

Systematic placement of the Turkish endemic genus *Ekimia* (Apiaceae) based on morphological and molecular data

Dmitry LYSKOV^{1*}, Galina DEGTJAREVA², Tahir SAMIGULLIN³, Michael PIMENOV²

¹Department of Higher Plants, Biological Faculty, Moscow State University, Moscow, Russia

²Botanical Garden, Biological Faculty, Moscow State University, Moscow, Russia

³Department of Evolutionary Biochemistry, A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia

Received: 30.05.2014

Accepted/Published Online: 01.02.2015

Printed: 30.07.2015

Abstract: The systematic position of the monotypic genus *Ekimia* H.Duman & M.F.Watson (Apiaceae), a narrow endemic to Turkey, was evaluated on the basis of morphological data and nrDNA ITS sequences. *Ekimia bornmuelleri* (Hub.-Mor. & Reese) H.Duman & M.F.Watson was initially described in *Prangos* Lindl. Due to the unique fruit morphology uncommon for this genus it was later shifted to an independent genus. In the Bayesian and most parsimonious trees, *E. bornmuelleri* is sister to *Laserpitium petrophilum* Boiss. & Heldr and *Laserpitium glaucum* Post within the Daucinae clade. This result is consistent with its morphology: the presence of the primary and secondary ribs of *E. bornmuelleri* fruits brings the species closer to *Laserpitium* rather than *Prangos*.

Key words: Apiaceae, carpology, Daucinae, *Ekimia*, internal transcribed spacer (ITS), *Laserpitium*, molecular phylogeny, Turkey

1. Introduction

Ekimia bornmuelleri (Hub.-Mor. & Reese) H.Duman & M.F.Watson is a narrow endemic to the Turkish province Burdur in Central Anatolia, where it was collected for the first time in 1938. Huber-Morath and Reese described this species in the genus *Prangos*, due to resemblance of immature fruits to fruits of *Prangos lophoptera* Boiss. (Huber-Morath, 1945). The authors, however, paid attention to notable distinction of this species from the other members of *Prangos*. According to Huber-Morath and Reese, it differs from other congeners by fleshy brush-like blue-green leaves, small number of umbel rays, broadly elliptic to orbicular bracteoles, and winged secondary ribs of immature mericarps. The authors of the taxonomic treatment of *Prangos*, Herrnstadt and Heyn (1972, 1977), adhered to the same opinion; they expressed reasonable doubts about the generic attribution of this species based on the morphology of fruits, umbels, and leaves, which is not typical to *Prangos*. Although Herrnstadt and Heyn considered that this species should be excluded from *Prangos*, they indicated that a study of mature fruits was needed to decide on the generic placement of the species.

Duman and Watson (1999) collected new material

with mature fruits and well-developed flowers during their fieldwork in Turkey. They examined the cross-sections of mature fruits, presented a detailed description of plant morphology, and placed the species in the independent monotypic genus *Ekimia*, noting its similarity to *Prangos*. The difference between genera was supported by the analysis of pollen morphology (Pehlivan et al., 2009).

The objectives of the present research were to: (1) reassess in detail the carpological characters of *Ekimia*; and (2) ascertain its phylogenetic placement using sequences of nrDNA ITS, since it has been shown to be the most suitable instrument to clarify relationships of genera and species in Apiaceae (Spalik and Downie, 2007; Downie et al., 2010).

2. Materials and methods

2.1. Morphology and micromorphology

Four species from three genera were chosen for carpological analysis. *Prangos ferulacea* (L.) Lindl. is the type species of the genus *Prangos* and has the typical anatomy of mericarps for this genus. *Prangos lophoptera* is a species with which *E. bornmuelleri* was initially compared (Huber-Morath, 1945). *Laserpitium petrophilum* and *Laserpitium glaucum*

* Correspondence: df.lyskov@yandex.ru

Post form the clade with *Ekimia* in molecular analysis and so the choice of *L. petrophilum* for carpological analysis was justified by the results of our preliminary molecular studies. Close examination of *L. glaucum* Post would have been more desirable, but we failed to obtain specimens of this species. The material was collected during our expedition to Turkey or taken from herbaria; the origin of the material is indicated in Figure 1. Fruits were examined under a light microscope. Mericarps were crosscut in the middle with a hand razor and cross-sections were then treated with phloroglucinol (Erdmann et al., 1986). We used standard terms to describe the fruit and other parts of the plant (Kljuykov et al., 2004). Microstructure of the fruit surface was studied by scanning electron microscopy (SEM). Fruits were coated with a 25-nm layer of Au-Pd using an Eiko IB-3 sputter coater. Micrographs were taken at 15 kV using a CamScan S-2 microscope at Moscow State University. Micromorphological features were described in the terms used by Ostroumova et al. (2010).

2.2. Molecular phylogenetic analyses

For molecular phylogenetic study, nrDNA ITS sequences of *Ekimia bornmuelleri* and a close relative to *Prangos*, *Bilacunaria microcarpa* (M.Bieb.) Pimenov & V.N.Tikhom., were generated and analyzed along with a selection of sequences retrieved from GenBank. Total DNA was extracted from a herbarium specimen using a NucleoSpin Plant II kit (Macherey-Nagel, Germany) according to the protocol. Primers and PCR conditions conformed to those described in Valiejo-Roman et al. (2002). PCR products were purified using the DNA cleaning kit (Evrogen, Moscow, Russia) as indicated by the manufacturer's instructions. Direct sequencing was performed on an automated DNA sequencer ABI Prism 3100-Avant (Applied Biosystems, Foster City, CA, USA) using an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit for cycle sequencing reactions in accordance with the manufacturer's instructions. The newly obtained sequences were deposited in GenBank (see Appendix; on the journal's website).

The initial set of taxa to be compared with *Ekimia* was selected using the BLAST option of the GenBank database. This search suggested that taxa belonging to the clade currently known as Daucinae (Downie et al., 2010) were most alike. The coherence in nrDNA ITS sequences was highest between *Ekimia* and *Laserpitium* species with 95%–93% values of identity. Other species from the Scandiceae and Cachrys clade were appended to the matrix to demonstrate relationships between groups. After preliminary analysis, a dataset of 99 species (see GenBank numbers in Appendix) including representatives of *Laserpitium*, *Prangos*, and allied taxa was compiled.

The trees were rooted with *Physospermum cornubiense* DC. in reliance upon the results obtained previously (Downie et al., 2010). A total set of 100 species, including an outgroup, were analyzed. GenBank accession numbers for the studied taxa are listed in the Appendix. Sequences were aligned using MUSCLE (Edgar, 2004) followed by manual adjustment in BioEdit (version 5.0.9; Hall, 1999). The nrDNA ITS data matrix was analyzed using both maximum parsimony (MP) and Bayesian inference (BI).

Maximum parsimony (MP) analyses were performed using PAUP* (version 4.0b08, Swofford, 2003) with equal weighting of characters and TBR branch swapping. For each heuristic search, 1000 random sequence additions replicates were run and all the shortest trees were saved. Bootstrap analysis (Felsenstein, 1985) was performed to assess the degree of support for particular branches on the tree, and bootstrap values were calculated using 1000 replicates with TBR branch swapping and random addition of taxa; the one thousand most parsimonious trees from each replicate were saved. For assessment of bootstrap support (BS) we considered 85%–100% as strong, 75%–84% as moderate, and 50%–74% as weak (Kress et al., 2002).

A Bayesian analysis was conducted using MrBayes (version 3.2.2; Ronquist et al., 2012) with the GTR+G model. The model was selected by the Akaike Information Criterion using MrModeltest (Nylander, 2004). The analysis was performed with two parallel runs; four Markov chains were used for each run. 20,000,000 generations were performed; trees were sampled every 1000 generations. The number of generations to be discarded was determined using the cold chain log likelihood examination in Tracer v. 1.5 (Rambaut and Drummond, 2007). After discarding the first 500 (2.5%) trees as burn-in, the remaining trees were used for building the majority-rule consensus tree to provide posterior probabilities (PP). Because PP in Bayesian analyses are not equivalent to BS and are generally much higher (Ericson et al., 2003), we interpreted values >0.95 PP as a strong support. In addition, AWTY (Nylander et al., 2008) was used to verify topological convergence among chains.

In all analyses, gaps (indels) were treated as missing data. Visualization of trees was performed by TreeView (Page, 1996).

3. Results

3.1. Morphological and anatomical study

Below we provide a detailed description of the morphology and anatomy of *E. bornmuelleri* and some representative species of *Prangos* and *Laserpitium* for comparison. Key carpological characteristics are listed in the Table.

Table. Carpological characters that differentiate the four studied species.

| | <i>Ekimia bornmuelleri</i> | <i>Laserpitium petrophilum</i> | <i>Prangos ferulacea</i> | <i>Prangos lophoptera</i> |
|--------------------------------------|--|---|---|---|
| Fruits (separation at maturity) | hardly separated into mericarps | separated into mericarps | separated into mericarps | separated into mericarps |
| Fruits (shape/outline) | elliptic to ovate | elliptic to ovate | ovate to broad- ovate | narrowly-ovate to cylindrical |
| Mericaip length, mm | 3–6 | 3.5–6 | 15–18 | 5–12 |
| Mericaips width, mm | 2–4 | 2–3 | 12–15 | 2–5 |
| Carpophore | entire | entire | bifurcate down to the base | bifurcate to the base |
| Mericaips (in cross-section) | slightly compressed dorsally | slightly compressed dorsally | terete | slightly compressed dorsally |
| Mericaip ribs | primary and secondary ribs | primary and secondary ribs | only primary ribs | only primary ribs |
| Shape of ribs | all ribs are alike, winged and wavy | primary ribs are keeled, secondary ribs are winged | all ribs are alike, winged and wavy | all ribs are alike, winged and wavy |
| Shape of stylopods | flat with wavy margin | conical | flat | flat |
| Exocarp | of large cells | of large cells | of small cells | of small cells |
| Mesocarp tissue | in ribs consisting of parenchymatous lignified cells | in secondary ribs consisting of parenchymatous lignified cells | divided into outer “epimesocarp” and inner mesocarp | divided into outer “epimesocarp” and inner mesocarp |
| Vascular bundles | compact, broad, placed in the base of each primary rib | compact, broad, placed in the base of each primary rib | thin, situated in the inner mesocarp layer | thin, situated in the inner mesocarp layer |
| Secretory ducts/ vittae | rib secretory ducts placed in the bases of secondary rib; two commissural vittae | rib secretory ducts placed in the bases of secondary ribs; two commissural vittae | vittae multiple, near endocarp, vallecular and commissural vittae, no rib secretory ducts | vittae multiple near endocarp, vallecular and commissural vittae absent; rib secretory ducts solitary |
| Endosperm shape | with broad groove at commissural side | with broad groove at commissural side | with mushroom-like groove on commissural side | with mushroom-like groove on commissural side |
| Cell borders on the mericaip surface | indistinct | distinct | distinct | distinct |
| Cuticle | papillose tuberculate | striate, seldom knotted | smooth, rare striate | striate, rare smooth |
| Epicuticular secretions | present | present | absent | absent |

Ekimia bornmuelleri (Hub.-Mor. & Reese) H.Duman & M.F.Watson, 1999, Edinb J Bot 56: 200. (Figures 1A–1C). Polycarpic herbaceous plant with basal and reduced cauline leaves. Caudex without branches, rarely with short branches. Stems up to 150 cm tall and up to 3.5 mm in diameter at the base, solid, erect, terete, striate, glabrous, with dichotomous branches in the middle or upper stem part. Basal leaves 10–15 cm long, with flattened petioles, emarginate at adaxial side, broadened at the base, gradually merging into sheaths; leaf blades ovate or rhombic, 3–4 ternately divided into filiform lobes, glabrous. Ultimate segments about 10 mm long and 0.5 mm wide. Primary and secondary segments of the leaf usually petiolulate. Upper leaves reduced to lanceolate, cuspidate sheaths with membranous margins. Umbels with 2–4 equal, terete, glabrous rays 4–10 cm long, 0–2 glabrous bracts similar to upper leaves. Umbellets with 10–15 flowers and 5–7

lanceolate or elliptic glabrous, cuspidate bracteoles with membranous margins. Pedicels papillose, 2–3 mm in fruit. Calyx teeth inconspicuous. Petals yellow. Fruits not separating into mericarps; elliptic to ovate; 3–6 mm long; 2–4 mm wide; carpophore entire; beak absent; mericarps homomorphic, glabrous, slightly compressed dorsally, with primary and secondary ribs; all ribs are alike, winged and wavy, with entire margin; stylopods flat with wavy margin; commissure of intermediate width. Cell borders of mericaip surface indistinct, hairs and stomata absent. Cuticle papillose tuberculate. Epicuticular secretions are present. Exocarp of large cells; mesocarp in ribs consists of parenchymatous lignified cells, other parts of mesocarp are nonlignified; vascular bundles compact, broad, in the base of each primary rib; rib secretory ducts broad, in the base of each secondary rib; two commissural vittae; other vittae solitary, small, usually situated in primary ribs lateral

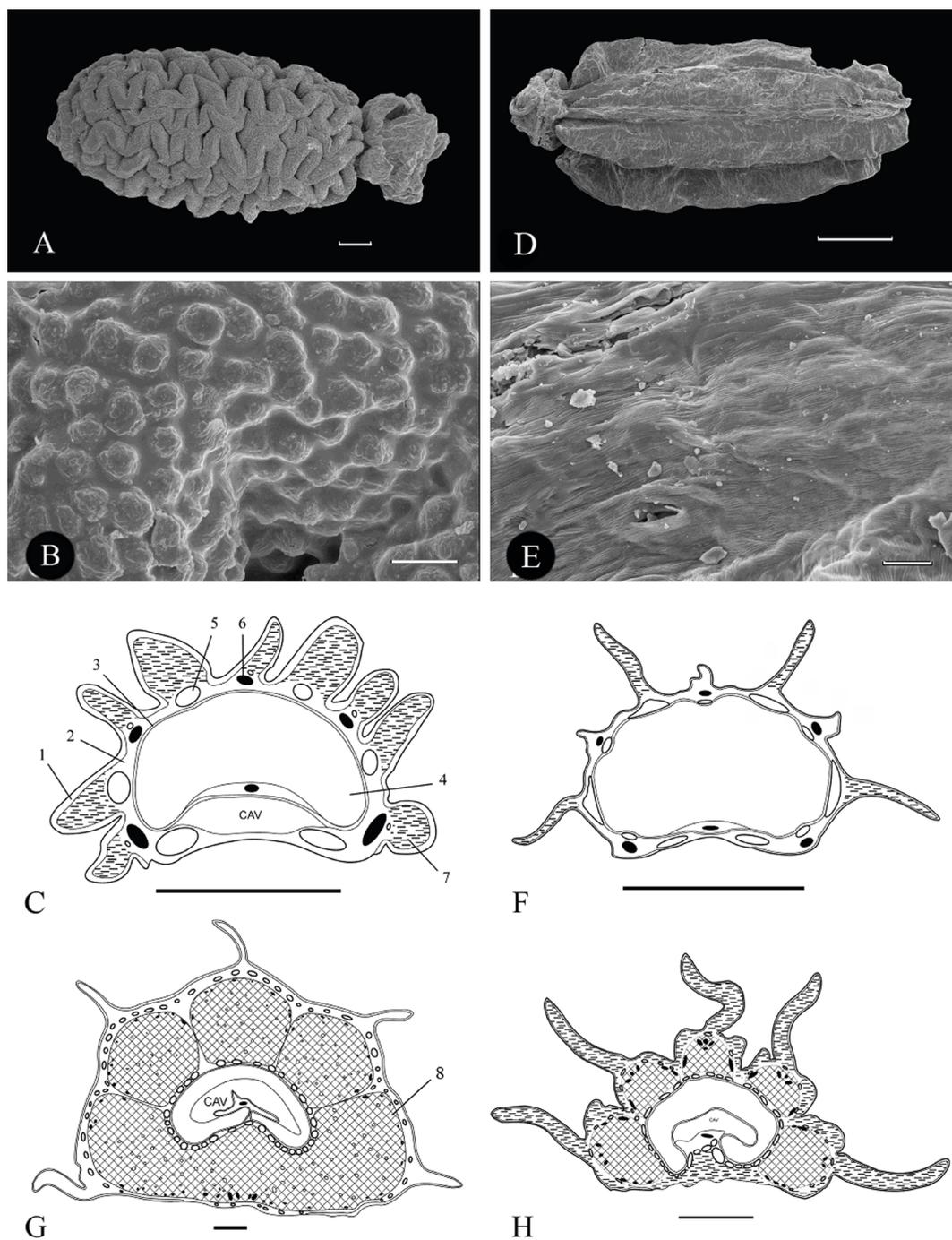


Figure 1. Fruit morphology and anatomy of the studied species. A–C: *Ekimia bornmuelleri* (Pimenov & Kljuykov 65, MW, Turkey, Burdur Province, 03.08.2007). (A) general view of mature mericarp, scales = 300 μ m, (B) details of fruit surface in the middle part of the fruit (SEM), scale = 30 μ m, and (C) transect of mericarp, scale = 1 mm. D–F: *Laserpitium petrophilum* (Hartwig 23613, EGE, Turkey, Antalya, Tahtali Dag). (D) general view of mature mericarp, scales = 1000 μ m, (E) details of fruit surface in the middle part of the fruit (SEM), scale = 30 μ m, and (F) transect of mericarp, scale = 1 mm. G: *Prangos ferulacea* (Pimenov & Kljuykov 79, MW, Turkey, Konya Province, 15.08.2008), transect of mericarp, scale = 1 mm. H: *Prangos lophoptera* (Pimenov et al. 276, MW, Armenia, Erevan prov., 07.06.1977), transect of mericarp, scale = 1 mm. 1 - exocarp, 2 - mesocarp, 3 - endocarp, 4 - endosperm, 5 - secretory duct, 6 - vascular bundle, 7 - parenchyma cells of mesocarp with lignified pitted walls, 8 - mesocarpic aerenchyma, CAV - seed cavity.

of vascular bundles; endocarp and spermoderma of small cells; crystals in pericarp absent; endosperm with broad groove at commissural side.

Laserpitium petrophilum Boiss. & Heldr., 1849, Diagn. Pl. Orient. ser. 1, 10: 46. (Figures 1D–1F). Polycarpic herbaceous plant with basal and cauline leaves. Caudex without branches, rarely with short branches. Stems up to 80 cm tall and to 3 mm in diameter at the base, solid, erect, terete, striate, glabrous, with dichotomous branches in the middle or upper stem part. Basal leaves 5–20 cm long, with flattened petioles, emarginate at adaxial side, broadened at the base, gradually merging into sheaths; leaf blades ovate or rhombic, 3 pinnate or ternate, ultimate segments elliptic to ovate, 10–15 mm long and 3–6 mm wide, divided into lanceolate lobes, glabrous. Leaf primary and secondary segments usually petiolulate. Upper leaves reduced to lanceolate, cuspidate sheaths with membranous margins about 1.5–2.5 cm long. Umbels with 7–12 equal, terete, glabrous rays, 3–5 cm long, with 5–7 glabrous bracts similar to upper leaves. Umbellets with 5–15 flowers and 5–7 lanceolate or linear-lanceolate cuspidate bracteoles with hairs on membranous margins. Pedicels papillose, 2–3 mm in fruit. Calyx teeth inconspicuous. Petals yellow. Fruits divided into mericarps; elliptic or ovate; 3.5–6 mm long; 2–3 mm broad; carpophore entire; beak absent; mericarps homomorphic, glabrous, slightly compressed dorsally, with primary and secondary ribs; primary ribs are keeled, secondary ribs are winged, with entire margin; stylopods conical; commissure of intermediate width. Cell borders of mericarp surface distinct only on secondary wings and in surface depressions, hairs and stomata absent. Cuticle on primary ribs striate, seldom knotted, epicuticular secretions are present. The cells on the surface of secondary ribs and depressions are isodiametric; anticlinal walls convex, outer walls concave, seldom flat. Cuticle smooth, seldom knotted. Epicuticular secretions are present in depressions of the surface. Exocarp of large cells; mesocarp in secondary ribs consist of parenchymatous lignified cells, other parts of mesocarp are nonlignified; vascular bundles compact, broad, situated in the base of primary ribs; rib secretory ducts broad, situated in the base of secondary ribs; two commissural vittae; other vittae solitary, large, usually situated in primary ribs medial of vascular bundles; endocarp and spermoderma of small cells; crystals in pericarp absent; endosperm with broad groove at commissural side.

Prangos ferulacea (L.) Lindl., 1825, Quart. J. Sc. 19:7. (Figure 1G). Polycarpic herbaceous plant with basal and reduced cauline leaves. Caudex without branches, seldom

with short branches and covered with petiole remains, with thick tap-root. Stems up to 150 cm tall and 2 cm in diameter at the base, solid, erect, terete, minutely striate, glabrous, with branches, in the middle or upper stem parts. Basal leaves 30–50 cm long, with flattened petioles, emarginate at adaxial side, broadened at the base, gradually merging into sheaths; leaf blades obovate to ovate, 3–4 pinnate, glabrous, divided into filiform lobes. Leaf primary segments petiolulate. Umbels with 12–16 equal, terete, glabrous rays, 8–10 cm long, with 5–8 glabrous bracts similar to upper leaves. Umbellets with 10–15 flowers and 5–7 lanceolate or linear-lanceolate cuspidate and glabrous bracteoles. Pedicels glabrous, 2–3 mm. Calyx teeth inconspicuous. Petals yellow. Fruits divided into mericarps; ovate or broad-ovate; 15–18 mm long; 12–15 mm wide; carpophore bifurcate down to the base; beak absent; mericarps homomorphic, terete, elliptic, glabrous; only primary ribs are present; ribs are equal, winged and wavy, with entire margin; furrows between ribs broad, stylopods flat; commissure of intermediate width. Cell borders of mericarp surface distinct, hair and stomata absent. Cells area in outline shape isodiametric or elongated; anticlinal walls convex; outer walls flat, seldom concave. Cuticle smooth, seldom striate. Exocarp of small cells; mesocarp divided into an outer “epimesocarp” and inner mesocarp; inner mesocarp divided into 5 completely separated parts; inner mesocarp consist of parenchymatous cells with lignified walls; “epimesocarp” consist of nonlignified cells; vascular bundles thin, situated in the inner mesocarp layer; vittae broad, multiple, forming cycle near endocarp, vallecular and commissural vittae thin, multiple; rib secretory ducts absent; endocarp and spermoderma of small cells; endosperm with mushroom-like groove at commissural side.

Prangos lophoptera Boiss., 1844, Ann. Sci. Nat., sér. 3, Bot. 2: 82. (Figure 1H). Polycarpic herbaceous plant with basal and reduced cauline leaves. Caudex without branches, rarely with short branches and covered with petiole remains, with thick tap-root. Stems up to 100 cm tall and to 8 mm in diameter at the base, solid, erect, terete, minutely striate, glabrous, with branches in the upper part. Basal leaves 35–60 cm long, with flattened petioles, emarginate at adaxial side, broadened at the base, gradually merging into sheaths; leaf blades obovate to triangular in outline, 3–4 pinnate, glabrous, divided into narrowly linear lobes. Leaf primary segments petiolulate. Umbels with 12–15 equal, terete, glabrous rays up to 4 cm long, 6–9 glabrous bracts similar to upper leaves. Umbellets with 8–10 flowers and 5–7 filiform or linear-lanceolate

glabrous bracteoles. Pedicels glabrous, 5–10 mm long. Calyx teeth inconspicuous. Petals yellow. Fruits are divided into mericarps (Figure 1 G); narrowly-ovate to cylindrical; 5–12 mm long; 2–5 mm wide; carpophore bifurcate down to the base; beak absent; mericarps homomorphic, slightly compressed dorsally, lanceolate, glabrous; only primary ribs present; ribs are equal, winged and wavy, with entire margin; furrows narrow, with small outgrowths; stylopods flat; commissure of intermediate width. Cell borders of mericarp surface distinct, hairs and stomata absent. The surface of cells isodiametric or elongated; anticlinal walls convex or with narrow groove; outer walls concave, seldom flat. Cuticle striate, seldom smooth. Exocarp of small cells; mesocarp divided into an outer “epimesocarp” and inner mesocarp; inner mesocarp divided into 5 parts, fully separated; inner mesocarp consist of parenchymatous cells with lignified walls; “epimesocarp” consist of nonlignified cells in proximal layer and of large cells with lignified porous walls in distal layers and ribs; vascular bundles thin, situated in the inner mesocarp layer; vittae broad, multiple, forming cycle near endocarp, vallecular vittae thin, multiple; commissural vittae absent; rib secretory ducts solitary, small, present in all ribs; endocarp and spermoderma of small cells; endosperm with mushroom-like groove at commissural side.

The results of the morphological study highlight the difference between *Ekimia* and *Prangos*. The most demonstrative features were found in vegetative parts. The small number of rays and particular bracts and bracteoles with membranous margins separate *Ekimia* from both *Prangos* and *Laserpitium*. Sharp distinctions were also found in key characteristics of their fruits. Endosperms of *Ekimia bornmuelleri* and *Laserpitium petrophilum* have a broad groove on the commissural side, whereas the groove on this side in *Prangos* species is mushroom-like. Mesocarp tissue of *P. ferulacea* and *P. lophoptera* split into “epimesocarp” and inner mesocarp with different structures. *Ekimia* and *Laserpitium* lack such bipartition. Vascular bundles of *Ekimia* and *Laserpitium* are compact and located only at the base of each primary rib, whereas *Prangos* species have thin vascular bundles placed cyclically in the inner mesocarp layer.

Secretory ducts of *Ekimia* and *Laserpitium* run at the base of each secondary rib; there are two commissural vittae. By contrast, *Prangos* species have cyclic vittae near endocarp and in mesocarp, vallecular and commissural vittae, and lack rib secretory ducts. *Prangos* species have no secondary ribs, which are present in *Laserpitium* and *Ekimia*.

3.2. Molecular phylogenetic analysis

The aligned matrix of nrDNA ITS data had 629 characters; 46 ambiguous and gap-rich positions were excluded, 305 positions were parsimony-informative, 224 characters were constant, and 54 variable, but parsimony-uninformative. Maximum parsimony analyses recovered 956 shortest trees of 761 steps (CI = 0.391, RI = 0.803). Because tree topologies do not contradict each other, we present here only the Bayesian tree (Figure 2).

The topologies of both MP and BI trees strongly support affinity of *Ekimia bornmuelleri* to *Laserpitium*, but not to *Prangos* species. *Ekimia bornmuelleri* groups with *Laserpitium glaucum* and *L. petrophilum* with high support (BS 100%, PP 1.00), this clade being nested within the Daucinae clade. The remaining *Laserpitium* species form a few separate clades of unresolved relationship.

The genus *Prangos* forms a separate clade (BS 84%, PP 1.00) both in the Bayesian and parsimony analyses, where it is nested together with the related genera *Bilacunaria*, *Cachrys*, *Ferulago*, and *Diplotaenia*. All these species form a strongly supported group designated as the Cachrys clade according to Downie et al. (2010).

4. Discussion

Our studies of carpology and nrDNA ITS analysis have shown that *Ekimia* is a close relative to *Laserpitium* rather than to *Prangos*. This conclusion agrees with the results of the morphological analysis that showed dissimilarity between *E. bornmuelleri* and *Prangos* species in key carpological characteristics. The fruit structure of both *Prangos ferulacea* and *P. lophoptera* is entirely different. In addition, *Ekimia* lacks specific bipartition of mesocarp into “epimesocarp” and inner mesocarp, which is a unique feature for fruits of the Cachrys clade species.

Carpological features favor the placement of *Ekimia* in the Daucinae clade, since all its members have secondary ribs, which is a unique trait of the representatives of the Torilidinae and Daucinae clades with few exceptions. *Laserpitium petrophilum*, a close relative of *Ekimia* according to nrDNA ITS data, shows endosperm of similar shape. Moreover, the size of commissure and exocarp cells, as well as the number, size, and locality of vascular bundles and main secretory ducts in mericarps of these two species are identical. It should be noted that these characters are of special taxonomic importance (Kljuykov et al., 2004). In addition, the geographical distribution of *E. bornmuelleri* matches that of *L. petrophilum*.

In vegetative and flowering parts, *Ekimia* has unique traits, such as fleshy blue-green leaves, small number of

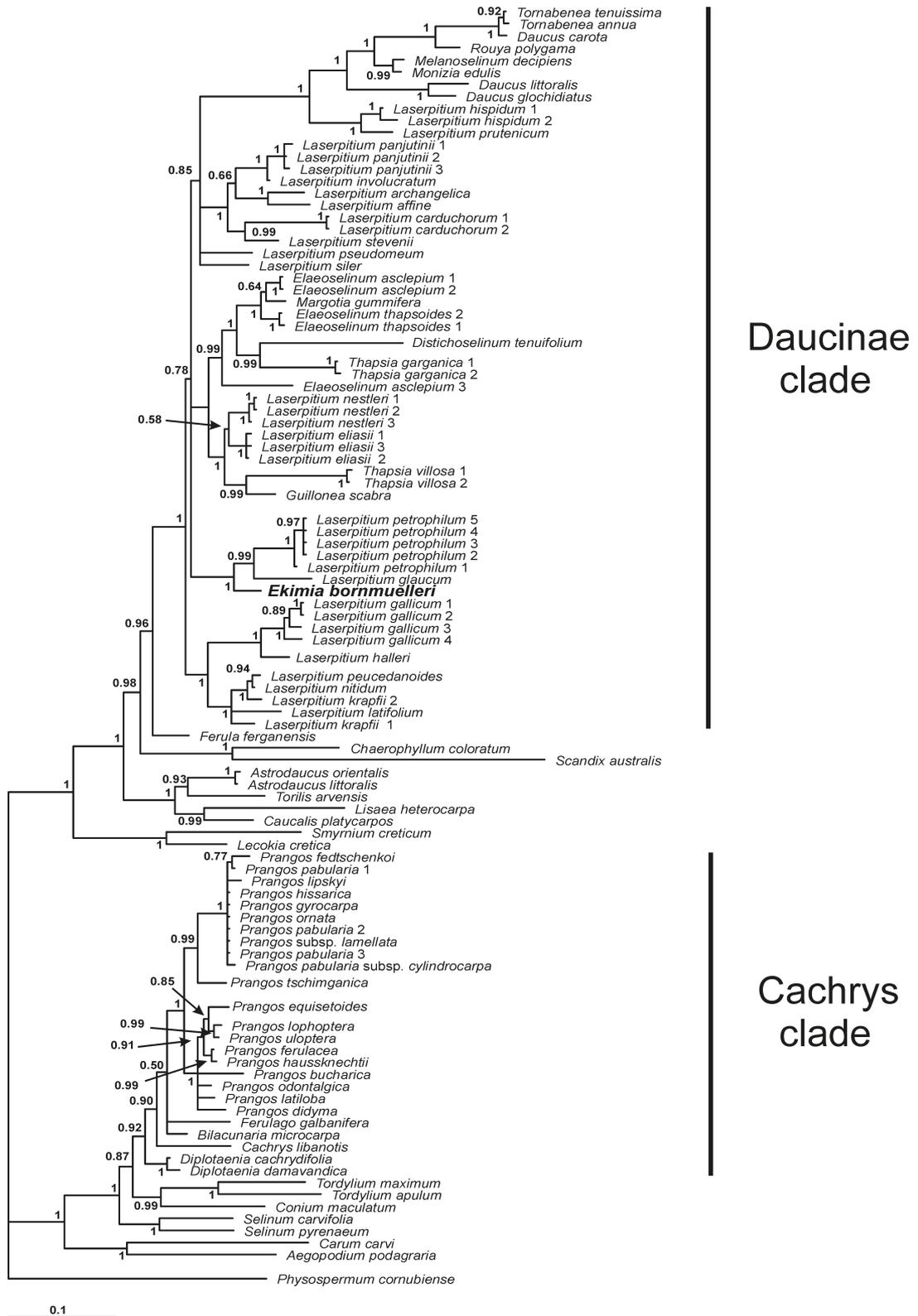


Figure 2. Bayesian phylogenetic tree of the nrDNA ITS nucleotide sequences of the studied Apiaceae taxa. Posterior probabilities higher than 0.5 are shown on the corresponding branches.

umbel rays, and broadly elliptic to orbicular bracteoles, which are different from those found in *Laserpitium* and *Prangos*. *Ekimia* fruit surfaces also show unique features as papillose tuberculate cuticle and presence of epicuticular secretions, which are not characteristic of *Prangos* or *Laserpitium* (see results for a broader description of the four studied *Ekimia*, *Prangos*, and *Laserpitium* species).

Thus, available molecular and morphological data provide ample evidence against any close affinity between *Ekimia* and *Prangos*. However, *Laserpitium petrophyllum* has never been considered to be a close relative of *Ekimia*. Molecular analysis evidences the paraphyly of the genus

Laserpitium; other *Laserpitium* species could be close relatives to *Ekimia*. *Laserpitium gallicum* (the type of genus *Laserpitium*) falls in another clade of the tree. Further investigation with the use of broad sampling of *Laserpitium* taxa would be required to specify the taxonomic status of *Ekimia* and its allies.

Acknowledgements

The study was carried out with the support of the Russian Foundation for Basic Research grant N 13-04-00648. We are grateful for suggestions made by the reviewers and editor.

References

- Downie SR, Spalik K, Katz-Downie DS, Deborah S, Reduron J-P (2010). Major clades within *Apiaceae* subfamily *Apioideae* as inferred by phylogenetic analysis of nrDNA ITS sequences. *Plant Syst Evol* 128: 111–136.
- Duman H, Watson MF (1999). *Ekimia*, a new genus of *Umbelliferae*, and two new taxa of *Prangos* Lindl. (*Umbelliferae*) from southern Turkey. *Edinburgh Journal of Botany* 56: 199–209.
- Edgar RC (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32: 1792–1797.
- Erdmann B, Hoffann P, Wiedenroth EM (1986). Changes in the root system of wheat seedlings following root anaerobiosis I. Anatomy and respiration in *Triticum aestivum* L. *Ann Bot* 58: 597–605.
- Ericson P, Sennblad B, Britton T, Oxelman B (2003). Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic Biol* 52: 665–673.
- Felsenstein J (1985). Confidence limits on phylogenetics: an approach using the bootstrap. *Evolution* 39: 783–791.
- Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acid S* 41: 95–98.
- Herrnstadt I, Heyn CC (1972). *Prangos* Lindl. In: Davis PH, editor. *Flora of Turkey and the East Aegean Islands*. Vol. 4. Edinburgh, Scotland: University Press, pp. 382–388.
- Herrnstadt I, Heyn CC (1977). A monographic study of the genus *Prangos* (*Umbelliferae*). *Boissiera* 26: 1–91.
- Huber-Morath A (1945). *Novitae Elorae Anatolicae* IV. *Candollea* 10: 147–154 (article in German).
- Kljuykov EV, Liu M, Ostroumova TA, Pimenov MG, Tilney PM, van Wyk B-E (2004). Towards a standardized terminology for taxonomically important morphological characters in the *Umbelliferae*. *S Afr J Bot* 70: 488–496.
- Kress WJ, Prince LM, Williams KJ (2002). The phylogeny and a new classification of the gingers (*Zingiberaceae*): evidence from molecular data. *Am J Bot* 89: 1682–1696.
- Nylander JAA (2004). MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Nylander JAA, Wilgenbusch JC, Warren DL, Swofford DL (2008). AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* 24: 581–583.
- Ostroumova TA, Pimenov MG, Ukrainskaja UA (2010). Micromorphological diversity of hairs and emergences on fruits in the *Umbelliferae* and its taxonomic value. *Botanicheskii Zhurnal* 95: 1219–1231 (article in Russian with an abstract in English).
- Page RDM (1996). TREEVIEW: An application to display phylogenetic trees on personal computers. *Comput Appl Biosci* 12: 357–358.
- Pehlivan S, Başer B, Cabi E (2009). Pollen morphology of 10 taxa belonging to *Prangos* Lindl. and *Ekimia* H. Duman & M.F. Watson (*Umbelliferae*) from Turkey and its taxonomic significance. *Bangl J Plant Taxon* 16: 165–174.
- Rambaut A, Drummond AJ (2007). Tracer. Website: <http://beast.bio.ed.ac.uk/tracer> [accessed 22 September 2014].
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. *Systematic Biol* 61: 539–542.
- Spalik K, Downie SR (2007). Intercontinental disjunctions in *Cryptotaenia* (*Apiaceae*, *Oenantheae*): an appraisal using molecular data. *J Biogeogr* 34: 2039–2054.
- Swofford DL (2003). PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods): Version 4. Sunderland, MA, USA: Sinauer Associates.
- Valiejo-Roman CM, Terentjeva EI, Samigullin TH, Pimenov MG (2002). Relationships among genera in *Saniculoideae* and selected *Apioideae* (*Umbelliferae*) inferred from nrITS sequences. *Taxon* 51: 91–101.

Appendix. List of species and their GenBank accession numbers (ITS nrDNA sequences) used in this study. Voucher data are given for the two accessions studied by the authors. Data are listed as follows: Taxon name, country, province, collector(s), herbarium acronym, collector's number, GenBank accession number. For the samples from GenBank only accession numbers are given.

Aegopodium podagraria L.: JQ792200. *Astrodaucus littoralis* Drude: FJ415109. *A. orientalis* Drude: FJ415108. *Bilacunaria microcarpa* (M.Bieb.) Pimenov & V.N.Tikhom.: Iran, prov. Theran, M.G. Pimenov, E.V. Kljuykov, A.K. Sytin, F. Ghahremani-nejad, MW-86, KJ700298. *Cachrys libanotis* L.: KJ395460. *Caucalis platycarpus* L.: FJ415106. *Carum carvi* L.: JQ792211. *Chaerophyllum coloratum* L.: FJ415105. *Conium maculatum* L.: GU266024. *Daucus carota* L.: FJ415158. *D. glochidiatus* (Labill.) Fisch., C.A.Mey. & Avé-Lall.: FJ415160. *D. littoralis* Sm.: FJ415159. *Diplotaenia cachrydifolia* Boiss.: EU169258. *D. damavandica* Mozaff., Hedge & Lamond: EU169259. *Distichoselinum tenuifolium* (Lag.) García-Martin & Silvestre: FJ415140. *Ekimia bornmuelleri* (Hub.-Mor. & Reese) H. Duman & M.F. Watson: Turkey, prov. Burdur, M.G. Pimenov, E.V. Kljuykov, MW-65, KJ680158. *Elaeoselinum asclepium* Bertol.: FJ415135 (1), FJ415136 (2), FJ415138 (3). *E. thapsoides* DC.: FJ415141 (1), FJ415142 (2). *Ferula ferganensis* Lipsky ex Korovin: DQ379401. *Ferulago galbanifera* W.D.J.Koch: AF077889. *Guillonea scabra* Coss.: FJ415150. *Laserpitium affine* Ledeb.: FJ415151. *L. archangelica* hort. ex Link: FJ415153. *L. carduchorum* Hedge & Lamond: FJ415116 (1), FJ415117 (2). *L. eliasii* Sennen & Pau: FJ415118 (1), FJ415119 (2), FJ415120 (3). *L. gallicum* L.: FJ415126 (1), FJ415127 (2), FJ415128 (3), FJ415129 (4). *L. glaucum* L.: FJ415115. *L. halleri* Crantz: FJ415130. *L. hispidum* M. Bieb.: FJ415154 (1), FJ415155 (2). *L. involucreatum* Koso-Pol.: JQ305147. *L. krapffi* Crantz: FJ415124 (1), FJ415125

(2). *L. latifolium* L.: FJ415131. *L. nestleri* Soy.-Will.: FJ415121 (1), FJ415122 (2), FJ415123 (3). *L. nitidum* Zanted.: FJ415132. *L. panjutinii* (Manden. & Schischk.) M.Hiroe: JQ305148 (1), JQ305149 (2), AF008645 (3). *L. petrophilum* Boiss. & Heldr.: JQ305150 (1), JQ305151 (2), JQ305152 (3), FJ415114 (4), AF073567 (5). *L. peucedanoides* Brot.: FJ415133. *L. prutenicum* L.: FJ415156. *L. pseudomeum* Orph.Heldr. & Sart.: FJ415134. *L. siler* L.: FJ415112. *L. steveni* Fisch., C.A.Mey. & Trautv.: FJ415152. *Lecokia cretica* DC.: EU169294. *Lisaea heterocarpa* Boiss.: FJ415107. *Margotia gummifera* Lange: FJ415139. *Melanoselinum decipiens* (Schrad. & J.C.Wendl.) Hoffm.: FJ415161. *Monizia edulis* Lowe: AF073569. *Prangos bucharica* Fedtsch.: KJ395461. *P. didyma* (Regel) Pimenov & V.N.Tikhom.: KJ395463. *P. equisetoides* Kuzmina: KJ395466. *P. fedtschenkoi* (Regel & Schmalh.) Korovin: KJ395469. *P. ferulacea* (L.) Lindl.: KJ395459. *P. gyrocarpa* Kuzmina: KJ395458. *P. haussknechtii* Boiss.: KJ395465. *P. hissarica* Korovin: KJ395468. *P. latiloba* Korovin: KJ395462. *P. lipskyi* Korovin: KJ395453. *P. lophoptera* Boiss.: KJ395455. *P. odontalgica* (Pall.) Herrnst. & Heyn: KJ395464. *P. ornata* Kuzmina: KJ395456. *P. pabularia* Lindl.: KJ395454 (1), KJ395450 (2), KJ395448 (3). *P. pabularia* Lindl. subsp. *cylindrocarpa* (Korovin) Pimenov & V.N.Tikhom.: KJ395451. *P. pabularia* Lindl. subsp. *lamellata* (Korovin) Pimenov & V.N.Tikhom.: KJ395452. *P. uloptera* DC.: KJ395457. *Physospermum cornubiense* DC.: AF077904. *Rouya polygama* Coincy: FJ415157. *Scandix australis* L.: FJ415104. *Selinum carvifolia* (L.) L.: AY179028. *S. pyrenaicum* Gouan: AY179027. *Smyrniium creticum* Mill.: FJ415103. *Thapsia garganica* L.: FJ415143 (1), FJ415145 (2). *T. villosa* L.: FJ415147 (1), FJ415148 (2). *Tordylium apulum* L.: EU169329. *T. maximum* L.: DQ996585. *Torilis arvensis* (Huds.) Link: FJ415110. *Tornabenea annua* Bég. ex A.Chev.: DQ516356. *T. tenuissima* (A.Chev.) A.Hansen & Sunding: DQ516357.