

Resurrection of *Ornithogalum brevipedicellatum* (Asparagaceae) with morphological and molecular data

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Abstract – This study evaluates *Ornithogalum brevipedicellatum*, which was previously accepted as a synonym of *O. oligophyllum*, as a separate distinct species and discusses the similarities and differences between *O. brevipedicellatum* and its related species (*O. oligophyllum* and *O. pamphylicum*). Similarities and differences among these species were identified by morphological and molecular studies. The leaf morphology and inflorescence of *O. brevipedicellatum* and *O. pamphylicum* are similar to each other, and in terms of these features, they show differences from *O. oligophyllum*. Some diagnostic characteristics are quite different in *O. brevipedicellatum* and *O. pamphylicum*, such as the size of tepals, length of fruiting pedicels and style. Morphological data were supported by the results obtained from molecular studies. According to a dendrogram obtained by molecular studies, *O. brevipedicellatum* and *O. pamphylicum* are similar. *O. oligophyllum* is more closely related to *O. pyrenaicum* used as an out-group. Additionally, the seeds of *O. brevipedicellatum* were examined with the use of scanning electron microscopy.

Key words: Asparagaceae, endemic, molecular, morphology, *Ornithogalum*, scanning electron microscopy, synonym, Turkey.

Introduction

Asparagaceae Juss. (1789) (including Agavaceae Dumort., Aphyllanthaceae Burnett, Hesperocallidaceae Traub, Hyacinthaceae Batsch ex Borkh., Laxmanniaceae Bubani, Ruscaceae M. Roem., Themidaceae Salisb.) include 143 plant genera (APG III 2009). Hyacinthaceae can be treated as subfamily Scilloideae of Asparagaceae, and the subfamilies Hyacinthoideae, Ornithogaloideae, Urgineoideae and Oziroëoideae of Hyacinthaceae are then treated as tribes Hyacintheae, Ornithogaleae, Oziroëeae and Urgineeae (e.g. APG III 2009). Ornithogaloideae treated as one of the subfamilies in Hyacinthaceae show a distribution through Europe, south-west Asia and Africa, and include about 280 species (Speta 1998). Nowadays, as a result of molecular phylogenetic studies on this subfamily, 19 monophyletic genera are accepted within Ornithogaloideae: *Albuca*, *Avonsera*, *Battandiera*, *Cathissa*, *Coilonox*, *Dipcadi*, *Eliokarmos*, *Elsiea*, *Ethesia*, *Galtonia*, *Honorius*, *Loncomelos*, *Melomphis*, *Neopaterosonia*, *Nicipe*, *Ornithogalum*, *Pseudogaltonia*, *Stellarioides* and *Trimelopter* (Martínez-Azorín et al.

2011). Recently, molecular tools have gained importance in identifying taxonomic relations. In Ornithogaloideae, *matK*, *trnL* intron, *trnL-F* spacer, and *rbcL* plastid DNA sequences have been used for phylogenetic analysis (Manning et al. 2009, Martínez-Azorín et al. 2011) because plastid sequences comprise an important source for phylogenetic reconstruction, mostly at interspecific or higher taxonomic levels (Clegg and Zurawski 1992, Cameron 2004, Kress and Erikson 2007).

In Flora of Turkey (Cullen 1984), the species of the genus *Ornithogalum* L. were evaluated under 4 subgenera, subg. *Beryllis* (Salisb.) Baker, subg. *Ornithogalum*, subg. *Myogalum* (Link) Baker and subg. *Caruelia* (Parl.) Baker. The recent arrangements recombined *Beryllis* subg. as *Lancomelos* Raf., *Myogalum* subg. as *Honorius* Gray and *Caruelia* subg. as *Melomphis* Raf. (Martínez-Azorín et al. 2011).

Anatolia is an important area for the distribution of the genus *Ornithogalum* in Asia (Uysal et al. 2005). Since the last revision by Cullen (1984) for the Flora of Turkey, nu-

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merous new taxa, records and combinations of *Ornithogalum* have been introduced from Turkey (e.g. Davis et al. 1988, Özhatay 2000, Düşen and Sümbül 2002, 2003, Düşen and Deniz 2005, Uysal et al. 2005, Özhatay and Kültür 2006, Bağcı et al. 2009, 2011, Özhatay et al. 2009, Yıldırım 2009, Koca and Yıldırım 2010, Özhatay et al. 2011, Mutlu and Karakuş 2012). A total of 61 species of the genus *Ornithogalum* are distributed in Turkey, 31 of them endemic to the country (Uysal 2012).

The first specimens of *Ornithogalum brevipedicellatum* in Turkey were collected by Bourgeau in 1860 in the higher mountain steppes of the Elmalı (Antalya) district, the region known as ‘Lycia’ in antiquity. These specimens were evaluated under the name of “*O. brevipedicellatum*” by Boissier, and subsequently were introduced to science in 1873 by Baker (Boissier 1884, Cullen 1984). *O. brevipedicellatum* was reported as the synonym of the *O. oligophyllum* species in the Flora of Turkey (Cullen 1984), the Plant List of Turkey (Uysal 2012) and in “The Plant List” database. *O. oligophyllum* has a substantially wide area of occupancy in Turkey. It was previously reported in The Flora of Turkey that the specimens of the species *O. oligophyllum*, which were collected in Antalya, Isparta and Konya, would be classified as *O. brevipedicellatum* provided that the distinguishing determinant characteristics were supported by a sufficient number of samples (Cullen 1984).

Materials and methods

Plant samples and morphological studies

The plant specimens of the genus *Ornithogalum* were collected by field studies between 2012 and 2014 in the Muğla and Antalya Provinces. During the field studies, GPS locations of *O. brevipedicellatum* were taken, and the individuals of its populations were numbered. This data was used to determine the threat category of *O. brevipedicellatum* according to the Categories and criteria of IUCN

(Version 11, 2014). The extent of occurrence (EOO) and area of occupancy (AOO) values were calculated.

In the present study, *Ornithogalum brevipedicellatum* known as a synonym of *O. oligophyllum* is morphologically described in detail and compared with its related species, *O. oligophyllum* and *O. pamphylicum* O.D.Düşen & Sümbül, with the data obtained from morphological and molecular studies. The individuals of these species were observed during field studies and the morphological evaluations were done both in the field and in laboratory. Specimens collected and used in molecular studies within the scope of this study, are shown in Tab. 1. The digital isotype photographs obtained from MNHN (Museum National d’Histoire Naturelle) of *O. brevipedicellatum* were also examined. Additionally, a number of herbarium specimens of *O. oligophyllum* collected from different parts of Turkey in the ISTE (Istanbul University, Herbarium of the Faculty of Pharmacy) and GAZI (Gazi University Herbarium) were morphologically examined (see Appendix).

Seed micromorphology of *Ornithogalum brevipedicellatum* was investigated using scanning electron microscopy (SEM) techniques. For SEM study, the seeds were treated with gold conjugate on stub. The microphotographs were taken with a Zeiss LEO-1430 scanning electron microscope.

Molecular study: DNA isolation, PCR amplification and sequencing

Complete DNA of *Ornithogalum brevipedicellatum* and *O. pamphylicum* was extracted from the leaves of herbarium specimens using the cetyl trimethyl ammonium bromide (CTAB) method of Doyle and Doyle (1990). DNA concentration and quality were tested with 1% agarose gel against a DNA standard. Two different plastid regions were used for molecular analysis to identify the phylogenetic similarities of the species. Amplification of these regions was conducted with the universal primers used in previous

Tab. 1. Locality data of collected specimens of *Ornithogalum* for the current study.

Species	Locality	Collection data
<i>O. brevipedicellatum</i>	C2 Muğla, Fethiye, Seki, 6 km from Seki to Yuva, Ak Mountain, steppe, 1980 m, 27.iv.2012.	C. Aykurt 3071 (AKDU)
	Muğla, Fethiye, Seki, 4–6 km from Seki to Yuva, Ak Mountain, 1900–1980 m, steppe, 4.v.2012.	C. Aykurt 3084 (AKDU)
	Muğla: Fethiye, Seki, 4–6 km from Seki to Yuva, Ak Mountain, 1900–1980 m, steppe, 22.v.2012.	C. Aykurt 3137* (AKDU)
	Antalya: Kaş, Gömbe, between Gömbe-İkizgöller, Subaşı environs, plateau, 2080 m, 25.05.2012.	C. Aykurt 3156 (AKDU)
	Muğla, Fethiye, Seki, 6 km from Seki to Yuva, Ak Mountain, steppe, 1975 m, 30.04.2013.	C. Aykurt 3741* (AKDU)
	Muğla, Fethiye, Seki, 6 km from Seki to Yuva, Ak Mountain, steppe, 1827 m, 30.04.2013.	C. Aykurt 3742* (AKDU)
	Muğla, Fethiye, Seki, 6 km from Seki to Yuva, Ak Mountain, steppe, 1825 m, 23.05.2013.	C. Aykurt 3868 (AKDU)
	Muğla, Fethiye, Seki, 6 km from Seki to Yuva, Ak Mountain, steppe, 1975 m, 23.05.2013.	C. Aykurt 3869 (AKDU)
<i>O. pamphylicum</i>	Antalya, above Fesleğen Plateau, Sakarpınar, 1850–1900 m, calcereous slopes, 4.v.2012.	C. Aykurt 3087 (AKDU)
	Antalya, Elmalı, İmecik Plateau, Bey Mountains, 1800 m, 29.04.2012.	C. Aykurt 3089* (AKDU)
	Antalya, Elmalı, Kızlarsivrisi Mountain, Karakuyu district, 1900 m, openings in <i>Cedrus libani</i> forest, calcareous slopes, 09.v.2012.	İ.G. Deniz 4528 (AKDU)
	Antalya: Çalbalı Mountain, 1700 m, calcareous slopes, 15.v.2014.	İ.G. Deniz 5548 (AKDU)
<i>O. oligophyllum</i>	C3 Antalya: Sarıçınar, 36 S 274566, 4096808, 1380 m, 14.iv.2014.	C. Aykurt 3882 (AKDU)
	C2 Antalya: Finike, West side of Finike, under <i>Cedrus libani</i> , 1200 m, 24.iv.2014.	C. Aykurt 3911 (AKDU)

studies. The *trnL* intron and *trnL* spacers were amplified with c (5'-CGAAATCGGTAGACGCTACG) and f (5'-ATTGAACTG-GTGACACGAG) primers described in Taberlet et al. (1991). Amplification of the *rbcL* gene was performed using a forward primer (427F), 5'-GCTTATTCAAAAACTTTCCAAGGCCCGCC, and a reverse primer (724R), 5'-TCGCATGTACCTGCAGTTGC (Lledó et al. 1998). Polymerase chain reaction (PCR) for both *trnL* and *rbcL* regions contained 2 mM MgCl₂, 0.2 mM of each dNTP, 10 pM μL⁻¹ of each primer, 1 U of Taq DNA polymerase (Fermentas Life Sciences, Burlington, Canada) with supplied reaction buffer at 10× concentration, and 40 ng of template DNA. The amplifications were performed on a programmable thermocycler (BIONEER, MyGenie™) with the following program: one cycle of 4 min at 94 °C, 28 cycles of 1 min at 94 °C, 30 s at 48 °C for *rbcL*, or 1 min at 50 °C for *trnL*, 1 min at 72 °C, and for final extension one cycle of 7 min at 72 °C. PCR products were cleaned up using the GeneJET gel extraction kit (Thermo Scientific Fermentas, Vilnius, Lithuania). Sequencing process was carried out at Iontek Laboratory in Istanbul, Turkey as direct sequencing from PCR products. The sequences of other species (*O. oligophyllum* and *O. pyrenaicum*) were retrieved from GenBank databases at the National Center for Biotechnology Information (NCBI) and compared with *O. brevipedicellatum trnL* and *rbcL* sequences. A phylogenetic tree was constructed using the software MEGA 5. Bootstrap values are displayed at tree nodes (Tamura et al. 2011).

Results

Morphological studies

Ornithogalum brevipedicellatum Boiss. ex Baker, J. Linn. Soc., Bot. 13: 263 (1873) (Figs. 1–3)

Geophyte, 5–10(–15) cm high; bulb 1.3–1.8(–2) × 1.3–1.8(–2) cm, ovoid-spherical, without bulblets; outer tunics cream to pale-brown. Leaf synanthous, 4–6(–7), 9–37 × 2.3–0.5 cm, linear-lanceolate, canaliculate, with a whitish median line, gradually narrowing towards the base, longer than scape, margins entire, glabrous. Scape below the ground level, 3–12 cm, slender. Inflorescence congested racemose, racem borne at ground level, dense, 2–6 × 3–4.5 cm, erect, with (1–)5–15(–18) flowers. Bracts lanceolate, long acuminate, (12–)15–23 × 3–5 mm, membranous, longer than pedicels. Flowers sessile or subpedicellate up to 3 mm at anthesis and fruiting period. Tepals elliptic to ovate-elliptic, obtuse to acute at apex; outers 12–18 × 4–5 mm; inners 13–18 × 4.5–5 mm, white inside, green with narrowly white margins outside. Filaments white, conspicuously tapering towards the apex, 4–6 × 1–2 mm; anthers medifixed, milky white, 2–2.2 mm length. Ovary ovoid, 3.5–5 × 2–3.5 mm, pale green, longer than style; style 1.5–2 mm length; stigma capitate. Capsule 10–15 × 10–15 mm, ovoid, erect, distinctly winged, pale brown. Seeds numerous, 1.8–2 × 1.2–1.8 mm, elliptic-subglobose to globose, black, strongly apiculate; testa reticulate, with reticules formed by prominent crests, testa cells irregular.

Hitherto *Ornithogalum brevipedicellatum* was evaluated as the synonym of *O. oligophyllum*; however the species

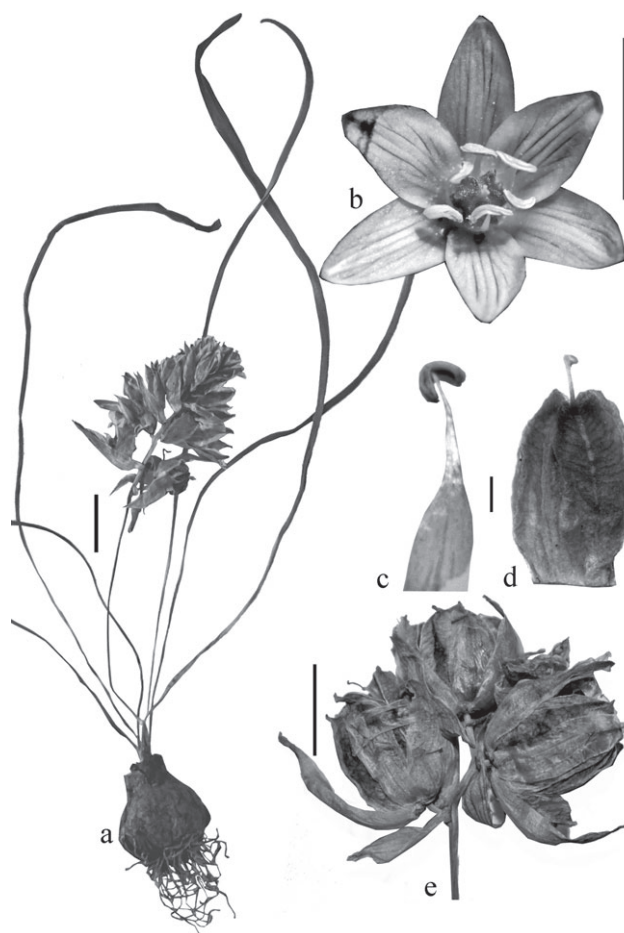


Fig. 1. *Ornithogalum brevipedicellatum* (from C. Aykurt 3071): a) habit, b) flower, c) stamen, d) pistil, e) capsule. Scale bars: 1 cm (a,b,e), and 1 mm (c,d).

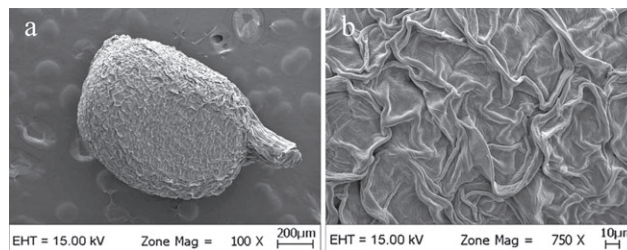


Fig. 2. SEM photographs of the seeds of *Ornithogalum brevipedicellatum* (from C. Aykurt 3137): a) seed, b) seed surface.

is morphologically more related to *O. pamphylicum*, described in 2002. The inflorescence type of *O. brevipedicellatum* is similar to *O. pamphylicum*. The raceme of *O. brevipedicellatum* is very condensed in flowering and fruiting period and borne at ground level. Its flowers are sessile or the floral or fruiting pedicels are up to 3 mm long. The inflorescence of *O. pamphylicum* resembles *O. brevipedicellatum*, but the internodes are more elongated and the pedicels are flaccid and longer at fruiting time. On the other hand, the lower pedicels of *O. oligophyllum* are longer than the flowers; fruiting pedicels are 10–30 mm long. Therefore, the inflorescence of *O. oligophyllum* can be evaluated as corymbose or pseudocorymbose. Moreover, the inflorescence of *O. brevipedicellatum* is different due to its sessile or subpedicellate flowers.



Fig. 3. *Ornithogalum brevipedicellatum* on Ak Mountain (Elmalı, Antalya).

The other morphological differences of *O. brevipedicellatum*, *O. oligophyllum* and *O. pamphylicum* are shown in Tab. 2.

Habitat, distribution and conservation status

Ornithogalum brevipedicellatum shares its habitats with several herbaceous plants such as *Cerastium macranthum* Boiss., *Ceratocephala falcata* (L.) Pers., *Corydalis erdelii* Zucc., *Crocus biflorus* Mill. subsp. *isauricus* (Siehe ex Bowles) B. Mathew, *Fumaria asepala* Boiss., *Gagea villosa* (M. Bieb.) Sweet subsp. *hermonis* Dafni & Heyn, *Scilla bifolia* L., *Tussilago farfara* L., *Viola heldreichiana* Boiss.

An old specimen collection record was documented in Flora Orientalis indicating that specimens of the *Ornithogalum brevipedicellatum* species were collected on Mount Trodos in southern Cyprus (Boissier 1884). However, recent data supporting these dubious records was not documented and for that reason, the species *O. brevipedicellatum* was evaluated as endemic to Turkey.

The conservation status of *Ornithogalum brevipedicellatum* is described below according to the rules of citation. Considering the IUCN (2014) categories and criteria (Ver-

sion 11), the species should be placed in the EN (endangered) category, according to the criteria for critically endangered, endangered and vulnerable.

The subpopulations of the species were identified in Seki (Muğla) and Gömbe (Kas, Antalya) (Fig. 4). No artificial factors endangering the development of the species in its area of occupancy were present. Therefore, it was projected that the population of the species in terms of percentage did not decrease through time as a result of anthropological effects. Item A cannot therefore be evaluated. The distance between the two locations of the species is 25 km. The EOO value of the species was determined as 130 km² (EN B1b(iii)) taking both locations of occupancy and the area contained within the shortest continuous imaginary boundary. The AOO value in this area, where the species was identified was calculated as 13 km² (EN B2b(ii)). The number of individuals identified in the subpopulations of the species was determined as 400 at the Seki location and 200 at the Gömbe location (EN C).

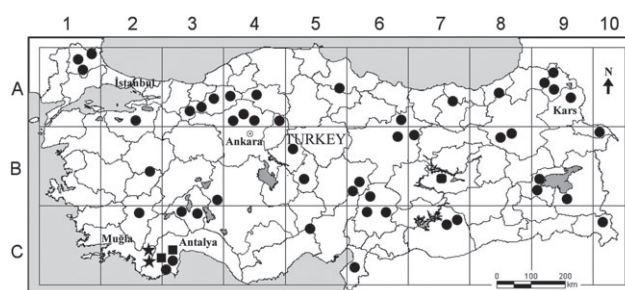


Fig. 4. Distribution areas of *Ornithogalum brevipedicellatum* (★), *Ornithogalum oligophyllum* (●) and *Ornithogalum pamphylicum* (■) in Turkey.

Molecular studies

Individuals of *Ornithogalum brevipedicellatum* and *O. pamphylicum* were collected from different localities for molecular analysis (Tab. 1). The sequences of these specimens were compared and evaluated with *O. oligophyllum*

Tab. 2. Comparison of the morphological characters of *Ornithogalum brevipedicellatum*, *O. oligophyllum* and *O. pamphylicum*.

Features	<i>O. brevipedicellatum</i>	<i>O. oligophyllum</i>	<i>O. pamphylicum</i>
Scape length (cm)	3–12 (borne at ground level)	4–15	3–15
Number of leaves	4–6(–7)	2–3 (rarely 4)	(3–)4–11(–13)
Leaves	with a whitish median line	without a whitish median line	with a whitish median line
Leaves width	2–5 mm	5–20 mm	1–4 mm
Inflorescence	congested racemose	corymbose or pseudocorymbose	laxly racemose (internodes more elongated)
Number of flowers	(1–)5–15(–18)	3–10(–20)	3–25
Fruiting pedicels	Capsules sessile or subpedicellate (up to 3 mm long), erect	10–30 mm, become flaccid at base	Capsules pedicellate (6–12 mm), erect
Flowers	odour	odourless	odourless
Tepals (mm)	12–18 × 4–5	–	20–30 × 5–9
Style length (mm)	1.5–2	2–3.5	4–5

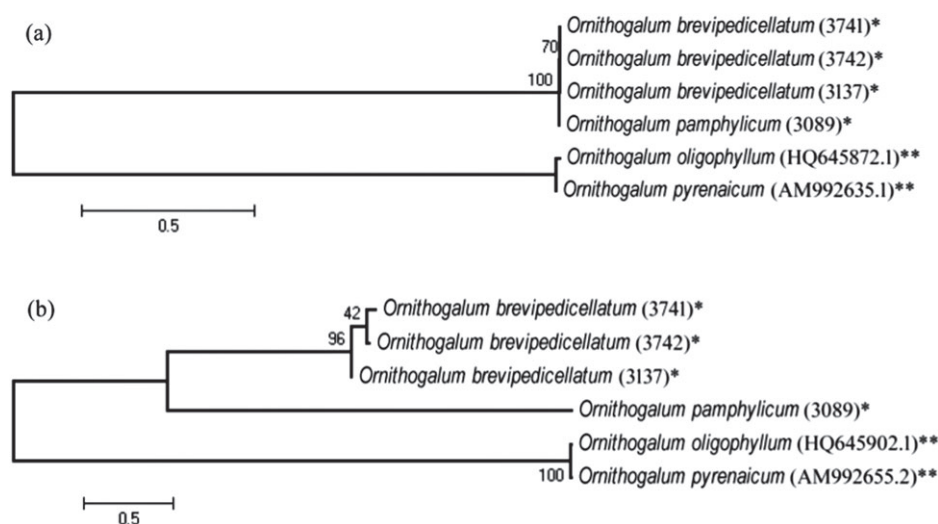


Fig. 5. Phylogenetic trees of *trnL* (a) and *rbcL* (b) sequences from six *Ornithogalum* specimens. Locations (*) and GenBank accession numbers (**) are given next to the *Ornithogalum* name.

and *O. pyrenaicum* retrieved from the GenBank database. To amplify the *trnL* and *rbcL* region of *O. brevipedicellatum* and *O. pamphylicum* species, c/f and 427F/724R primer pairs were used, respectively, as described in methodology section. About 1200 bp amplicons for *trnL* and 300 bp amplicons for *rbcL* were yielded in PCR assays. These amplicons were sequenced and compared with those of *O. oligophyllum* species (Fig. 5) available in the GenBank using the BLAST similarity search tool. In the *trnL* tree, two groups were identified. All of the individuals of *O. brevipedicellatum* were aligned in the first group. *O. pamphylicum* was the closest species to *O. brevipedicellatum* while *O. oligophyllum* and *O. pyrenaicum* were more distant from this group. Similarly, three individuals of *O. brevipedicellatum* were located together in the *rbcL* tree. Although *O. pamphylicum* was placed in a different branch, it was the closest species to *O. brevipedicellatum* while *O. oligophyllum* and *O. pyrenaicum* species were positioned in another group.

Discussion

The *Ornithogalum* genus, previously classified into numerous subcategories by several different researchers, was rearranged recently in the light of the novel molecular phylogenetic studies conducted on the Ornithogaloideae subfamily (Martínez-Azorín et al. 2011). Moret and Galland (1992) indicated that many taxa have been described in subg. *Ornithogalum* which is a taxonomically problematic subgenus on the basis of subtle morphological variations, usually with little biological significance. The complex taxonomy of subg. *Ornithogalum* may be due to intraspecific variations, the plasticity of individuals, their habits being strongly dependent on environmental factors and poor conservation of the types (Moret and Galland 1992).

Martínez-Azorín et al. (2010) reported that some authors have used the inflorescence structure and/or the length of the floral bracts relative to their pedicels as diagnostic characteristics. When *Ornithogalum brevipedicellatum*, *O.*

pamphylicum and *O. oligophyllum* species are evaluated with respect to the inflorescence type and length of the pedicels, it is seen that the specimens of *O. brevipedicellatum* are distinct; distinguished from the other species by the lack of pedicels, or the presence of very short pedicels- either during flowering or fruiting periods. The presence of very short internodes between the flowers in the subpedicellate or the sessile flowers allowed the inflorescence of the species to be evaluated as congested racemose, rather than corymb or pseudocorymb. Furthermore, the habitus of these three species show differences, while the first flowers of *O. brevipedicellatum* and *O. pamphylicum* are at ground level but the inflorescence of *O. oligophyllum* has generally a scape above ground level. The pedicels of *O. oligophyllum* are distinctly longer than the pedicels of *O. brevipedicellatum* and *O. pamphylicum* in flowering and fruiting periods.

Ornithogalum brevipedicellatum and *O. pamphylicum* are similar to each other with respect to their leaf morphology and inflorescence in flowering time. They have narrower leaves from *O. oligophyllum*. We observed the white line on the leaf surface of *O. pamphylicum* and the ovary of the species is distinctly winged, contrary to the description of the species by Düşen and Sümbül (2002). Therefore, *O. pamphylicum* was included in subg. *Ornithogalum* in the present study but, on the contrary, in subg. *Myogalum* by Düşen and Sümbül (2002). The closer morphologic proximity between *O. brevipedicellatum* and *O. pamphylicum* as opposed to *O. oligophyllum* was supported by the data obtained from molecular studies. Considering to the phylogenetic tree obtained from *trnL* and *rbcL* sequences, the individuals of *O. brevipedicellatum* were separated from *O. oligophyllum* in two different dendograms. *O. brevipedicellatum* are closer to *O. pamphylicum* compared with *O. oligophyllum*.

The most remarkable morphological differences between *O. brevipedicellatum* and *O. pamphylicum* can be defined as follows: (1) The capsules of *O. brevipedicellatum* are generally sessile, sometimes subpedicellate but they are pedicellate and pendant in *O. pamphylicum*; (2) lengths of

the style are quite different; (3) *O. brevipedicellatum* has more congested inflorescence while the internodes are elongated in *O. pamphylicum*; (4) The tepals are more narrow in *O. brevipedicellatum* (Tab. 2).

The seed size, shape, color, surface ornamentation and shape of the cells, raphe, micropylar and chalazal poles are useful diagnostic characteristics for seed micro-morphological studies conducted on the genus *Ornithogalum* (Bednorz and Czarna 2008, Çitak et al. 2015). Moret et al. (1990) and Martínez-Azorín et al. (2010) reported that subg. *Ornithogalum* shows globose and apiculate seeds with reticulate testa. Similarly, the seeds of *O. brevipedicellatum* are elliptic-subglobose to globose, distinctly apiculate with reticulate testa. The results obtained from the present study clearly indicate that *O. brevipedicellatum* is a distinct species and cannot be claimed to be a synonym of *O. oligophyllum*.

Acknowledgements

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Appendix

Additional specimens examined

O. oligophyllum: A1(E) Kırklareli: Demirköy, Mahyadağ, Mareşal road, *Fagus* gap, 860 m, 22.5.1974, *A. Baytop* et *E. Tuzlacı* (28271 ISTE). Kırklareli: Demirköy, Mahyadağ, 1030 m, 22.5.1974, *A. Baytop* et *E. Tuzlacı* (28304 ISTE). Kırklareli: Merkez, between Dereköy and boundary, 1.5.1973, *G. Ertem* (24281 ISTE). Kırklareli: Centre, woodlands in Dereköy, Kocakaynak environs, 26.4.1974, *N. Özhatay*, *E. Özhatay* (27642 ISTE), Kırklareli: Demirköy, between Yeniceköy and Velika, 1 km to Haydut Mountain, 24.4.1988, *G. Dalgiç*, *N. Başak* (59757 ISTE). Kırklareli: Centre, Dereköy, boundary, under *Fagus*, 23.4.1988, *G. Dalgiç*, *N. Başak* (59758 ISTE). Kırklareli: Demirköy, Velika, Balaban village picnic areas, under woodlands, 24.4.1988, *G. Dalgiç*, *N. Başak* (59766 ISTE). **A3** Bolu: between Bolu and Mudurnu, under *Quercus*, 1250–1350 m, 03.05.1993, *Z. Aytaç* 5789 et al. (GAZI). Bolu: Kale, Tenekeci River, streamside, woodlands, 800 m, *İ. Kılınç* 1029 (GAZI). **A4** Ankara: Kızılcahamam, Soğuksu National Park, Çakmaklı environs, 1450–1500 m, 12.05.1990, *Ö. Eyüboğlu* (GAZI). Ankara: Çubuk, between Ovacık and

Saraycık villages, Borucağın Hill, shrubs, 1250–1380 m, 20.5.1992, *E. Dündar* 1193 (GAZI). Çankırı: Atkaracalar, Dumanlı Mountain, Atkaracalar plateau, Abaza River environs, steppe, 1300–1450, 09.05.1992, *A. Duran* 1516 (GAZI). Ankara: Ayaş, Ayaşbeli, Çal Hill, steppe, 1300–1380 m, 13.04.1986, *M. Vural* 4029 (GAZI). Kırıkkale: Koçubaba Town, 1200 m, 31.03.1990, *A.A. Dönmez* 1696 (GAZI). **A9** Kars: Arpaçay, Karakale Village, under *Rumex* shrubs, 2250 m, 16.06.1984, *H. Ocakverdi* 1714 (GAZI). Ardahan: Posof, Baykent village, birch woodlands, 1900 m, 25.6.2007, *Sezgin Esen* (87083 ISTE). Ardahan: Çataldere village birch woodlands, 9.6.2008, *Sezgin Esen* (87275 ISTE). **B4** Kırşehir: Çiçek Mountain, Halaçlı Village, Almanderebaşı environs, *Quercus* shrubs, slopes, 1550 m, 09.05.1993, *F.A. Karavelioğulları* 1026 et al. (GAZI). **B5** Nevşehir: between Uçhisar and Göreme, erosional dry slopes, 1250 m, 19.04.1989, *M. Vural* 4497 et al. (GAZI). **B6** Kayseri: Sarız, Yalak environs, Binboğa Mountain, steppe, 1800–2200 m, 07.05.1991, *Z. Aytaç* 3709 et al. (GAZI). Kahramanmaraş: Binboğa Mountain, west of Kaşvut Village, alpine steppe, under *Cedrus libani*, cliffs, 1750–2250 m, 08.05.1991, *Z. Aytaç* 3733 et al. (GAZI). Kayseri: Sarız, Binboğa Mountain, Doğankonak, Karagöz, 1450–1900 m, 21.04.1992, *Z. Aytaç* 4486 et al. (GAZI). Kayseri: Pınarbaşı, south of Aşağıbeyçayırı village, eroded west slopes, 29.4.2004, *B. Yıldız*, *T. Arabacı* (89931 ISTE). **B7** Malatya: Kubbe Mountain, Kubbe plateau, mountain steppe, 1700 m, 1.6.1992, *B. Yıldız* 9378 (ISTE). **B8** Erzurum: between Aşkale and Tercan, Kükürtlü environs, slopes and shrubs, 1950 m, 23.5.2004, *M. Koyuncu*, *M. Firat*, *M. Armağan* (88864 ISTE). **B9** Bitlis: Adilcevaz, Süphan Mountain hillside, Süte plateau, meadows, 1900 m, 04.06.1993, *Y. Altan* 4796 (GAZI). Van: Gevaş, west slopes of Çadır Mountain, steppe, 2300 m, 06.06.1993, *Y. Altan* 5077 (GAZI). **C3** Isparta: Barla, Gelincik Mountain, Tahtacıçukuru Hills environs, *Pinus nigra*, 1800–1900 m, 09.05.2009, *H. Duman* 9921 (GAZI). **C6** Kahramanmaraş: Engizek Mountain, Küçükcerit Village, under *Quercus*, 1400–1500 m, 21.04.1987, *H. Duman* 2486 (GAZI). Kahramanmaraş: Engizek Mountain, south of Şaç Rocks, wetlands, 2400–2500 m, 20.05.1987, *H. Duman* 2680 (GAZI). Kahramanmaraş: Erince Mountain, under *Quercus*, 1300–1400 m, 22.04.1987, *H. Duman* 2507 (GAZI). Kahramanmaraş: between Göksun and Geben, Kayranlı Mountain, east slopes, 30.4.2014, *B. Yıldız*, *T. Arabacı* (89959 ISTE). **C7** Şanlıurfa: Karacadağ, 2 km from Karabahça to Dağ, Reme River environs, stony areas, 1400 m, *İ. Eker* 181-b (GAZI).

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