belbasi, 1500 m.s.m	GB
*15061	Zeynep Aydin	19	Osmaniye, Fevzipasa, Open area with oak trees, 1180 m.s.m	GB
*15066	Zeynep Aydin	21	Hatay, Hassa to Dörtyol 12 km., 1332 m.s.m	GB
*15067	Zeynep Aydin	21	Hatay, Hassa to Dörtyol 12 km., 1332 m.s.m	GB
*15077	Zeynep Aydin	22	Hatay, Hassa to Dörtyol 13.km, 1466 m.s.m	GB
*15087	Zeynep Aydin	24	Osmaniye, Osmaniye to Zorkun 5 km., 675 m.s.m	GB
*15096	Zeynep Aydin	25	Osmaniye, Zorkun, 1650 m.s.m	GB
*15106	Zeynep Aydin	26	Osmaniye, Osmaniye to Zorkun 16 km., 1340 m.s.m	GB
*15119	Ömer Faruk Kaya		Mersin, Guzel Yayla, kizilbag village, 1220 m.s.m	GB
15121	A. Selcuk Ertekin		Mardin, Zinar Wineyard	GB
*15123	Zeynep Aydin	27	Antalya, Gebiz, Gebiz to Pinargözu, 405 m.s.m	GB
15146	Zeynep Aydin	30	Antalya, Altinyaka, , 1140 m.s.m	GB
15192	Zeynep Aydin	39	Antalya, From Kemer to Kumluca 7 km. to Beycik., 960 m.s.m	GB
*15194	Zeynep Aydin	40	Sanliurfa, Birecik, , 487 m.s.m	GB
15195	Zeynep Aydin	40	Sanliurfa, Birecik, , 487 m.s.m	GB
*15200	A. Selcuk Ertekin		Siirt, Sirvan, Stepp area 3km. to Madenköy	GB
*15214	Zeynep Aydin	45	Konya, Ermenek, Daran Valley	GB
*15223	Zeynep Aydin	46	Mersin, Kuzucubelen, road side, 650 m.s.m	GB
*15238	Zeynep Aydin	47	Mersin, Findikpinari, , 1365 m.s.m	GB
15239	Zeynep Aydin	47	Mersin, Findikpinari, , 1365 m.s.m	GB
15241	Zeynep Aydin	48	Mersin, Findikpinari, , 1195 m.s.m	GB
*15247	Zeynep Aydin	48	Mersin, Findikpinari, , 1195 m.s.m	GB
15256	Zeynep Aydin	50	Siirt, Aydinlar, Cultivated area with grape and pistachio, 1190 m.s.m	GB
15263	Zeynep Aydin	52	Siirt, Siirt to Eruh, Oak area by the road side in Akarsu Village	GB
15269	Zeynep Aydin	53	Siirt, Siirt to Sirvan, 984 m.s.m	GB
15281	Zeynep Aydin	58	Sanliurfa, Birecik, Pistachioyard by the road side, 487 m.s.m	GB
*15294	Zeynep Aydin	59	Mersin, Arslankoy, Rocky slopes, 725 m.s.m	GB
15311	Zeynep Aydin	64	Mersin, Civanyaylagi, , 117 m.s.m	GB

Sample ID	Collector	Collector No	Locality	Herbarium
*15318	Zeynep Aydin	65	Gaziantep, Nizip, Nizip to Gaziantep 18 km., 655 m.s.m	GB
*15348	Zeynep Aydin	10	Antalya, Gazipasa, Cayiryakasi, 1880 m.s.m	GB
*15349	Zeynep Aydin	10	Antalya, Gazipasa, Cayiryakasi, 1880 m.s.m	GB
*15350	Zeynep Aydin	30	Antalya, Altinyaka, , 1140 m.s.m	GB
*15353	Zeynep Aydin	33	Antalya, Gebiz, , 890 m.s.m	GB
*15354	Zeynep Aydin	33	Antalya, Gebiz, , 890 m.s.m	GB
*15355	Zeynep Aydin	34	Antalya, Gebiz, , 1150 m.s.m	GB
*15356	Zeynep Aydin	39	Antalya, From Kemer to Kumluca 7 km. to Beycik., 960 m.s.m	GB
*15357	Zeynep Aydin	50	Siirt, Aydinlar, Cultivated area with grape and pistachio, 1190 m.s.m	GB
*15358	Zeynep Aydin	52	Siirt, Siirt to Eruh Oak area by the road side in Akarsu Village	GB
*15359	Zeynep Aydin	53	Siirt, Siirt to Sirvan, 984 m.s.m	GB
*15360	Zeynep Aydin	55	Gaziantep, Islahiye, Islahiye to Hassa 10 km. , pistachioyard	GB
*15361	Zeynep Aydin	56	Hatay, Hassa, Yukari Bucak Village, stony slopes, 690 m.s.m	GB
*15363	Zeynep Aydin	60	Mersin, Mersin to Arslanköy., 860 m.s.m	GB
*15364	Zeynep Aydin	61	Mersin, Gözne to Arslanköy; Yavsa Village, 1140 m.s.m"	GB
*15365	Zeynep Aydin	62	Mersin, Mersin to Arslanköy; Yenikoy Village, 970 m.s.m"	GB
*15366	Zeynep Aydin	63	Mersin, Mersin to Gözne, 320 m.s.m	GB
*15367	Zeynep Aydin	66	Antalya, Gebiz, Bozburun Dag, 1150 m.s.m	GB
*15368	Zeynep Aydin	66	Antalya, Gebiz, Bozburun Dag, 1150 m.s.m	GB
*15369	Zeynep Aydin	66	Antalya, Gebiz, Bozburun Dag, 1150 m.s.m	GB
15370	A. Selcuk Ertekin		Siirt, Sirvan, Oak area 7km. to Madenköy	GB
15388	Kemal Yildiz		Lefkosa, Alevkayasi (Halevga), 800 m.s.m	GB
17702	Zeynep Aydin		Mardin, Nusaybin / Akarsu village, rocky slope, meadow	GB
17703	Zeynep Aydin		Mardin, Nusaybin / Akarsu village, rocky slope, meadow	GB
17895	A. Selcuk Ertekin		Mardin, Zinar Bağları Çevresi	GB
17896	A. Selcuk Ertekin		Mardin, Zinar Bağlari Çevresi	GB
*17253	Kalheber	80-2024	Germany,Hessen, Kreis Limburg-Weilburg, Weilburger Lahntal, Weiburg, Graben an der Strasse nach Hasselbach	GB
*2406	Bengt Oxelman	2200	Sweden, Hisingen, Kornhall	GB
*7566	Bengt Oxelman	GB-0149042	Sweden,Cultivated in Göteborg Botanical Garden	GB
*17254	T F Hewer	H.4022	Iran, E. Mazandaran Province, Golidagh. At edge of woodland	GB
*2488	Bengt Oxelman	2278	Sweden, Cultivated in private garden	GB
*13789	Polevova and Stefantchuk		Turkmenistan, Karakalinskiy rayon. 8 km NNW of Kara-Kala[?], southern slope of mount Isaac	MW

Sample ID	Collector	Collector No	Locality	Herbarium
*7823	Erixon	72	Sweden, Uppsala, Behind EBC, Kåbovägen 4	UPS
*15351	Zeynep Aydin	31	Antalya, From Kemer to Kumluca 7 km. to Beycik., 960 m.s.m	GB
*15352	Zeynep Aydin	28	Antalya, Altinyaka, Ovaçik Köyu Yolu, 1160 m.s.m.	GB
*15178	Zeynep Aydin	36	Antalya, Antalya to Altinyaka 30 km., 941 m.s.m	GB
*15345	Bengt Oxelman	2541	Greece, Rodos, around the Thari monastery, sandy gravel, 265 m.s.m.	GB
*1115	Bengt Oxelman	1628	Antalya, Road Finike-Elmalı Km 38, c 2km NW Arif. Garigue on limestone.700 m.s.m.	GB
*14849	Bengt Oxelman	2504	Turkey, Road east from Saribelen towards Gökçeören, 900 m.s.m.	GB
*14858	Bengt Oxelman	2513	Turkey, W Gömbe, dirt track towards Yesilgöl, 1620 m.s.m.	GB
*14835	Bengt Oxelman	2492	Turkey, Datça peninsula, 6 km E Emeçik, virgin gravel	GB
*14840	Bengt Oxelman	2662	Turkey, Roadside gravel near main road Fethiye-Mughla	GB
*14838	Bengt Oxelman	2493	Turkey, Dry river bed near the main road Fethiye-Mughla, just by the turn-off to Köysegizselale waterfalls	GB
*12625	Larsen, Larsen and Jeppesen	196	Germany,At Sandkrug W of Lauenburg. Dry sandy slopes along the river Elben together with Festuca rubra etc.	S
*2614	Anja Rautenberg	3	Cameroun, Mont Cameroon, above Buea	UPS
*14829	Magnus Popp		Ethiopia, Simen, Chennek, Grassland 3 alt: 3748 m.	GB
*1348	Bengt Oxelman	1887	Algeria, 2 km NW Medea. Margins of vine-field.	GB
*12609	Peter Schönswetter and Andreas Tribsch	T598	Russia, aimyrski Autonomous Okrug, Anabarskoje Ploskogorje (Anabar-Plateau): Valley of river Kotuy, c. 55 km SSE Khatanga, 8-10 km WSW Kayak.	
*7615	Magnus Popp	1053	Cultivated, Botanical garden, Uppsala	GB
*2474	Bengt Oxelman	2264	Cultivated in private Garden in Säve, Göteborg. Plant obtained in 1992 from Tagene plantskola.(Iran??)	GB
*1019	Bengt Oxelman and Lars Tollsten	946	Greece, Lakonias, Mount Taygetos, Forest road Paleopanagia - Krioneri - Anavriti Km 6. Phrygana.	GB
*2404	Bengt Oxelman	2198	Sweden, Bohuslän, Hisingen, Kornhall	GB
*14320	Bengt Oxelman	2488	Sweden, Stora Amundön, W part, rock crevices	GB
*20287	Melilia Mesbah		Algeria, Targa Ouzemour.	
*27036	Melilia Mesbah		Algeria, Champs cultivée Zlazel -Machrouha Souk Ahras	GB
*14830	Magnus Popp		Ethiopia,Simen, Dirni Gate, Rock outcrop 1, alt: 3731. Horse shoe formed plot covering both sides of the gorge.	GB
*19029	A. Gholipour		Iran, Mazandaran, 3370 m.s.m	SPNH
*7099	Harry Smith	7208	China, Shansi, Yün-ting-shan	UPS
*7329	Egger	431	USA, Alaska East of Chicken along Taylor highway	WTU
*7610	Magnus Popp	1045	Sweden, Gotland, Cult. in Uppsala Bot. Garden from seeds (B. Pettersson 58) collected in Gotland Endre: Ölbäck	GB
*17755	Bengt Oxelman	2619	Turkey, Summer village N of Islamlar	GB
*15252	Zeynep Aydin	49	Turkey, Mersin, Findikpınari	GB
*15372	Raus	9700	Greece, Dodekanisou, Island of Karpathos. Mount Kollas, Berg Kollas, Ostflanke. Kalkfelsdurchragte, krautreiche Kleinstrauchvegetation unter lockerem Pinus brutia-Schirm. 700 m.s.m.	UPS
*12851	Greimler	2	Georgia, Kartli	WU

Sample ID	Collector	Collector No	Locality	Herbarium
*14210	H.C. Prentice	L178:2	Germany, Wesendahl/Märkisch-Oderland	GB
*26869	Babali	1	Algeria, Naama, Djebal Aissa	ENSA
*19042	Joharchi		Iran, Golestan, 1150-1200 m.s.m	FUMH
*13547	P. Schönswetter and B. Frajman	11439	Spain, Navarra/Pyrénées Atlantiques, W of Candanchú: Collado de Causiat - La Callaberisa - Ibón (Lago) de Estanés, 1520-1850 m.s.m	LJU
*13312	B. Frajman		Austria, Carinthia, Hohe Tauern: Mallnitz, on the way from Häusler Alm to Lonza Mt, 1980 m.s.m	LJU
*KP849829 Silene fraudatrix		Prieto-Benítez et al. 2015		
*KP849828 Silene fuscata		Prieto-Benítez et al. 2015		
HBG-503531	W. Schimper	1932	Algeria	
MA-00031723		Medina and Aedo, 2022	Portugal	
G00545050				G-BOIS
G00545053				G-BOIS
G00545054				G-BOIS
not recorded	Haradjian, M	4549	Syrian Arab Republic, Kurd Dağı, (1913).	G
not recorded	Haradjian, M	1077	Syrian Arab Republic, Kurd Dağı, (1907)	G
KEW	Brot. (1928)		Palestine, Ramath-Gan, Sandy fields,	K
HUB 3704	H.Sümbül		Antalya, Gazipaşa, Çimbiti Yaylası, 1650 m, 18 .v. 1983	
Celal Bayar Ünv. Herb.	K. Yıldız 0381-1, E. Minareci ve M. Kuh		Hassa-Islahiye 3 km batı yamaç, 500 m ve sonrası, 06. v .2012	
AIBU	H.Sümbül		Antalya, Gazipaşa, Macar köyü yaylası, Sarımazı alanı mevkii, 2000-2100 m,13 vii 1983	AIBU

* indicates samples used for DNA sequence analysis