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Ekimia ozcan-secmenii (Apiaceae), a new species from Southwest Anatolia, Turkey

Serdar Gökhan ŞENOL*, Volkan EROĞLU, Nazlı Bahar PELİT, Duygu BOZYEL
Ege University Botanical Garden and Herbarium Research and Application Center, İzmir, Turkey

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Abstract: *Ekimia ozcan-secmenii* Şenol & Eroğlu sp. nov. (Apiaceae) is described from SW Turkey. *E. ozcan-secmenii* differs from its close affinity, *E. bornmuelleri*, by life form (monocarpic), petal and bracteole morphology, and mericarp features. These data were more supported by the results of nrDNA ITS sequences. The ML tree depicted based on phylogenetic studies aimed to reveal the closeness of the *Laserpitium* and *Prangos* species as well as the location the affinity of species in the genus *Ekimia*. The taxonomic situation of *Prangos hulusi* was also evaluated based on nrDNA ITS sequences. Since *E. ozcan-secmenii* is distributed in Sami Soydam Sandalcık hydroelectric power plant in Acıpayam, Denizli, the population is threatening by flooding. Therefore, conservation programs are urgently recommended.

Key words: Apiaceae, *Ekimia*, internal transcribed spacer (ITS), *Laserpitium*, new species, phylogeny, *Prangos*, taxonomy, Turkey

1. Introduction

Apiaceae is one of the largest plant families in the world. Turkey is one of the largest centers of biodiversity for the family among Asian countries, with about 160 endemic species included in 44 genera (Bilgili et al., 2016). The flora of Turkey consists of 19 monotypic endemic genera (Pimenov and Leonov, 1993; Güner et al., 2012; Vural et al., 2012; Uysal et al., 2014). Five of the known monotypic genera belong to the family Apiaceae. One of these genera, *Microsciadium* Boiss. (1844), is not endemic and grows in the East Aegean Islands. *Aegokeras* Raf. (1840), *Crenosciadium* Boiss. & Heldr. ex Boiss. (1849), *Postiella* Kljuykov (1985), and *Ekimia* Duman & Watson (1999) are endemic (Güner and Ekim, 2014).

Ekimia bornmuelleri (Hub.-Mor. & Reese) Duman & Watson (1999) has a quite small habitat around Lake Salda, between Tefenni and Burdur. It is considered a critically endangered (CR) species. Due to nonmature fruits, it was previously considered within *Prangos* Lindl. (1825) as *Prangos bornmuelleri* Hub.-Mor. & Reese. It was reported to be an ambiguous species as it differs from *Prangos* in its relatively fewer and long umbel rays (Herrnstadt and Heyn, 1977; Pimenov and Tikhomirov, 1983; Herrnstadt and Heyn, 1987).

During fieldwork for the "Turkish Endemic Plants Project" in June 1993, the specimens with mature fruits species were collected that were already known as *Prangos bornmuelleri* due to nonmature fruits. Careful examination showed that the species did not show similarities to *Prangos* species either in the characteristics mentioned above or its fruit characteristics. In 1999, *Ekimia* was defined as a new genus and the species was re-introduced as *E. bornmuelleri* (Hub.-Mor. & Reese) H.Duman & M.F.Watson (Duman and Watson, 1999).

Phylogenetic relationships among genera and species of the family Apiaceae have been evaluated using molecular data, which showed major controversy with traditional classifications (Downie et al., 1998, 2010; Menemen et al., 2016). Lyskov et al. (2015), based on these data, found that *Ekimia* is closer to *Laserpitium* than *Prangos*. Banasiak et al. (2016) defined the taxonomic situation of species within the subtribe Daucinae using the fruit morphological characters and nrDNA ITS sequences. They considered *Ekimia bornmuelleri* in *Laserpitium petrophilum* Boiss. & Heldr (1849) and *L. glaucum* Post (1896) in the clade Ekimia.

In June–August 2013, during a field expedition to serpentine screes slopes and open forests of *Pinus brutia* Ten. around Sami Soydam Sandalcık hydroelectric power plant upon Dalaman Stream (630–700 m asl), SW of Turkey, a new species of *Ekimia* was discovered by Serdar G. Şenol and Volkan Eroğlu. Careful examination of herbarium specimens showed some differences from its close affinity, *E. bornmuelleri*. In addition, these results were supported by the results of molecular phylogenetic analysis using nrDNA ITS regions. We found that the

* Correspondence: sgsenol@yahoo.com

species was considered as a new species belonging to *Ekimia*, and therefore the number of endemic species of this genus in Turkey increased to two.

2. Materials and methods

2.1. Morphology and anatomy

During field works, samples were collected in different seasons at the vegetative and reproductive stages. Herbarium specimens were deposited at EGE. All available literature (Herrnstadt and Heyn, 1977; Pimenov and Tikhomirov, 1983; Herrnstadt and Heyn, 1987; Duman and Watson, 1999) and specimens in GAZI and EGE herbaria were carefully studied.

For mericarp morphology, 50 mature mericarps were measured under a Dino-lite AM423U digital microscope. Mature mericarps were hand-sectioned by razors and kept in glycerin. Then they were examined under an Olympus SZ50 stereomicroscope mounted with a Dino-Lite AM413 digital camera.

Micromorphology of the mericarp and pollen was studied by Philips XL 30S FEG scanning electron microscope (SEM). Live material was studied by Nikon D 5000 digital camera.

The distribution area and geographical location of the species were determined using Magellan Mobile Mapper 6 GPS.

2.2. Molecular phylogenetic analyses

Molecular phylogenetics were studied based on nrDNA ITS regions of *Ekimia ozcan-secmenii*, *E. bornmuelleri*, *Laserpitium*, and *Prangos*. The leaf samples were taken from herbarium samples (EGE) and also the NCBI GenBank database (28 taxa, see Appendix). Analyses on samples with total DNA were conducted following a modified CTAB protocol (Doyle and Doyle, 1987; Cota-Sánchez et al., 2006). As indicated in the protocol, primers ITS4 and ITS5 were used in PCR amplification (White et al., 1990; Downie and Kazt-Downie, 1996). Results of the PCR amplification were checked with agarose gel electrophoresis. A DNA sequencing cleanup kit was used in the purification, and DNA sequences were performed with an AB1 prism 3100-Avanr DNA sequencer. Total nrDNA sequences of 35 species belonging to 4 genera were obtained from NCBI GenBank. *Physospermum cornubiense* (L.) DC. was selected as outgroup. The sequences were aligned using the clustalW and Bioedit version 7.2.5 (Hall, 1999). To decide on the best modeling technique, Mega 6 program was used, and based on the BIC (Bayesian modeling) results, Kimura-2 model was selected (Tamura et al., 2013). The maximum parsimony and likelihood phylogenetic trees were constructed. MP analysis were performed using software Mega 6 following tree bisection reconnection (TBR) methodology. For each heuristic search, 1000 additional randomly aligned sequences were added and the shortest tree was recorded. To evaluate the support level given to certain branches, bootstrap analysis was performed. After each replicate, thousands of parsimonious trees were recorded.

3. Results

3.1. Taxonomy

Ekimia ozcan-secmenii Şenol & Eroğlu **sp. nov.** (Figure 1)
Type:—Turkey, C2 Denizli: Between Karaismailler-Suçatı villages, Acıpayam, serpentine scree slopes, *P. brutia* open forests, around Dalaman stream; 37°06′62.2″N, 29°05′49.824″E, 650 m, 03.06.2013, S.G. Şenol & V. Eroğlu 3560 (holotype: EGE42490!).

Diagnosis: Perennial monocarpic. Bracteoles 8–9, narrowly elliptic-cuspidate, 1-nerved. Mericarps with reticulate-alveolate surface and 9 gyrose-moniliform wings (Figure 2).

Description: Perennial herbaceous. Root fusiform to cylindrical, vertical, fleshy, up to 29 × 3 cm. Stems dichotomously branched, slender, 60–130 cm; branches up to 60 cm long, virgate, arranged in a spreading corymb, finely striate. Basal leaves numerous, glaucescent; lamina up to 6 × 6 (–7) cm, herbaceous (not rigid), 3-ternate; ultimate segments filiform, 4–7 × 0.5 mm, acute; petiole stick and flexuous, up to 10 cm long, broadening to a sheath at base. Upper stem leaves much reduced to semiamplexicaul lanceolate sheaths, 0.7 × 0.3–0.4 cm, mucronate to cuspidate, membranous margins, with a glaucous bloom. Umbels 2–4(–5) rayed; rays 4–10 cm, subequal; umbellules 1–1.5 cm diameter, raylets 12–26, glabrous to papillose, up to 3 mm in fruiting. Bracts 0–1, very similar to upper stem leaves, lanceolate-cuspidate, 4–7 × 2–3 mm, membranaceous margin, 8–10 nerved; bracteoles equal or slightly shorter than flowers, 8–9, mm, narrowly elliptic-cuspidate 4–5 × 1–1.5 mm, with membranaceous margin, green, 1 nerved. Calyx teeth minute. Petals broadly elliptic or ovate, 1.8 × 0.7–0.8 mm, tip inflexed, pale whitish yellow and greenish at bottom suffused red at apex, glabrous, dorsal ribs scabrous. Fruits easily separated into homomorphic mericarps, ovoid oblong, 5–6.5 × 2–4 mm, symmetrical with same primary and secondary ribs; mericarps straw colored with 9 gyrose-moniliform wings; ribs width 0.5–0.7 mm, surface reticulate-alveolate, cuticle striate. Exocarp of large cells; mesocarp in ribs consists of parenchymatous cells; vascular bundles compact broad, in the base of each primary rib; commissural vittae 2, dorsal vittae 4, vittae running unbranched the full length of the fruit; endosperm with broad groove at commissural side; stylopodium undulate at the margin. Styles divaricate, 2–3 mm.

Etymology: The species is dedicated to Prof Dr Özcan Seçmen, who was an outstanding botanist and ecologist, especially regarding wetland and conservation biology, for many years in Turkey.

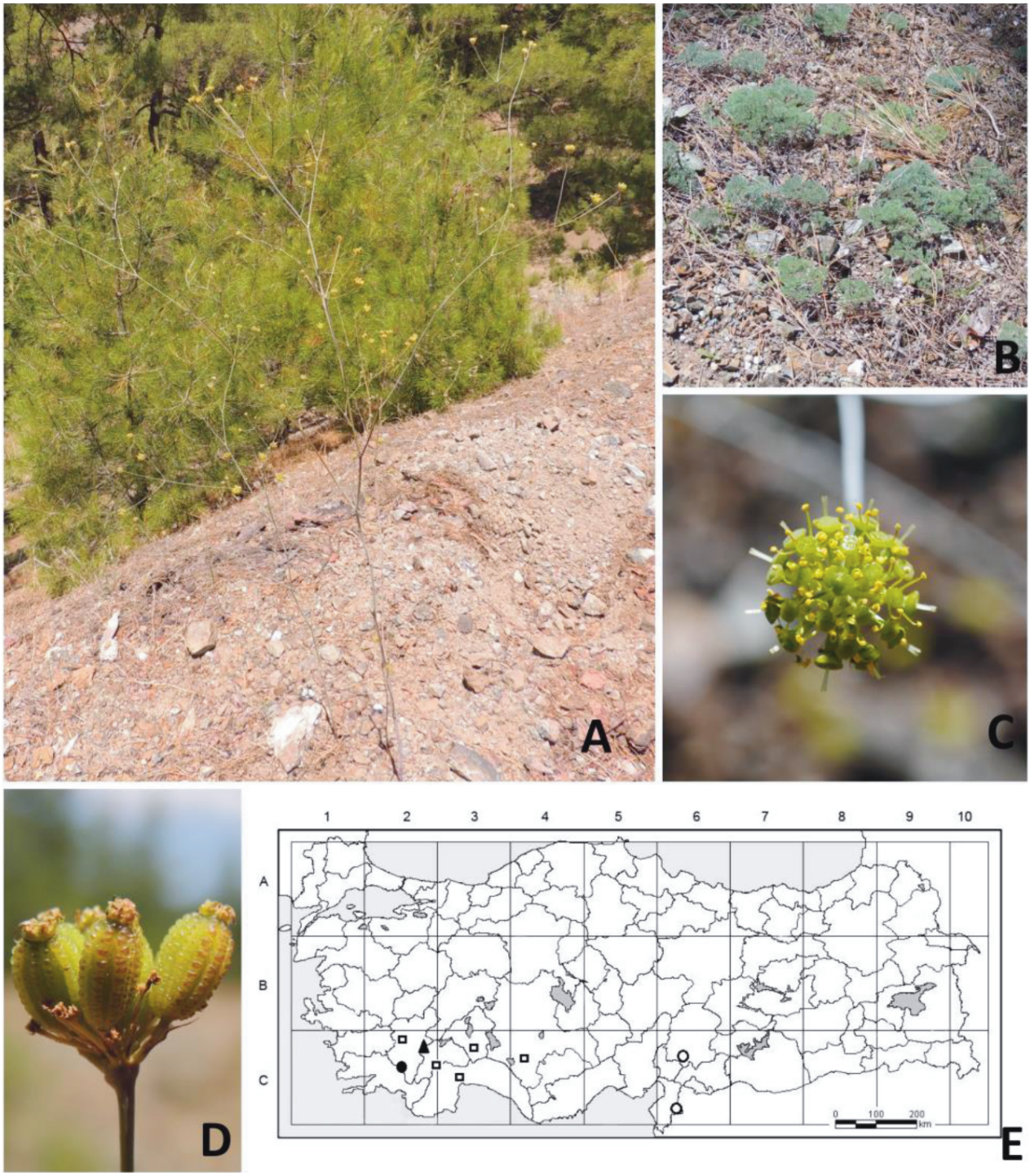

Figure 1. A. *E. ozcan-secmenii* habit form; B. Basal leaves; C. Umbellule; D. Fruits; E. *E. ozcan-secmenii* (●), *E. bornmuelleri* (▲), *L. petrophillium* (□), *L. glaucum* (○) distribution in Turkey.

3.2. Distribution and ecology

The new species is endemic to W Turkey (a small area of Acıpayam region, SW Anatolia), which is a part of the E Mediterranean floristic region. It grows on serpentine areas intermixed with *Pinus brutia* forest at altitudes of 630–700 m a.s.l. The reproductive period starts in June and continues to August.

Suggested conservation status: *E. ozcan-secmenii* is distributed in a very small area. The surface of the habitat is 0.28 km^2, where only 4200 individuals were counted. Of

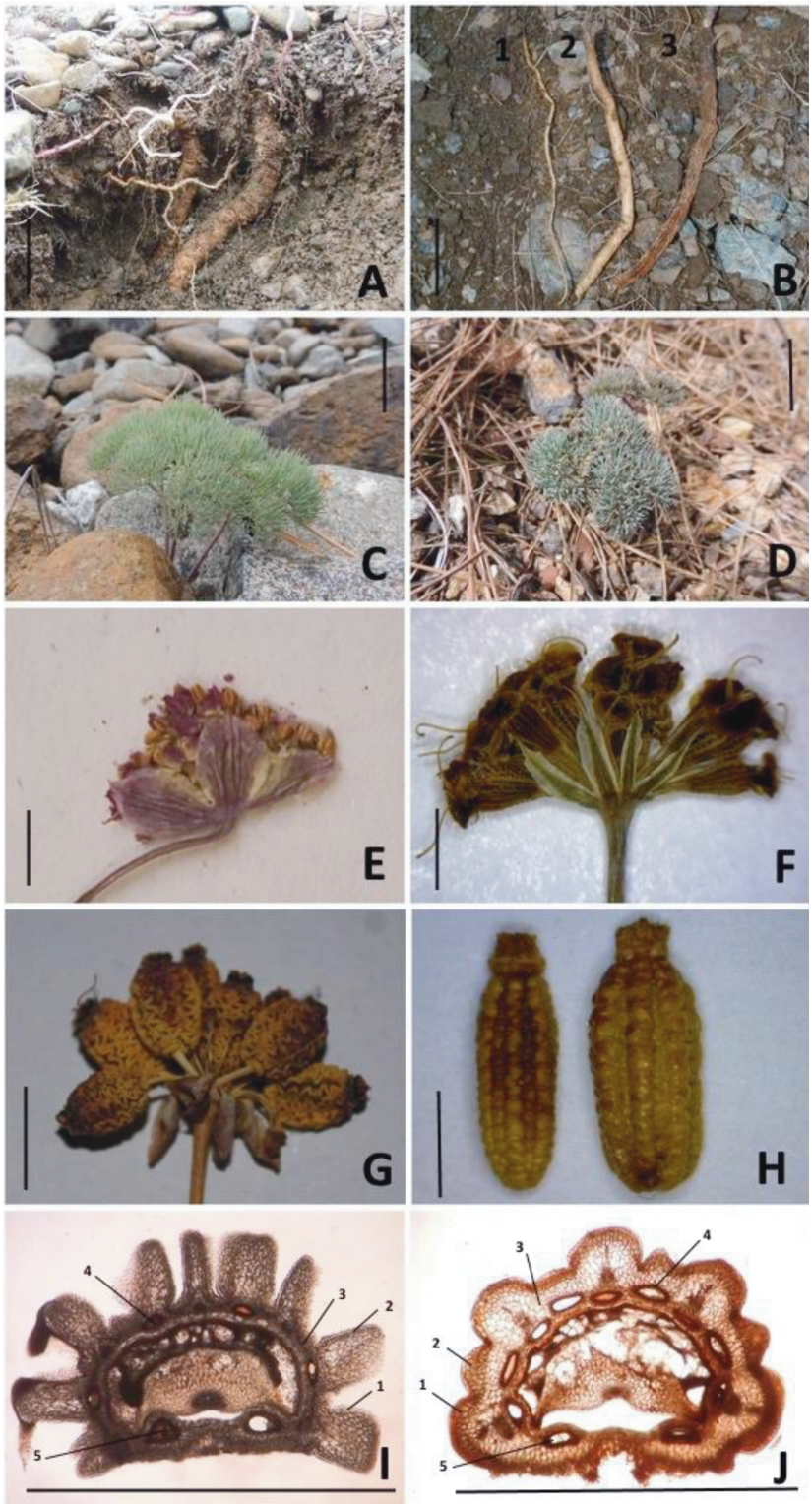

Figure 2. Comparison of morphological and anatomical characters of *E. bornmuelleri* (A. Roots, scale = 10 cm; C. Basal leaf, scale = 5 cm; E. Bracteoles, scale = 5 mm; G. Fruit, (GAZI-H.Duman5071), scale = 5 mm; I. Mericarp cross-section (1. Primary ribs, 2. Secondary ribs, 3. Mesocarp tissue between the primary and secondary ribs, 4. Dorsal vitta, 5. Commisural vitta), scale = 4 mm) and *E. ozcan-secmenii* (B. Roots, scale = 10 cm, (1. 2–3 years old, 2. 5–6 years old, 3. 8–9 years old (dead root after fruiting)); D. Basal leaf, scale = 3 cm; F. Bracteoles, scale = 3 mm; H. Fruit, scale = 3 mm; J. Mericarp cross-section, (1. Primary ribs, 2. Secondary ribs, 3. Mesocarp tissue between the primary and secondary ribs, 4. Dorsal vitta, 5. Commisural vitta), scale = 3 mm).

these, 200 individuals were at the flowering stage, while the rest were at the vegetative stage. The habitat of the new species, Sami Soydam Sandalcık hydroelectric power plant site, can be flooded by the dam lake. Moreover, this area is highly threatened by new road projects from 2016. Based on the data and according to IUCN (version 3.1), the species should be considered in category CR.

3.3. Palynology and micromorphology

The pollen is tricolporate with a tectate perforate surface. Monads are prolate, polar axis (*P*) = 27.6 ± 1 µm, equatorial axis (*E*) = 11.9 ± 1.14 µm, *P/E* = 2.32 (Figure 3A).

Mericarps straw colored, sculpture ornamentation reticulate-alveolate. The ornamentation is characterized by surface cells with mostly polygonal or orbicular walls and many striae between lateral walls (Figures 3B and 3C).

3.4. Molecular phylogeny

Alignment matrices of nrDNA ITS regions of the studied taxa resulted in a total of 525 characters. Of these, 289 characters were preserved, 229 variables, 166 parsimony-informative, and 63 singleton regions. Based on the data, a MP tree with length of 419 was depicted. Bootstrap results (CI = 0.642, RI = 0.868) of 85%–100% were evaluated as strong, 75%–84% as moderate, and 50%–74% as weak (Kress et al., 2002). ML analyses were performed using the Kimura-2 method and the ML tree was analyzed with the highest log value (–2856.13). Based on the results of the conducted analysis, the relationship between *E. bornmuelleri* and *E. ozcan-secmenii* was confirmed. The relationship was measured as MP 88% and ML 82% (BS support values) and analysis data were consistent with each other. At the same time, the affinity of the genus *Ekimia* to *Laserpitium* and *Prangos* was studied using two different methods. These results show that *Ekimia* is more closely related to *Laserpitium* than to *Prangos* (MP 98%, ML 95% BS support values) (Figure 4).

4. Discussion

The genus *Ekimia* was already known as a monotypic and endemic genus in Turkey (Duman and Watson, 1999; Güner et al., 2014). Pollen morphology, fruit anatomy, and some preliminary taxonomic relationships of the genus were previously studied (Pehlivan et al., 2009; Lyskov et al., 2015).

The new species differ from its close affinity, *E. bornmuelleri*, by life form; fruit, petal, and bracteole morphology; and pollen and mericarp features. While *E. ozcan-secmenii* is perennial monocarpic, *E. bornmuelleri* is a perennial polycarpic species (Figures 2A and 2B).

Mericarps of *E. bornmuelleri* are hardly jointed and consist of 9 gyrose-plicate wings while in *E. ozcan-secmenii* are very loose and contain 9 moniliform wings (Figures 2E–2F).

Other notable properties are petals. Petals of *E. ozcan-secmenii* differ in their ovate shape; they have no red tips and have greenish bottoms and scabrous hair on their dorsal surface. In addition, *E. ozcan-secmenii* has 8–9 bracteoles that are 4–5 × 1–1.5 mm in size, one-nerved, with narrowing elliptic shape becoming cuspidate at the apex with hyalinus margins and green color (Table).

The fruit anatomy of the new species also shows some differences with the affinities. Epimesocarp structure in genus *Prangos* species is not observed in *Ekimia* and *Laserpitium*. While vascular bundles in *Prangos* were placed in the inner mesocarp layer, these structures in *Ekimia* and *Laserpitium* were placed at the bottom of primary ribs (Lyskov et al., 2015).

In the new species, the tissue between the primary and secondary ribs is clearly thicker than in *E. bornmuelleri*. The structure of the cuticle in the new species is reticulate-alveolate and striate but in *E. bornmuelleri* it is papillose-tuberculate.

The family Apiaceae is a large family, represented by 455 genera and 3600–3751 species. Of the five subfamilies,

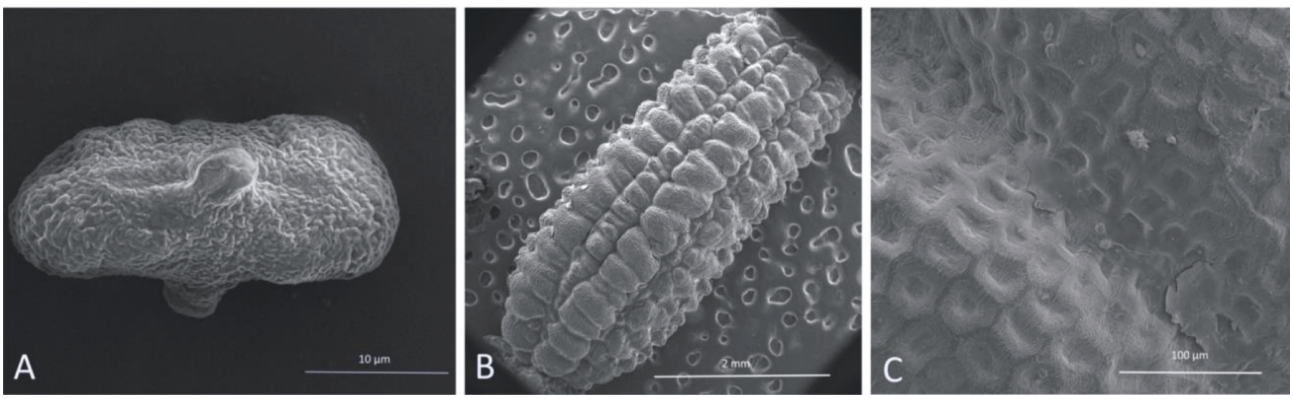

Figure 3. Scanning electron micrograph of *Ekimia ozcan-secmenii*. A. Pollen; B. General view of mature mericarp; C. Details of mericarp surface.

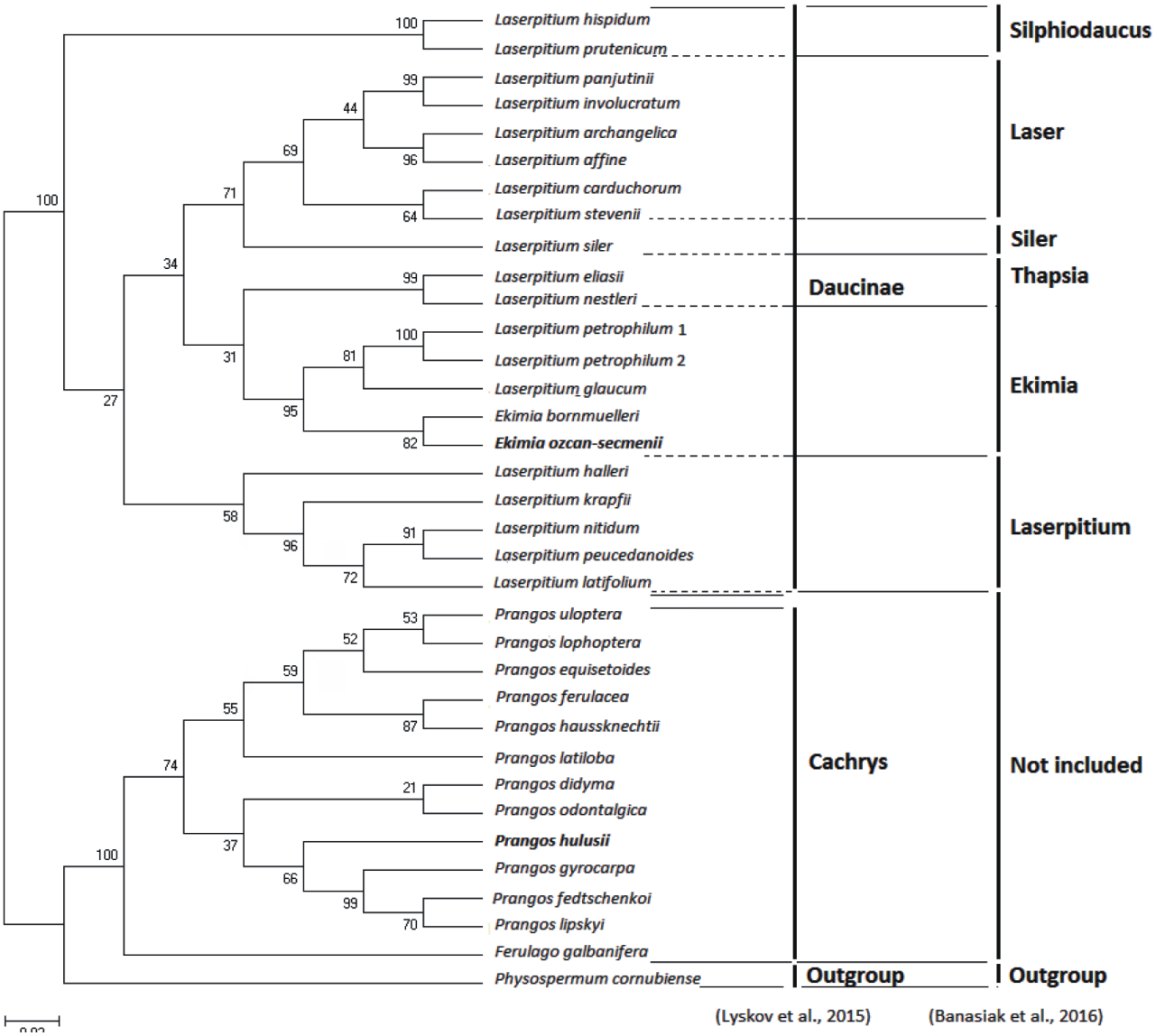

Figure 4. ML phylogenetic tree of the nrDNA ITS nucleotide sequences of *E. ozcan-secmenii* and related species and comparison with previous literature.

Apioideae is the largest one, represented by 404 genera and 2827–2935 species (Pimenov and Leonov, 1993). In recent molecular studies of the subfamily, 41 main clades were identified; among those, 21 of them are viable at tribe or subtribe levels. Traditionally, the genus *Prangos* was placed in the tribe Scandiceae. This genus was considered a close affinity to *Ekimia*, which was placed in the clades Cachrys and *Laserpitium* (Downie et al., 2010). Lyskov et al. (2015), based on ITS data, found that *Ekimia* should be placed in the subtribe Daucinae. In the present study, phylogenetic findings confirmed the relationship between *E. bornmuelleri* and *L. petrophilum*. These data also showed that *E. ozcan-secmenii* was placed in the same clade (subtribe *Daucine*) as *L. petrophilum* and *L. glaucum*. These findings are also supported by the previous results. In addition, *Prangos hulusii* Şenol, Yıldırım & Seçmen showed relationships with other *Prangos* species (Figure 4). Banasiak et al. (2016) found that this species shared the same position as *L. petrophilum* and *L. glaucum* and therefore considered them as *Ekimia petrophila* (Boiss & Heldr) and *E. glauca* (Post) (Banasiak, Baczyński & Spalik, 2016). These taxonomic changes were made based on ML trees obtained through cpDNA and nrDNA, which mapped with mericarp characters (obsolete, winged, spiny, keeled). Nevertheless, *Ekimia*, with its distinct vegetative and reproductive characteristics (i.e. having no petiole

Table. Comparison of diagnostic characters of *E. ozcan-secmenii* and *E. bornmuelleri.*

Characters	*E. ozcan-secmenii*	*E. bornmuelleri*
Habit	monocarpic	polycarpic
Basal leaves dimension (cm)	up to 6 × 6 (–7) cm	up to 10 × 10 cm
Petiole length (cm)	up to 10	up to 15
Stem leaf dimensions (cm)	0.7 × 0.3–0.4	1–2 × 0.4–0.7
Umbels rays (no.; length)	2–4 (–5); 4–10 cm	2–4 (–5); 4–12 cm
Bracteoles shape, nr., dimension (mm), color, venetration	narrowly elliptic, 8-9, 4–5 × 1–1.5, green, 1 nerved	broadly elliptic or orbicular, 5–7, up to 6 × 4; purplish, 5–8 nerved
Flowers/umbellule	12–26	10–15
Fruits shape, dimension (mm), mericarps	ovoid oblong 5–6.5 × 2–4, easily separated	elliptic to ovate, 3–5 × 2.5–4, hardly separated
Mericarps wing and texture	9 moniliform wings, reticulate-alveolate and striate	9 gyrose – plicate wings, papillose tuberculate
Habitat	serpentine slopes in *Pinus brutia* forest, 630–700 m	serpentine slopes in *Quercus* scrub, 1000–1250 m

remnants, ternate blue-green, fleshy leaves, a few long umbel rays, and mericarps with 9 gyrose plicate wings) differs from *Laserpitium* and *Prangos*.

It can be concluded that *Ekimia* and *Laserpitium* are locally isolated. Both species of *Ekimia* dominate the scree slopes; therefore, the genus can be considered a local isolated genus. In contrast, *Laserpitium petrophilum* and *L. glaucum* prefer calcareous and gravel bedrocks. *L. petrophilum*, *E. bornmuelleri*, and *E. ozcan-secmenii* are distributed in transitional zones around Denizli, Turkey (Figure 1). The chain of mountains in this area meets the western Taurus Mountains and diverse bedrock types (serpentine, limestone, dolomite, marble, schist, etc.) in the region. The study suggests the importance of southwestern Anatolian districts of Denizli, Burdur, and Muğla as a biodiversity center.

In brief, the results of this study contradicted previous data on affinity of *L. petrophilum* and *L. glaucum* with the genus *Ekimia*. Therefore, two former species must remain in the genus *Laserpitium*.

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Appendix: List of species, their GenBank accession numbers and/or herbarium specimen numbers used in this study. Data are listed as follows: Taxon name, GenBank accession number or herbarium acronym, herbarium number.

***Ekimia bornmuelleri* (Hub.-Mor & Reese) H.Duman & M.F.Watson:** EGE42340. ***E.ozcan-secmenii* Şenol & Eroglu.:** KY563218, EGE42490. ***Ferulago galbanifera* W.D.J.Koch.:** AF077889. ***Laserpitium affine* Ledeb.:** FJ415151. ***L. archangelica* hort. ex Link.:** FJ415153. ***L. carduchorum* Hedge & Lamond.:** FJ415116. ***L. eliasii* Sennen & Pau.:** FJ415118. ***L. glaucum* L.:** FJ415115. ***L. halleri* Crantz.:** FJ415130. ***L. hispidum* M Bieb.:** FJ415154. ***L. involucratum* Koso-Pol.:** JQ305147. ***L. krapffi* Crantz.:** FJ415124. ***L. latifolium* L.:** FJ415131. ***L. nestleri* Soy.-Will.:** FJ415121. ***L. nitidum* Zanted.:** FJ415132. ***L. panjutinii* (Manden. & Schischk.) M. Hiroe.:** JQ305148. ***L. petrophilum* Boiss. & Heldr. (1):** EGE42255, **(2):** EGE35838. ***L. peucedanoides* Brot.:** FJ415133. ***L. prutenicum* L.:** FJ415156. ***L. siler* L.:** FJ415112. ***L. steveni* Fisch., C.A.Mey. & Trautv.:** FJ415152. ***Prangos didyma* (Regel) Pimenov & V.N.Tikhom.:** KJ395463. ***P. equisetoides* Kuzmina.:** KJ395466. ***P. fedtschenkoi* (Regel & Schmalh.) Korovin.:** KJ395469. ***P. ferulaceae* (L.) Lindl.:** EGE32743. ***P. gyrocarpa* Kuzmina.:** KJ395458. ***P. haussknechtii* Boiss.:** KJ395465. ***P.hulusii* Şenol, Yıldırım & Seçmen.:** KY463268, EGE42338. ***P. latiloba* Korovin.:** KJ395462. ***P. lipskyi* Korovin.:** KJ395453. ***P. lophoptera* Boiss.:** KJ395455. ***P. odontalgica* (Pall.) Herrnst. & Heyn.:** KJ395464. ***P. uloptera* DC.:** EGE28952. ***Physospermum cornubiense* DC.:** AF077904.