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A new species of *Cymbocarpum* (Apiaceae) from the Central Anatolia Region of Turkey and its phylogenetic position within Tordylieae

Yusuf MENEMEN^{1*}, Burçin ÇINGAY², Aşkın Öykü ÇİMEN³, Şeref ERTAŞ⁴

¹Department of Biology, Faculty of Science and Literature, Kırıkkale University, Yahşihan, Kırıkkale, Turkey

²Science Departments, Nezahat Gökyiğit Botanic Garden, Ataşehir, İstanbul, Turkey

³Department of Biology, Faculty of Science, Gazi University, Teknikokullar, Ankara, Turkey

⁴Institute of Science, Kırıkkale University, Yahşihan, Kırıkkale, Turkey

Received: 09.05.2017 • Accepted/Published Online: 30.09.2017 • Final Version: 11.01.2018

Abstract: A new species, *Cymbocarpum alinihatii*, is described from the Central Anatolia Region of Turkey. Diagnostic and morphological characteristics that distinguish it from allied species *C. anethoides*, *C. wiedemannii*, *C. amanum*, *C. erythraeum*, and *C. marginatum* and an identification key for all *Cymbocarpum* species are provided. To assess its phylogenetic placement, a maximum parsimony analysis of nrDNA ITS sequences from representative members of tribe Tordylieae was carried out. It shows that the new species nests within a well-supported clade comprising members of *Cymbocarpum* with strong bootstrap value, but it is distinguished from its most closely related species, *C. anethoides* and *C. wiedemannii*. In terms of stem and leaf hairiness, leaf type, and mericarp shape, *C. alinihatii* differs from *C. anethoides* by having sparsely to densely scabrid hairy stem and leaf (not glabrous), sometimes having ternate leaves and ovate mericarp shape (not elliptic to oblong). The distribution areas of these two species are also isolated in the high mountains occupying large areas of eastern Turkey. The new species clearly differs from *C. wiedemannii* by having 4–8 cm (not 20–40 cm) high stem, ca. 1–1.5 cm leaf lamina (not 3 cm), 1–2 cm petiole (not 5 cm), 2–4 mm ultimate leaf segments (not 5 mm), umbel with generally 4–7 (not 10–20) rays, and hairy 2.9–3.2 mm (not 4–5 mm) long mericarps. Based on characters of morphology, and concordant with the molecular phylogenetic results, we recognize the new species as belonging to the genus *Cymbocarpum*.

Key words: Ankara, *Cymbocarpum alinihatii*, endemic, phylogeny, Umbelliferae

1. Introduction

The genus *Cymbocarpum* was first described by de Candolle (1830) based on a nomenclaturally problematic species of *C. anethoides* (*Anethum? cymbocarpum*) and was later cited by Meyer (1831) as a monotypic genus. The distribution area of the genus covers Turkey, Caucasus, and Iran. Boissier (1872), in his *Flora Orientalis*, recognized *C. anethoides*, *C. erythraeum* (DC.) Boiss., *C. marginatum* Boiss., and *C. wiedemanni* Boiss. within the genus. Rechinger (1950) described *C. amanum* Rech.f. from the Amanos Mountains (Turkey), raising the number of species in the genus to five. Chamberlain (1972), in *Flora of Turkey*, recorded three certain (*C. amanum*, *C. anethoides*, *C. wiedemanni*) and a doubtful (*C. erythraeum*) species from Turkey. Davis et al. (1988) confirmed the existence of *C. erythraeum* Boiss. in eastern Anatolia. In his account of Turkish *Cymbocarpum*, Menemen (2012) reported four species growing in Turkey.

Alava (1975) transferred *C. marginatum* Boiss. to the new monotypic genus of *Kalakia* Alava. Ajani et al. (2008)

criticized the naturalness of this genus and stated that the construction of the monotypic genus *Kalakia* based on the thickened mericarp margin was not reliable. In their molecular study, they confirmed the monophyly of *Cymbocarpum* and ascertained its close relationship to *Ducrosia* and *Kalakia*; after giving the overall similarity between *Cymbocarpum* and *Kalakia* and their relatively low genetic divergence, they concluded that the genus *Kalakia* and its only species, *K. marginatum*, should be included in the genus *Cymbocarpum*, but they hesitated to transfer *Ducrosia* species to the genus *Cymbocarpum* due to the absence of *Ducrosia* species except for *D. anethifolia* (DC.) Boiss. in their study.

The name of *Cymbocarpum* comes from the Greek kymbe (Latin cymba) and karpon, meanings boat and fruit, respectively, referring to the shape of the mericarps (Shishkin, 1950). The genus is characterized by the features of being short annuals (rarely biennial) with a fetid smell of goat, 2 or more pinnate, ternate, or divaricately branched leaves; inconspicuous calyx teeth, white or rarely

* Correspondence: y_menemen@hotmail.com

violet (pink?) petals, generally lenticularly compressed fruit with narrow, thin tapering or slightly inflated margin, and thin, filiform, often inconspicuous ribs (Boissier, 1872; Shishkin, 1950; Chamberlain, 1972; Rechinger, 1987; Davis et al., 1988).

The aim of this study is to describe a new species of the genus *Cymbocarpum*, to determine the morphological differences between the new species and its allied species, and to assess its phylogenetic placement with a maximum parsimony analysis of nrDNA ITS sequences from representative members of tribe Tordylieae.

2. Materials and methods

Plant material belonging to the new species was collected from Ankara Province, Turkey, during the 2014 vegetation season. Herbarium specimens were prepared and deposited at NGBB (Nezahat Gökyiğit Botanic Garden Herbarium, İstanbul) and ADO (Anadolu Herbarium, Kırıkkale University). More than 10 specimens with both flowers and mature fruits were collected. The material was examined carefully using a stereomicroscope, and its features were compared to published literature on *Cymbocarpum* and its allies (Boissier, 1872; Shishkin, 1950; Chamberlain, 1972; Rechinger, 1987; Davis et al., 1988), to material found in the herbaria HUB and ISTE, to high magnitude images of *Cymbocarpum* specimens observed on websites, and to a high-quality photograph of the holotype specimen of *C. anethoides* sent by the head curator of G herbarium (see Examined material). The threat category assessment of the new species was defined according to the IUCN criteria (IUCN, 2016).

For the fruit anatomical studies, mature mericarps were boiled in distilled water and sections were made by hand, using a razor blade. After staining in alcian blue and safranin, these sections were examined under a light microscope.

Total genomic DNA was isolated from dried leaves of *C. alinihatii* using the modified CTAB procedure of Doyle and Doyle (1987). Double-stranded DNA of the complete ITS region in each genomic DNA was PCR-amplified and sequenced using primers ITS4 and ITS5 (White et al., 1990). Sequencing was carried out by PRZ BioTech (Ankara, Turkey). ITS sequences of all other taxa included in the phylogenetic analysis were obtained from GenBank (Table 1). *Conium maculatum* was used to root the trees, as this species is a sister group to tribe Tordylieae (Ajani et al., 2008).

Phylogenetic analyses were carried out initially using maximum parsimony (MP) as implemented in PAUP* Ver. 4.0b10 (Swofford, 2003). A set of most parsimonious trees was obtained through heuristic searches replicated 1000 times with random stepwise addition of taxa, tree bisection-reconnection (TBR) branch swapping, and saving multiple trees. Bootstrap (BS) values were calculated from 1000 replicate analyses using TBR branch swapping and random stepwise-addition of taxa.

3. Results and discussion

Cymbocarpum alinihatii Menemen & Çingay, sp. nov. (Figures 1–5)

Type: Ankara, Beypazarı, near Sekli village, roadside, scree rocks, 619 m a.s.l., 30.06.2014, 40.194850N, 31.707329E, B. Çingay, A. Ö. Çimen, R. Anşin, R. M.

Table 1. Voucher information (or reference, if voucher information was published previously) and GenBank accession numbers for taxa used in the ITS phylogenetic study. Taxon names in parentheses were those used by previous authors in their GenBank entries.

Taxon	Voucher information or reference	GenBank accession number
<i>Conium maculatum</i> L.	Logacheva et al., 2010	GU266037
<i>Cymbocarpum wiedemannii</i> Boiss.	Logacheva et al., 2010	GU291352
<i>Cymbocarpum erythraeum</i> Boiss.	Ajani et al., 2008	EU169254
<i>Cymbocarpum anethoides</i> DC.	Logacheva et al., 2010	GU190156
<i>Cymbocarpum anethoides</i> DC.	Ajani et al., 2008	EU169253
<i>Cymbocarpum marginatum</i> Boiss. (as <i>Kalokia marginata</i> Alava)	Ajani et al., 2008	EU169292
<i>Ducrosia anethifolia</i> (DC.) Boiss.	Khan S, Al-Qurainy F, Nadeem M, Tarroum M, Gaafar A, Alameri A “unpubl.”	KJ004352
<i>Ducrosia flabellifolia</i> Boiss.	Logacheva MD, Valliejo-Roman CM, Pimenov MG “unpubl.”	DQ427051
<i>Ducrosia assadii</i> Alava	Logacheva et al., 2010	DQ427043
<i>Cymbocarpum alinihatii</i> Menemen & Çingay	This paper	KY989959
<i>Zosima absinthifolia</i> (Vent.) Link	Ajani et al., 2008	EU169332
<i>Tordylium maximum</i> L.	Logacheva et al., 2008	DQ996585
<i>Tordylium apulum</i> L.	Ajani et al., 2008	EU169329



Figure 1. General habitat of *Cymbocarpum alinihatii*.

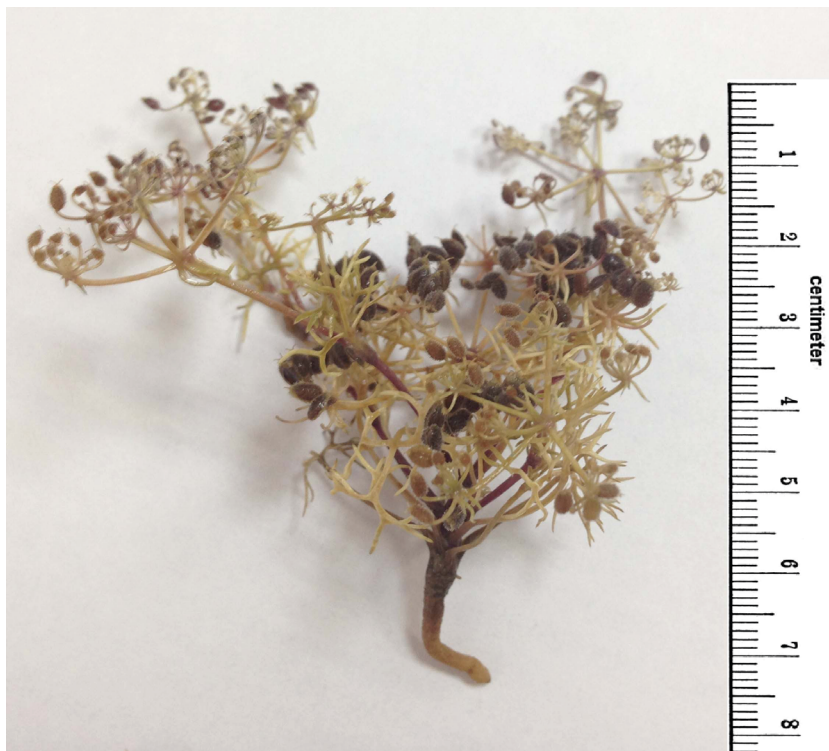


Figure 2. General habit of *Cymbocarpum alinihatii*, with root, leaves, and umbels.

Aydıncal, Ö. Demir (OBKA) 947 (holotype: NGBB 005901; isotypes: ADO, NGBB).

Annual tap-rooted plants. Stem 4–8 cm tall, branched at or near its base, striate or grooved, slender, 0.8–1.5 mm in diameter at base, dark violet in color almost throughout, and sparsely to densely scabrid. Basal leaves 2–3 pinnate or ternate with furcately divaricated segments, 2–3.5 cm; lamina 1–1.5 cm, ovate in outline; ultimate segments 2–4 × 0.3–0.5 mm, linear with violet mucronate or apiculate tips, glabrous to scabrid. Petioles 1–2 cm, with a sheath up to 4 mm. Cauline leaves similar

to basal leaves but smaller in size of lamina and petiole and less divaricated. Synflorescence composed of short-pedunculate (1–2.5 cm) compound umbels with unequal, ±glabrous to sparsely scabrid hairy, 4–7(–10) rays. Bracts 4–6.1 × 0.4–0.6 mm, simple, deflexed, filiform to linear, rarely bifid, glabrous to sparsely scabrid hairy. Bracteoles 2–5, deflexed, filiform to linear, 1–3 mm long, ±glabrous. Flowers 5–8 in each umbellule, polygamous, 1–1.5 mm in diameter, with 1–3 mm and longer fruiting, unequal, ±glabrous pedicels. Sepals obsolete or rarely minute. Petals white, ovate to obovate, strongly inflexed, with a



Figure 3. Basal and lower cauline leaf variation of *Cymbocarpum alinihatii*.



Figure 4. *Cymbocarpum alinihatii* mericarp dorsal (A), commissural surfaces and tubular and clavate hairs on dorsal surface (B).

shallowly emarginated truncate-retuse tip, and glabrous on the dorsal surface. Ovary and immature mericarp dark violet in color, densely tubular clavate or capitate glandular white hairy. Dried mature mericarp 2.9–3.2 × 1.5–1.8 mm,

ovate and lenticularly compressed in cross-section, with black vallecular and commissural area and light straw yellow ribs and lateral wings, sparsely to densely tubular clavate or capitate glandular white hairy on dorsal surface

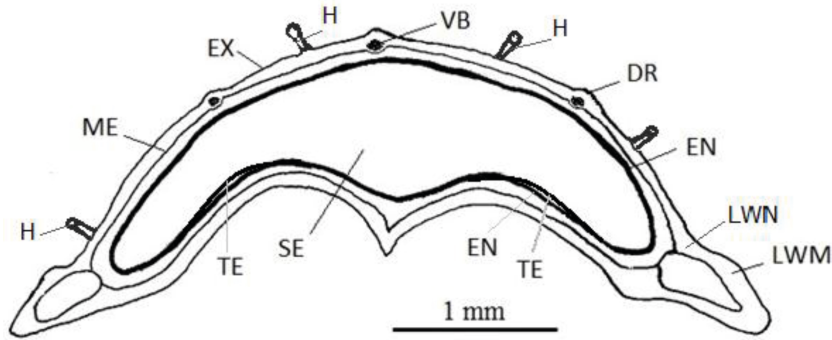


Figure 5. Diagrammatic images of a cross-section of a *Cymbocarpum alinihatii* mericarp showing absence of dorsal and commissural vittae. Vascular bundle (VB), exocarp (EX), mesocarp (ME), dorsal rib (DR), endocarp (EN), seed (SE), testa (TE), hair (H), lateral wing neck (LWN), and lateral wing margin (LWM).

including unthickened lateral margin; dorsal ridges filiform; lateral wings 0.25–0.30 mm; stylopodium short conical with undulate margin, glabrous; styles 0.5–0.8 mm long, slender, deflexed; stigma capitate; oil ducts absent on both dorsal and waxy commissural surfaces.

Flowering in May–June, fruiting in June–July.

Examined material

C. anethoides DC.

Turkey, B10 Iğdır: Aralık, Taze Köyü, 821 m a.s.l., 07.06.2008, *E. Altundağ* 521 (ISTE: 85495!); Azerbaijan, Karabagh, *A. J. Szovits* 221 (MNHN-P-P03222555 [web!] <http://mediaphoto.mnhn.fr/media/1445838262389lmTbLZEQFfbwf3VP> [accessed: 19.01.2017]); Azerbaijan, Karabagh orient., *A.J.Szovits* s.n.; Iran, Khosrowo, in arvis, 1884-06-09, *J. A. Knapp* s.n. (two labels with a single specimen) (JE, JE00022438 [web!] <http://plants.jstor.org/stable/10.5555/al.ap.specimen.je00022438> [accessed: 19.01.2017]); in aridis et arenosis circa Seid-khodzi Persiæ legit cl. *Szovits* (holotype: G00663559-photo!); t. 300 *Rech.* 40264. In: Hedge IC, Lamond JM, Rechinger KH (1987). Umbelliferae (Tabulae) Flora Iranica: flora des Iranischen Hochlandes und der umrahmenden gebirge: Persien, Afghanistan, Teile von West- Pakistan, Nord-Iraq, Azerbaidjan, Turkmenistan, no. 162. Graz, Austria: Akademische Druck-u Verlagsanstalt!

Cymbocarpum erythraeum (DC.) Boiss.

Turkey, Erzincan: Dağınık D., 3000 m a.s.l., 39°47'92"N, 39°28'19"E, 27 viii 2003, flowing slopes, *Kandemir* 5838 (ADO!, Erzincan University, Education Faculty Herbarium!)

C. wiedemannii Boiss.

Turkey, C5 Konya: Ereğli, Aydos Dağı, Delimahmutlu, Karasirt Mevki, kalker yamaç, bozkır, 1600 m a.s.l., 26 vi 1976, *S. Erik* 1620 (HUB!); ibid 17.07.1976 (HUB!); A7 Giresun: Bulacak, Bicik ormanları, ormanlık alanlar, 19.06.1995, *M. Arslan* (ISTE 72561!); A9 Iğdır: Tuzluca, Güzeldere Köyü, 12.06.2008, *E. Altundağ* 724 (ISTE 85625!).

Cymbocarpum amanum Rech.f.

Turkey, C5 Hatay: İskenderun, track to Akıncı Burnu, where the Kızıldağ range reaches sea (ca. 10–15 km S of Uluçınar [UTM YF52]) very exposed and windswept serpentine cliffs and cliff tops, 29.05.1996, 0–100 m a.s.l., *A. J. Byfield & D. Pearman* (B 2631) (ISTE73373!); Hatay: Amanus [Amanos], tere Bagtche [Derebahçe], 25/05/1933, Haßdrösm s.n (K-K000695939 [web!] <http://apps.kew.org/herbcat/getImage.do?imageBarcode=K000695939> [accessed: 19.01.2017]).

C. marginatum Boiss.

Iran: t.: *Iranshahr* 13980. In: Hedge IC, Lamond JM, Rechinger KH (1987). Umbelliferae (Tabulae) Flora Iranica: flora des Iranischen Hochlandes und der umrahmenden gebirge: Persien, Afghanistan, Teile von West-Pakistan, Nord-Iraq, Azerbaidjan, Turkmenistan, no. 162. Graz, Austria: Akademische Druck-u Verlagsanstalt!; Keredj: Hänge des Elburs, 16.06.1934, *E. Gauba* 1a. (type of *Ducrosia stenocarpa* Bornm. & Gauba) [web!] http://ww2.bgbm.org/herbarium/view_biocase.cfm?SpecimenPK=100229 [accessed: 19.01.2017]); Keredj: Hänge des Elburs. 11-16.06.1934, *E. Gauba* 1? (syntype of *Ducrosia stenocarpa* f. *leiocarpa* Bornm. & Gauba) [web!] http://ww2.bgbm.org/herbarium/view_biocase.cfm?SpecimenPK=100219 [accessed: 19.01.2017]); Teheran; Keredj; Vorberge d. Elburs. 03.06.1933, *E. Gauba* s.n. (syntype of *Ducrosia stenocarpa* f. *leiocarpa* Bornm. & Gauba) [web!] http://ww2.bgbm.org/herbarium/view_biocase.cfm?SpecimenPK=100220 [accessed: 19.01.2017]).

Etymology: *Cymbocarpum alinihatii* is named in honor of Ali Nihat Gökyiğit, founder and leading financial contributor of the Nezahat Gökyiğit Botanical Garden and ANG Foundation, for his continued contributions to the *Illustrated Flora of Turkey* and Turkish botany.

Proposed Turkish name for the new species according to Menemen et al. (2016): Ankara aşotu.

Habitat and ecology: The new species occurs on calcareous rocky slopes. It is restricted to elevations between 1750 and 1900 m. The collection site is in the Irano-Turanian phytogeographical region, with a relatively arid climate. Species growing in the vicinity include *Festuca ovina* L., *Aegilops neglecta* Req. ex Bertol., *Astragalus angustifolius* Lam., *Trifolium arvense* L., *Thymus sipyleus* Boiss., and *Galium vernum* Hook.f.

Distribution and proposed conservation status: *Cymbocarpum alinihatii* is endemic and is restricted to a very limited area of the type locality in Beypazari, Ankara. The species is known only from type gatherings and from an area of approximately 0.001 km². Because of farming effects and overgrazing, the habitat of this species is under threat, and this situation leads to a true potential reduction in the number of individuals. The population is currently in poor condition, and the number of individuals is estimated to be about 30–40. Therefore, the species should be categorized as Critically Endangered CR [B2ab(i,ii,iii,v), D], according to the IUCN categories (IUCN 2016).

Key to *Cymbocarpum* species

- 1a. Fruit margins clearly tumid and large.....2
- 1b. Fruit margins not tumid and narrow.....3
- 2b. Bracts present, mericarp oblong.....*C. marginatum*
- 2a. Bracts absent, mericarp elliptic.....*C. amanum*
- 3a. Fruit hairy.....4
- 3b. Fruit glabrous.....5
- 4a. Stem glabrous; mericarp elliptic.....*C. anethoides*
- 4b. Stem sparsely to densely scabrid; mericarp ovate...*C. alinihatii*
- 5a. Peripheral petals elongated, 2–2.5 mm long; umbel with 10–20 rays.....*C. wiedemannii*
- 5b. Peripheral petals not elongated, 1–1.5 mm long; umbel with 7–11 rays.....*C. erythraeum*

Here, we recognize 6 species belonging to the genus *Cymbocarpum*. A comparison of some diagnostic characters of these species is presented in Table 2. The taxa in the genus *Cymbocarpum* are fetid annual (rarely biennial) plants as in *C. erythraeum*, but *C. alinihatii* has

no unpleasant smell. Stem is glabrous in *C. anethoides*, *C. amanum*, *C. erythraeum*, and *C. marginatum*; sparsely papillate in *C. wiedemannii*; and sparsely to densely scabrid in *C. alinihatii*. Leaf is 2–3 pinnate or divaricately branched or 3-ternate in *C. marginatum* and sometimes in *C. alinihatii*, which has 2–3 pinnate or ternate with furcately divaricated segments (Figure 3). In terms of leaf hairiness and leaf type, *C. alinihatii* differs from its closest relative, *C. anethoides*, by having sparsely to densely scabrid hairy and sometimes ternate leaves.

The number of rays varies between 2 and 20 in the genus *Cymbocarpum*. *C. amanum* differs from all the other species by having 2–5 subequal rays. This species is the only species within the genus with no bracts or bracteoles. *C. alinihatii* has simple deflexed, filiform to linear, rarely bifid, glabrous to sparsely scabrid hairy bracts, and deflexed, filiform to linear, ±glabrous bracteoles. The flower color is white in all *Cymbocarpum* species except for *C. erythraeum* and *wiedemannii*, which sometimes have violet (pink?) corolla.

The mericarp in the species is dorsally lenticularly compressed and does not contain any vitta on either dorsal and commissural surfaces, and it ranges from elliptic or oblong to ovate in shape (Figures 4 and 6). Although thickness of mericarp margin is a good character to distinguish species within the genus, as for *C. marginatum* and *C. amanum* (Figure 6E), the use of this character to separate the genera was criticized by Ajani et al. (2008), who stated that the construction of the monotypic genus *Kalakia* based on the thickened mericarp margin is not reliable. In terms of mericarp size, hairiness, and margin, *C. alinihatii* shows an affinity to *C. anethoides* (Figures 4A and 4C, 6G, and 7), but differs by having an ovate mericarp. All *Cymbocarpum* species examined are more or less waxy on the commissural surface (Figures 4B and 6B, 6D, and 6F).

C. alinihatii is the only *Cymbocarpum* species detected in central Anatolia (Figure 8) except for *C. wiedemannii*, a specimen of which was unusually collected from the

Table 2. A comparison of some diagnostic characters of the six *Cymbocarpum* species.

Taxon	Stem	Ray numbers	Ray equality	Bract and bracteoles	Flower color	Mericarp shape	Mericarp margin	Mericarp hairiness
<i>C. anethoides</i>	Glabrous	6–13 (20)	Unequal	Present	White	Elliptic to oblong	Unthickened	Hairy
<i>C. wiedemannii</i>	Sparsely papillate	10–20	Unequal	Present	White/violet	Elliptic to ovate	Unthickened	Glabrous
<i>C. amanum</i>	Glabrous	2–5	Subequal	Absent	White	Elliptic	Thickened	Glabrous
<i>C. erythraeum</i>	Glabrous	9–11	Unequal	Present	White/violet	Ovate	Unthickened	Glabrous
<i>C. marginatum</i>	Glabrous	5–8(–10)	Unequal	Present	White	Oblong	Thickened	Hairy/glabrous
<i>C. alinihatii</i>	Scabrid	4–7(–10)	Unequal	Present	White	Ovate	Unthickened	Hairy

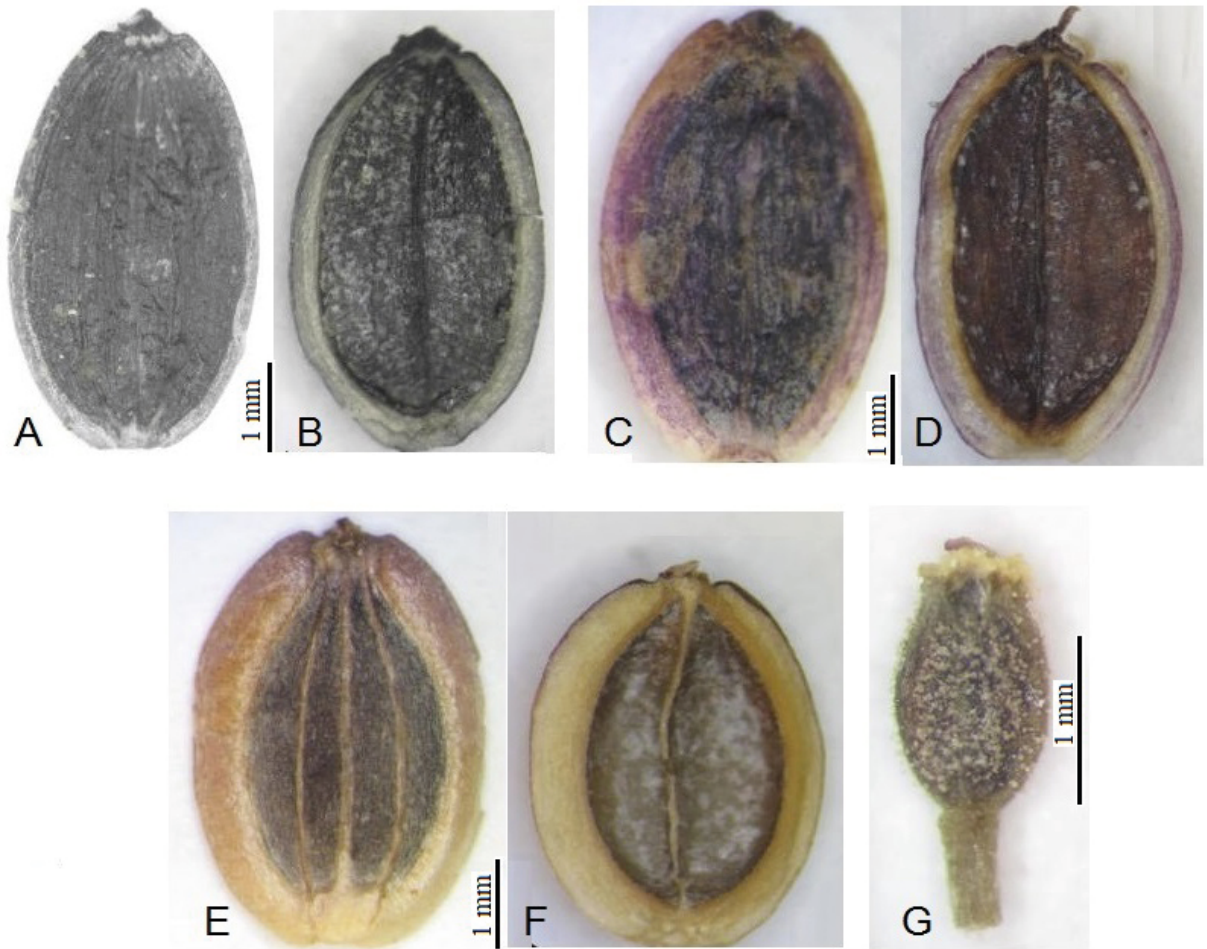


Figure 6. Mericarps of *Cymbocarpum* species distributed in Turkey except for *C. alinihatii*. Dorsal surfaces of mature mericarps of *C. wiedemannii* (A), *C. erythraeum* (C), and *C. amanum* (E); commissural surfaces of mature mericarps of *C. wiedemannii* (B), *C. erythraeum* (D), and *C. amanum* (F). Dorsal surface of an immature mericarp of *C. anethoides* (G), with tubular and clavate hairs similar to *C. alinihatii*.



Figure 7. General habit of *Cymbocarpum anethoides*, with root, leaves, umbels, and mature elliptic mericarps (from the holotype, G00663559).

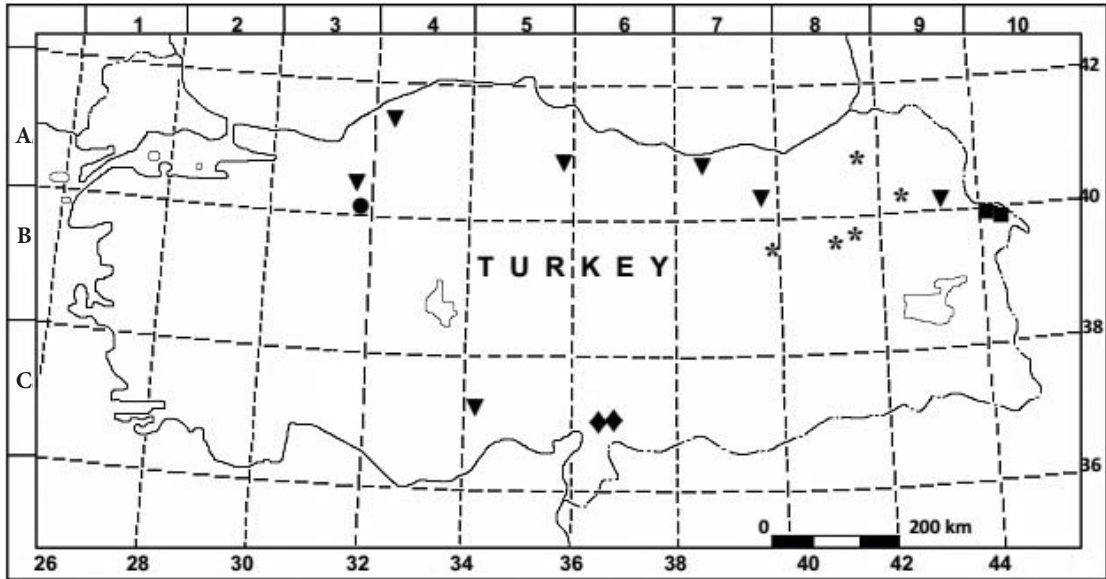


Figure 8. A distribution map of *Cymbocarpum* species distributed in Turkey: *C. alinihatii* ●; *C. anethoides* ■; *C. amanum* ◆; *C. wiedemannii* ▼; *C. erythraeum* ✱.

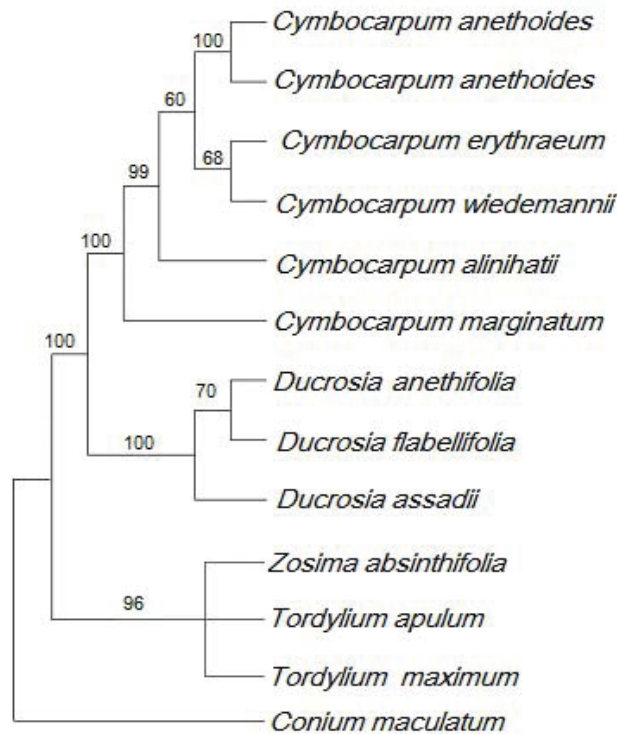


Figure 9. Strict consensus tree of shortest trees derived from maximum parsimony analysis of nrDNA ITS sequences from tribe Tordylieae and the outgroup *Conium maculatum*. MP bootstrap support values (above branches) are presented.

southern border of the region, isolated from other species. Despite the fact that *C. anethoides* (İğdır Province in Turkey) appears to be the closest relative species to *C. alinihatii* (Ankara Province), their habitats are very far from each other (Figure 8). The distribution area of the new species is very close to the distribution of *C. wiedemannii* in the Western Black Sea region. *C. wiedemannii* is clearly distinguished from the new species by having a 20–40 cm high stem, ca. 3 cm leaf lamina, 5 cm petiole, and 5 mm ultimate segments respectively, umbel with 10–20 rays, and hairless 4–5 mm long hairless mericarps.

To assess the phylogenetic placement of *C. alinihatii*, a maximum parsimony analysis of nrDNA ITS sequences from representative members of tribe Tordylieae was carried out. It shows that the new species nests within a well-supported clade comprising members of *Cymbocarpum* with a strong 100% bootstrap value (Figure 9). *C. alinihatii* takes place within the clade of *Cymbocarpum* as sister to a subclade that includes the species *C. anethoides*, *C. erythraeum*, and *C. wiedemannii*.

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- The phylogenetic analysis and our examination of the specimens collected from Ankara show that they are representatives of a new species belonging to the genus *Cymbocarpum*. After the introduction of this new species to science, the number of *Cymbocarpum* species is raised to 6 throughout the world, of which 5 are distributed in Turkey.

Acknowledgments

The authors thank the curators of GAZI, HUB, and ISTE herbaria for allowing them to study their Apiaceae collections, the head curator of G herbarium for sending a high-quality picture of the holotype specimen of *C. anethoides*, Prof Dr Ali Kandemir for providing his collection of *C. erythraeum* for examination, and Prof Dr Rahim Anşın, Rasim Murat Aydıncal, and Ömer Demir for their help during the fieldwork. We are also grateful to the Kırıkkale University Scientific Projects Coordination Unit (project no. 2013/18) and the Ali Nihat Gökyiğit Foundation for financial support.

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