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Fruit morphology of the genus *Pimpinella (Apiaceae)* in Turkey

Yeter Yeşil[1,*], Emine Akalın[2], Aşkın Akpulat[3] & Cem Vural[4]

[1,2]Istanbul University, Faculty of Pharmacy, Deparment of Pharmaceutical Botany, 34116 Istanbul, Turkey.
[3]Cumhuriyet University, Faculty of Education, Department of Biology Education, 58140 Sivas, Turkey.
[4]Erciyes University, Faculty of Arts and Sciences, Department of Biology, 38039 Kayseri, Turkey.

[*]Author for correspondence: yeteryesil@yahoo.com, https://orcid.org/0000-0002-4458-7881
[2]akaline@istanbul.edu.tr, https://orcid.org/0000-0002-0307-2128
[3]aakpulat99@yahoo.com, https://orcid.org/0000-0001-8394-2746
[4]vuralc@erciyes.edu.tr, https://orcid.org/0000-0001-9929-9935

Abstract. To explore if fruit morphology could aid in taxonomy of the genus *Pimpinella* L., we have undertaken a study of fruits from 26 Turkish taxa of *Pimpinella* using light and scanning electron microscopy—SEM—. A great deal of inter and intraspecific variation for both fruit shape and surface was observed. Fruit shapes of Turkish taxa of *Pimpinella* range from oblong-cylindrical to subglobose and indumentum when present can be strigose, hispid and may include hamate trichomes. Variation in fruit surface is also considerable and allows recognizing nine different ornamentation patterns. However, variation in shape, surface ornamentation and indumentum is not tightly associated since species with similar fruit shapes do not necessarily have similar surface ornamentation. To jointly analyse fruit morphology together with the most commonly used morphological characters of the whole plant and to compare morphological evidence with available phylogenetic hypotheses, a cluster analysis was also performed: the Turkish species of *Pimpinella* were clustered into two distinct groups, the second one subdivided in another two subgroups.

Keywords. *Apiaceae,* morphology, *Pimpinella,* taxonomy, Turkey.

Resumen. Para comprobar el valor diagnóstico de la morfología del fruto en la taxonomía del género *Pimpinella* L., hemos estudiado los frutos de 26 táxones mediante microscopía óptica y electrónica de barrido —SEM—. Se ha observado una gran variabilidad inter e intraespecífica en la forma y la superficie del fruto. Las formas del fruto de los táxones turcos de *Pimpinella* varían de oblongo-cilíndricas a subglobosas, así como el fruto puede ser de estrigoso a híspido y tener a veces tricomas hamosos. La variabilidad de la superficie del fruto también es considerable y permite reconocer nueve patrones de ornamentación diferentes. Sin embargo, las variabilidades de la forma, la ornamentación de la superficie y el indumento no están estrechamente asociadas, ya que las especies con frutos de forma similar no necesariamente tienen una ornamentación similar. Para analizar conjuntamente la morfología del fruto y los caracteres morfológicos más comúnmente utilizados y para comparar la morfológica con las hipótesis filogenéticas disponibles, también se ha realizado un análisis de grupos: las especies turcas de *Pimpinella* formaron dos grupos y el segundo se subdividió en otros dos.

Palabras clave. *Apiaceae,* morfología, *Pimpinella,* taxonomía, Turquía.

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INTRODUCTION

The c. 150 species constituting the genus *Pimpinella* L.—*Apiaceae* Lindl.—are distributed in temperate and subtropical regions of Eurasia and Africa including Madagascar. Turkey is one of the main centers of diversity for this genus along with Africa and Madagascar (Aksenov 1972; Abebe 1992). In his classical monograph, Wolff (1927) subdivided *Pimpinella* into three sections—*P.* sect. *Reutera* Boiss., *P.* sect. *Tragium* (Spreng.) DC. and *P.* sect. *Tragoselinum* (Mill.) DC.—based on petal color, fruit and petal indumentum, fruit ornamentation, and life form. *Pimpinella* sect. *Reutera* included species with yellow flowers and glabrous or hairy fruits; *P.* sect. *Tragium* included species mostly with white flowers and bristly or hairy, granular or tuberculate fruits, sometimes nearly glabrous or almost completely smooth; and *P.* sect. *Tragoselinum* included also white-flowered species with glabrous fruits. This classification has been widely adopted with some added characters. For instance, Pu & Watson (2005) in the *Flora of China* added calyx features. They recognized the first two of these sections: *P.* sect. *Tragium* including species with hairy or distinctly roughened fruits and obsolete calyx teeth, and *P.* sect. *Tragoselinum,* including species with glabrous fruits and obsolete or conspicuous calyx teeth. However, taxonomy of this genus, one of the most complex in the family, is relatively unsettled and phylogenetic studies have partly challenged Wolff's sections (Magee & al. 2010). To achieve a stable taxonomy for this genus, it is not only necessary to refine the description of morphological characters as well as their patterns of variation and distribution across species but also to test those characters against solid molecular phylogenetic analyses.

For Turkey specifically, Matthews (1972) recognized 23 species with no infrageneric subdivison. Subsequent modifications to such treatment have involved transfers to, or from, other genera as well as new species. *Pimpinella cruciata* Bornm. & H.Wolff, which was identified as one of two varieties of *P. anthriscoides* Boiss. by Matthews in the *Flora of Turkey,* has been recently transferred to *Tamamschjanella* Pimenov & Kljuykov (Zakharova & al. 2012). Conversely, two names, treated under the genus *Scaligeria* DC. in the *Flora of Turkey* (Stevens 1972), have been confirmed to be part of *Pimpinella,* namely *P. tripartita* Kalen. and *P. lazica* (Boiss.) M.Hiroe (Hand 2011). In the same work *P. affinis* Ledeb. and *P. squamosa* Karjagin have been considered to be synonyms of *P. peregrina* L. and *P. nudicaulis* Trautv., respectively (Hand 2011). Finally, two new species have been recently described, *P. ibradiensis* Çingilbel & al. (Çingilbel & al. 2015) and *P. enguezekensis* Yıldırım & al. (Yeşil & al. 2016), so that currently *Pimpinella* includes 25 species—30 taxa, 8 of them endemic—in Turkey (Matthews 1972; Ertekin & Kaya 2005; Göktürk 2008; Menemen 2012; Çinbilgel & al. 2015; Yeşil & al. 2016).

Fruit characters are considered crucial in taxonomy throughout the whole *Apiaceae* as can be seen in any identification key (Engler 1927). The possibility of better characterizing fruits by using both anatomical characters and micromorphological features using SEM has stimulated numerous studies across the family in genera such as *Bupleurum* L. (Özcan 2004), *Ferulago* Koch (Akalın & Kızılarslan 2013), *Ekimia* H.Duman & M.F.Watson (Lyskov & al. 2015), *Grammasciadium* DC. (Bani & al. 2016a, 2016b) and *Heracleum* L. (Liu & Downie 2017).

In *Pimpinella* there have been several anatomical studies confined to important regions such as Iran (Khajepiri & al. 2010), Russia (Aksenov & Tikhomirov 1972), Africa and Madagascar (Magee & al. 2010). In a previous work, we conducted an anatomical study on the Turkish species (Akalın & al. 2016) that led to the recognition of four groups defined on the basis of fruit anatomical structure. Specifically, those four groups differed on the number and size of vallecular vitae, fruit shape, and trichomes and were partly compatible with the sections of Wollf (1927).

The taxonomic uncertainties together with the interest of this genus both at the taxonomic and phytochemical levels have prompted several molecular phylogenetic studies assessing relationships within *Pimpinella.* Tabanca & al. (2005) sampled 26 Turkish species of this genus focusing on distribution patterns of essential oils. Magee & al. (2010) attempted to elucidate the phylogenetic position of the African and Malagasy species but included 26 species from Eurasia in their analyses. Focusing on the genus circumscription, Fereidounfar & al. (2016) analyzed 52 Southwest Asian species of *Pimpinella* within a considerable sample of species from the family and concluded that *P.* sect. *Reutera* as well as *Opsicarpium* Mozaff. fall within *Pimpinella* and should be included in this genus. All the three studies were based on nuclear ribosomal ITS sequences and the first and third one also used plastid DNA sequences. Even though the focuses are different and sampling are not comprehensive, the phylogenetic positions of the species of *Pimpinella* included in two or more of these studies are to a large part consistent and thus there is some basis for phylogenetic relationships, which can be considered when taxonomic uncertainties are addressed. However, more research is needed on several fronts to clarify the taxonomy of this complex genus at a fine level.

The main purpose of this carpological study is to provide a detailed description of fruit morphology of 26 Turkish *Pimpinella* taxa—c. 87% of the Turkish taxa—including both micromorphological characters assessed using SEM, to contribute to species delimitation and infrageneric classification and to explore concordance with existing phylogenetic studies. We aim to aid in taxonomic classification by examining the fit of fruit characters with existing phylogenetic studies and by analyzing fruit variation together with the morphology of other organs.

MATERIAL AND METHODS

Ripe fruits from *Pimpinella* corresponding to 26 taxa, 8 of them endemic, were obtained from specimens collected in different areas of Turkey (Table 1). Voucher specimens were deposited in ISTE—Herbarium of the Faculty of Pharmacy, Istanbul—. For the SEM micromorphological study, fruits were mounted on stubs using double adhesive tape and coated with gold-paladium. Specimens were examined under a JEOL Neoscope 5000 electron microscope at 10.00 kV. Macromorphological observations were made, and photograps were taken, with a LEICA DFC 295 stereo microscope with a digital camera. Measurements of mericarps, using LEICA software, were performed on at least five mature fruits from each of the 26 studied taxa. The main morphological features recorded are summarized in Table 2. For descriptions and terminology of our micromorphological observations, we follow Özcan (2004), Bani & al. (2016a, 2016b) and Liu & Downie (2017). Overall shapes of mericarps were classified according to *Botanical Latin* (Stearn 2005) and Aksenov & al. (1972). In addition, to explore phenetic similarity among the *Pimpinella* taxa, we performed a cluster analyses. Specifically a hierarchical agglomerative clustering analysis—method:ward.D—using the hclust function in R package v3.3.1. (R Development Core Team 2018) was run to construct a dendrogram. For this, the overlapping characteristics were previously eliminated (Wolf 1927; Abebe 1992) and catergorical variables were trasnformed into binary. Twenty-eight

Table 1. The list and collection numbers of studied Turkish taxa of *Pimpinella* L.

Taxon	Grid City	Location	Altitude m a.s.l.	Collection Number
P. affinis Ledeb.	B7 Erzincan	Kemaliye, Sançiçek Plateau, 13–VIII–2011, *E. Akalın* and *U. Uruşak* s.n. leg.	1790	ISTE 96851
P. anisetum Boiss. & Balansa	B7 Erzincan	Spikor mountain, Çayırlı road, 10 km after Erzincan, 14–VIII–2011, *E. Akalın* and *U. Uruşak* s.n. leg.	2293	ISTE 95807
P. anisum L.	A9 Ardahan	Kutul, Yalnızçam Forest, 4–IX–2010, *A. Akpulat* s.n. leg.	800	ISTE 96842
P. aromatica M.Bieb.	B7 Erzincan	Spikor mountain, Kolgeçmez pass, 14–VIII–2011, *E. Akalın* and *U. Uruşak* s.n. leg.	2360	ISTE 94693
P. aurea DC.	C10 Hakkâri	6 km after Hakkâri-Yüksekova turnout, 27–VII–2012, *E. Akalın* and *U. Uruşak* s.n. leg.	2185	ISTE 98881
P. cappadocica Boiss. & Balansa var. *cappadocica*	B7 Sivas	Mut-Kırobası, 7 km from Mut, 30–VI–2012, *A. Akpulat 4810* leg.	514	ISTE 10117
P. corymbosa Boiss.	B7 Erzincan	Spikor Mountain, Çayırlı road, 24 km from Erzincan, 14–VIII–2011, *E. Akalın* and *U. Uruşak* s.n. leg.	1318	ISTE 95805
P. cretica Poir. var. *cretica*	C1 Aydın	Priene ancient city, left side of entrance, 4–VI–2012, *E. Akalın* and *U. Uruşak* s.n. leg.	26	ISTE 98669
P. eriocarpa Banks & Sol.	B7 Şanlıurfa	Northwest of Korukezen village, 6–XI–2012, *E. Akalın* and *U. Uruşak* s.n. leg.	840	ISTE 98778
P. enguezekensis Yıldırım & al.	B6 Malatya	Darende District, Ergü road, Kilise location, 22–VII–2015, *H.Yıldırım HY3492* leg.	1420	ISTE 107588
P. flabellifolia (Boiss.) Benth. & Hook. ex Drude	B6 Sivas	Divriği, Arguvan-Divriği road, between Beldibi-Yeşilyol villages, 21–VII–2015, *H. Yıldırım HY3472* leg.	1451	ISTE 107580
P. ibradiensis Çinbilgel & al.	C3 Antalya	İbradı, Toka Yayla, 2–VII–2011, *Çinbilgel 7975* and *Eren* leg.	1527	ISTE 115057
P. isaurica V.A.Matthews subsp. *isaurica*	C4 Konya	Ermenek, around Keben fountain, 28–VIII–2011, *E. Akalın* and *U. Uruşak* s.n. leg.	1293	ISTE 95813
P. kotschyana Boiss.	B1 Manisa	Spil Mountain, Spil roadside, 5–VII–2011, *E. Akalın* and *U. Uruşak* s.n. leg.	306	ISTE 95735
P. lazica (Boiss.) M.Hiroe	A8 Rize	Çamlıhemşin, Boğaziçi village, Tunuslu town, 6–IX–2010, *A. Akpulat* and *M. Tekin 16* leg.	600	ISTE 96846
P. nephrophylla Rech.f. & Riedl	B8 Diyarbakır	Eğil, Eğil castle, 13–VIII–2011, *E. Akalın* and *U. Uruşak* s.n. leg.	900	ISTE 95784
P. nudicaulis Trautv.	B7 Erzincan	Tercan, Gahmut Plateau, 10–VIII–2009, *E. Akalın* and *U. Uruşak* s.n. leg.	1910	ISTE 101345
P. oliverioides Boiss. & Haussk n.	B9 Van	Van-Hoşap, Güzeldere pass, 18–VIII–1993, *Y. Altan 5552* leg.	2800	GAZI
P. paucidentata V.A.Matthews	B6 Malatya	Darende, Ağılbaşı town, Ergü road, Kilise location, 10–VIII–2017, *Y. Yeşil* s.n. leg.	1420	ISTE 115020
P. peregrina L.	B1 Manisa	Hatipler-Şatırlar, Hatipler village, 6–VII–2011, *E. Akalın* and *U. Uruşak* s.n. leg.	288	ISTE 95775
P. peucedanifolia Fisch.	B7 Erzincan	Spikor mountain, Mecidiye location, 13–VIII–2010, *E. Akalın* and *U. Uruşak* s.n. leg.	2310	ISTE 94695
P. puberula (DC.) Boiss.	C9 Hakkâri	Hakkâri-Van, 12 km after Hakkâri, 27–VII–2012, *E. Akalın* and *U. Uruşak* s.n. leg.	1446	ISTE 98878
P. rhodantha Boiss.	A9 Ardahan	Çataldere Plateau, 27–VII–2011, *B. Gürdal* and *S. Esen* s.n. leg.	1548	ISTE 97267
P. saxifraga L.	A6 Ordu	Koyulhisar-Mesudiye, 11–VIII–2010, *E. Akalın* and *U. Uruşak* s.n. leg.	1370	ISTE 94675
P. sintenisii H.Wolff	C8 Mardin	Darulzaferan Monastery, 11–VI–2012, *E. Akalın* and *U. Uruşak* s.n. leg.	1212	ISTE 98789
P. tragium subsp. *pseudotragium* (DC.) V.A.Matthews	B7 Erzincan	Spikor mountain, Kolgeçmez pass, 14–VIII–2011, *E. Akalın* and *U. Uruşak* s.n. leg.	2684	ISTE 95811

Table 2. Fruit measurements and features of the mericarps of Turkish taxa of *Pimpinella* L.

Taxon	Fruit length and width (mm)	Length/width ratio	Indumentum	Shape of fruit
P. affinis Ledeb.	1.65–1.75 × 0.77–0.8	2.06	pubescens	elliptic
P. anisetum Boiss. & Balansa	1.5–1.57 × 1–1.08	1.5	strigose	ovoid
P. anisum L.	3.9–4 × 1.5–1.53	2.6	strigose	ovoid-subglobose
P. aromatica M.Bieb.	1.85–1.9 × 1.21–1.25	1.52	strigose	ovoid
P. aurea DC.	2.23–2.25 × 1.58–1.6	1.40	pubescens	subglobose
P. cappadocica Boiss. & Balansa var. *cappadocica*	1.97–2 × 1.14–1.15	1.73	hispid	ovoid
P. corymbosa Boiss.	1.9–2 × 1.95–1	2	pubescens	ovoid-subglobose
P. cretica Poir. var. *cretica*	1.42–1.5 × 0.95–1	1.5	strigose	ovoid-globose
P. enguezekensis Yıldırım & al.	2.57–2.6 × 1.69–1.7	1.52	glabrous	oblong-ovoid
P. eriocarpa Banks & Sol.	1.57–1.6 × 0.78–0.8	2	hispid-subhamate	elliptic
P. flabellifolia (Boiss.) Benth. & Hook. ex Drude	3.9–4 × 2.25–2.3	1.73	rarely hispid	oblong-ovoid
P. ibradiensis Çinbilgel & al.	4–5.5 × 1–2	2.89	glabrous	oblong-cylindrical
P. isaurica V.A.Matthews subsp. *isaurica*	3.4–3.5 × 0.95–0.98	3.57	hirsute	oblong-cylindrical
P. kotschyana Boiss.	2.6–2.65 × 1.18–1.2	2.20	hispid	ovoid-subglobose
P. lazica (Boiss.) M.Hiroe	2.7–2.8 × 1.6–1.66	1.68	glabrous	oblong-ovoid
P. nephrophylla Rech.f. & Riedl	2.3–2.35 × 0.85–0.87	2.70	glabrous	oblong
P. nudicaulis Trautv.	3.6–3.65 ×1.3–1.35	2.70	glabrous	oblong-cylindrical
P. oliverioides Boiss. & Hausskn.	4.25–4.35 × 1.6–1.64	2.65	pubescens	oblong
P. peregrina L.	1.9–2 × 0.9–0.94	2.11	hispid	eliptic
P. peucedanifolia Fisch.	2.55–2.6 × 0.58–0,6	4.33	glabrous	oblong-cylindrical
P. paucidentata V.A.Matthews	2.15–2.2 × 0.7–0.71	3.07	glabrous	oblong
P. puberula (DC.) Boiss.	1.6–1.67 × 1.05–1.10	1.52	hamate	ovoid-globose
P. rhodantha Boiss.	2.9–3.1 × 1.8–1.92	1.61	glabrous	oblong-ovoid
P. saxifraga L.	2.1–2.2 × 1.7–1.78	1.23	glabrous	oblong-ovoid
P. sintenisii	1.8–1.95 × 0.65–0.7	2.76	glabrous	oblong
P. tragium subsp. *pseudotragium*	2.3–2.42 × 1.4–1.47	1.64	hamate	oblong-ovoid

binary characters—presence/absence—from the fruits and from other plant organs were included in the analysis. Fruit characters are size, shape, indumentum (Table 2) and the micromorphological ones described below under results. Morphological characters from other plant organs are flower color—white, yellow, pink, red—, fruit indumentum—hairy or glabrous—, leaf shape—simple or pinnate—, and bracts and bracteoles—presence/absence.

RESULTS

Macromorphology of fruits

Fruit shape of Turkish taxa of *Pimpinella* can be referred to the following categories: oblong-cylindrical, oblong, elliptic, ovoid-subglobose, oblong-ovoid, ovoid, ovoid-globose, and subglobose (Fig. 1). The ratio of fruit length to width varies between 4.33 and 1.5. The largest fruits—3.4–5.5 mm long—are found in *P. ibradiensis*—light microscopy photo not shown—, *P. oliveroides* Boiss. & Hausskn., *P. nudicaulis, P. anisum* L., *P. isaurica* V.A.Matthews subsp. *isaurica,* and *P. flabellifolia* (Boiss.) Benth. & Hook. ex Drude, whereas the smallest—1.42–1.5 mm—is found in *P. cretica* Poir. var. *cretica* (Table 2). Fruit indumentum has been assigned to the following states: pubescens, strigose, rarely hispid, hispid, hamate, hamate or glabrous. Tichome surface is always verrucate.

Micromorphology of fruit surface

The mericarp surface shows a variety of micromorphological patterns at the SEM (figs. 2–4). The following nine types of ornamentation were observed in this study:

Type 1, smooth-rugose: among the Turkish species, this distinct surface ornamentation pattern is only found in *P. cretica* var. *cretica.* The mericarp surface is covered by strigose hairs (fig. 2A, a).

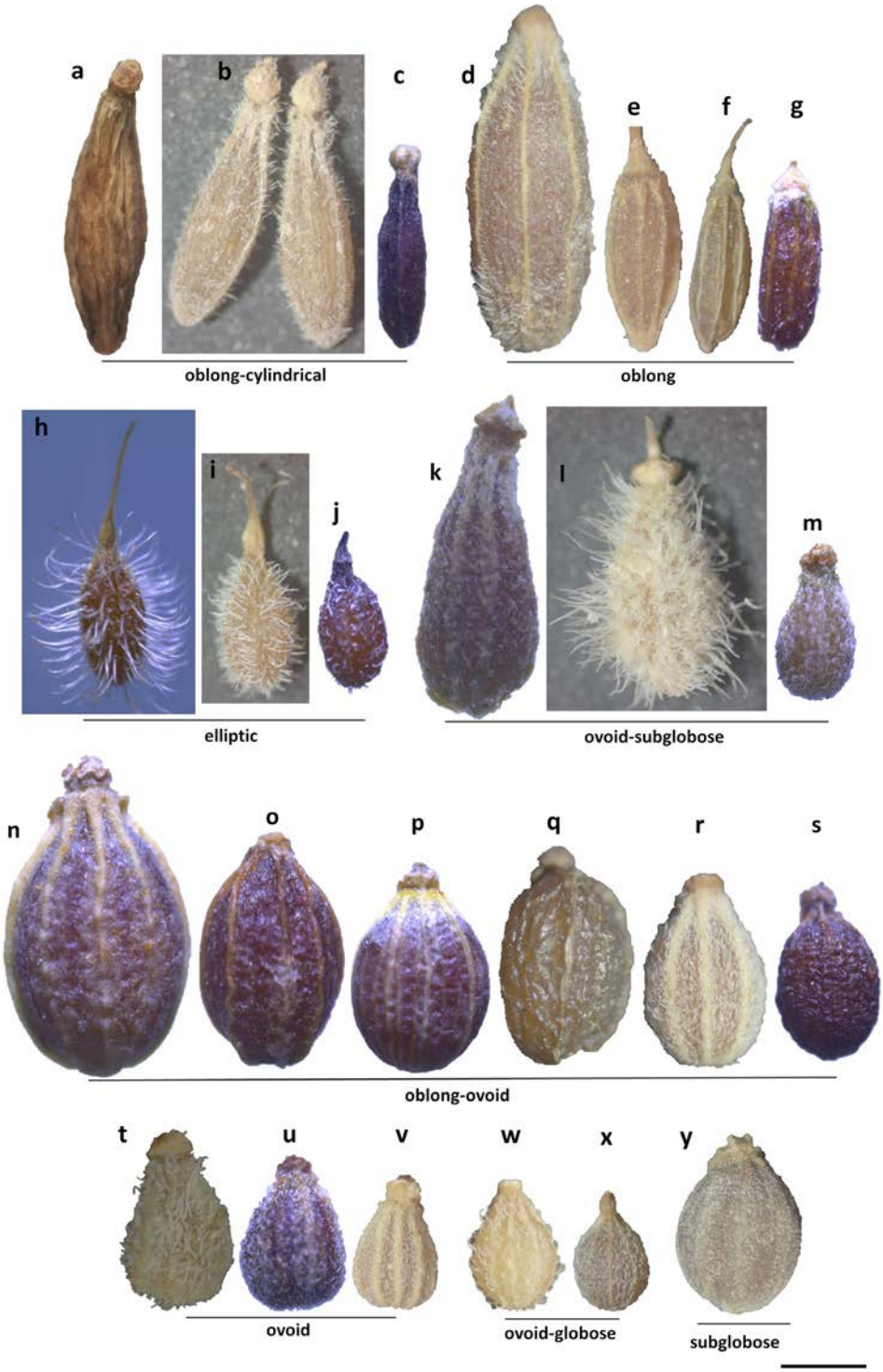

Fig. 1. Mericarps of the Turkish taxa of *Pimpinella* L.: **a,** *P. nudicaulis* Trautv.; **b,** *P. isaurica* V.A.Matthews subsp. *isaurica;* **c,** *P. peucedanifolia* Fisch.; **d,** *P. oliverioides* Boiss. & Hausskn.; **e,** *P. nephrophylla* Rech.f. & Riedl; **f,** *P. sintenisii* H.Wolff; **g,** *P. paucidentata* V.A.Matthews; **h,** *P. eriocarpa* Banks & Sol.; **i,** *P. peregrina* L.; **j,** *P. affinis* Ledeb.; **k,** *P. anisum* L.; **l,** *P. kotschyana* Boiss.; **m,** P. corymbosa Boiss.; **n,** *P. flabellifolia* (Boiss.) Benth. & Hook. ex Drude; **o,** *P. rhodantha* Boiss.; **p,** *P. enguezekensis* Yıldırım & al.; **q,** *P. lazica* (Boiss.) M.Hiroe; **r,** *P. tragium* subsp. *pseudotragium* (DC.) V.A.Matthews; **s,** *P. saxifraga* L.; **t,** P. cappadocica Boiss. & Balansa var. cappadocica; **u,** *P. aromatica* M.Bieb.; **v,** *P. anisetum* Boiss. & Balansa; **w,** *P. puberula* (DC.) Boiss.; **x,** *P. cretica* Poir. var. *cretica;* **y,** *P. aurea* DC. Scale bar: 1 mm.

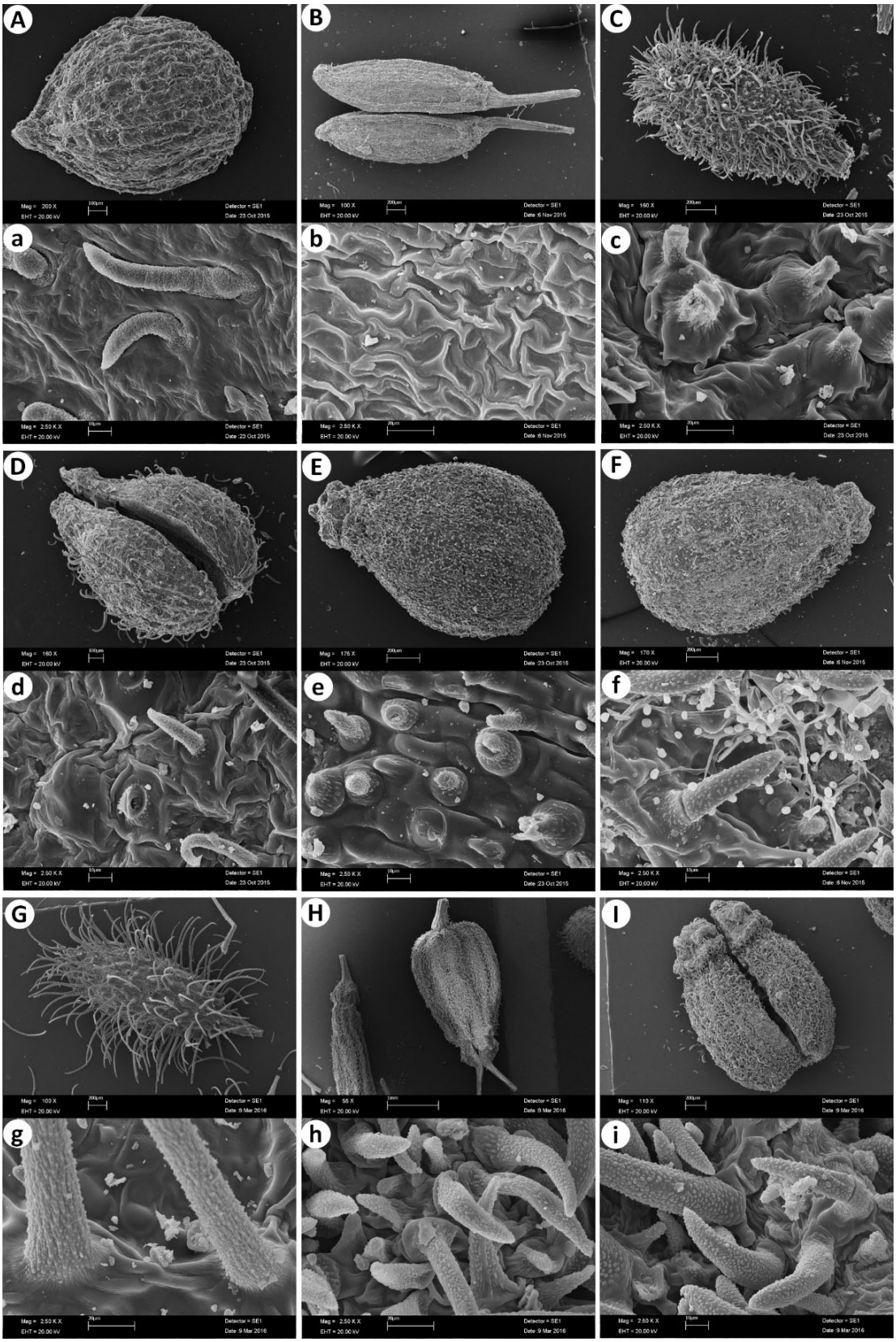

Fig. 2. SEM micrographs of mericarp coat surface in the genus *Pimpinella* L.: **A, a,** *P. cretica* Poir.; **B, b,** *P. nephrophylla* Rech.f. & Riedl; **C, c,** *P. peregrina* L.; **D, d,** *P. puberula* (DC.) Boiss.; **E, e,** *P. anisetum* Boiss. & Balansa; **F, f,** *P. aromatica* M.Bieb.; **G, g,** *P. eriocarpa* Banks & Sol.; **H, h,** *P. anisum* L.; **I, i,** *P. corymbosa* Boiss.

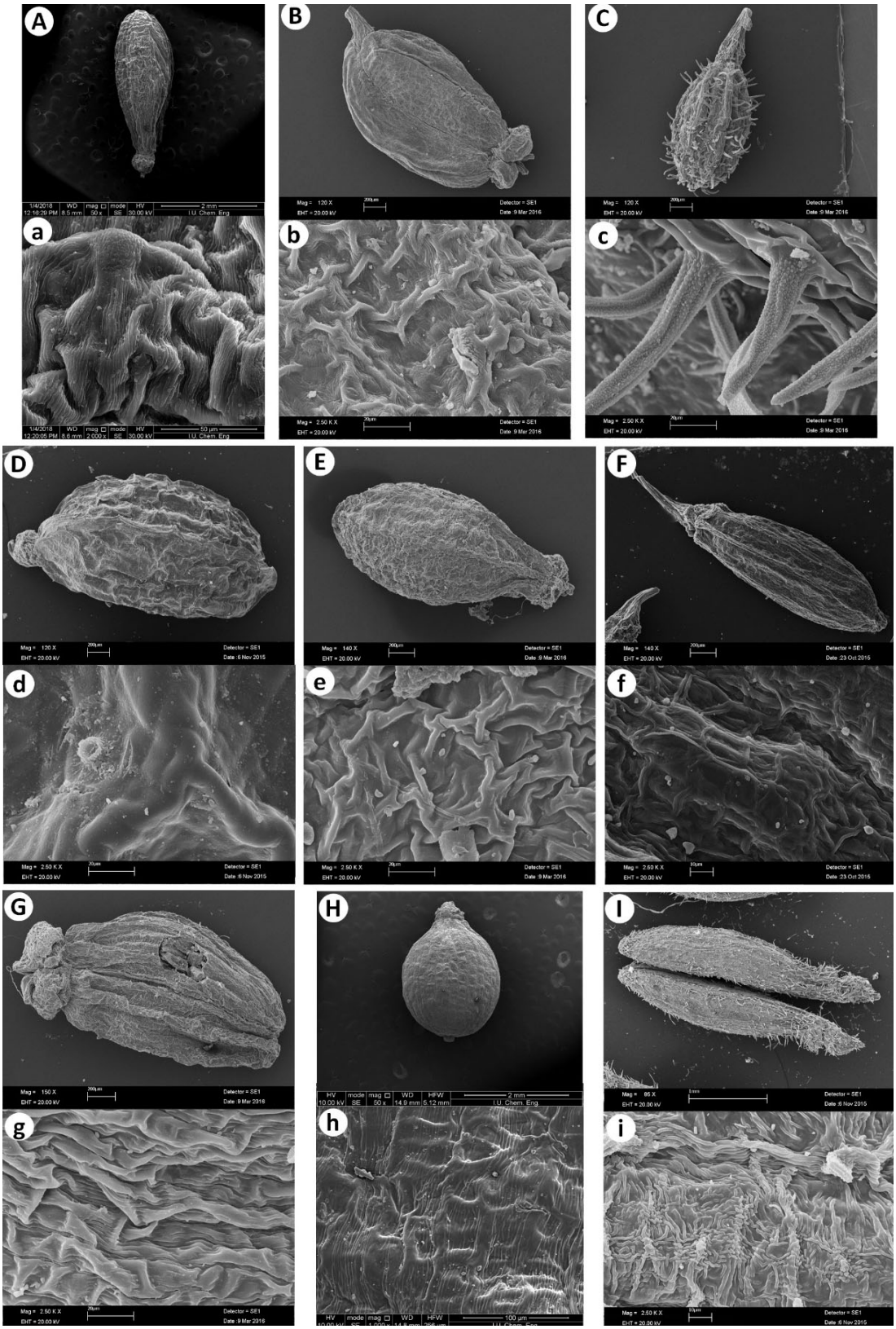

Fig. 3. SEM micrographs of mericarp coat surface in the genus *Pimpinella* L.: **A, a,** *P. nudicaulis* Trautv.; **B, b,** *P. peucedanifolia* Fisch. ex Ledeb.; **C, c,** *P. affinis* Ledeb.; **D, d,** *P. lazica* (Boiss.) M.Hiroe; **E, e,** *P. saxifraga* L.; **F, f,** *P. sintenisii* H.Wolff; **G, g,** *P. rhodantha* Boiss.; **H, h,** *P. enguezekensis* Yıldırım & al.; **I, i,** *P. isaurica* V.A.Matthews subsp. *isaurica.*

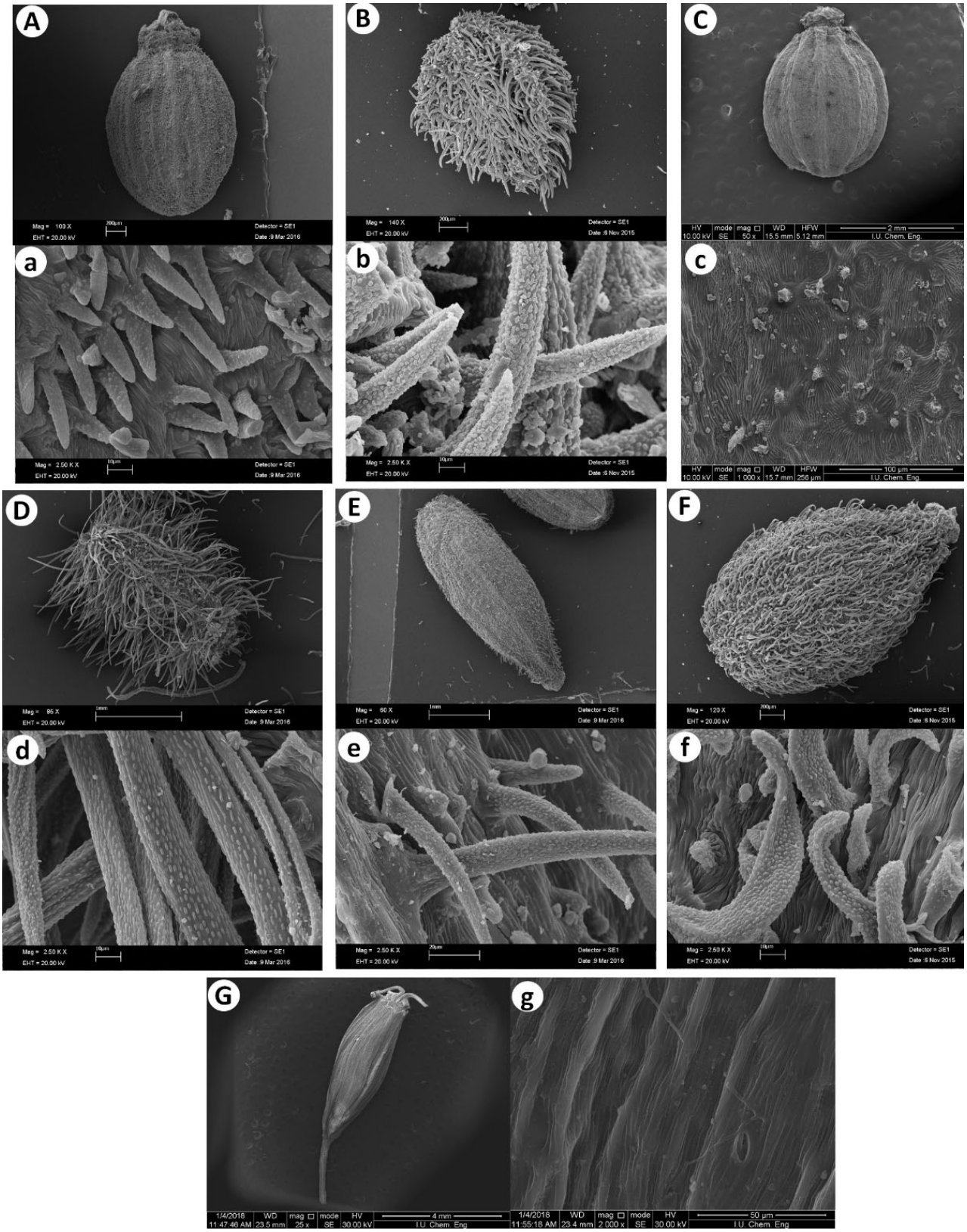

Fig. 4. SEM micrographs of mericarp coat surface in the genus *Pimpinella* L.: **A, a,** *P. aurea* DC.; **B, b,** *P. cappadocica* Boiss. & Balansa var. *cappadocica;* **C, c,** *P. flabellifolia* (Boiss.) Benth. & Hook. ex Drude; **D, d,** *P. kotschyana* Boiss.; **E, e,** *P oliverioides* Boiss. & Hausskn. ex Boiss.; **F, f,** *P. tragium* subsp. *pseudotragium* (DC.) V.A.Matthews; **G, g,** *P. ibradiensis* Çingilbel & al.

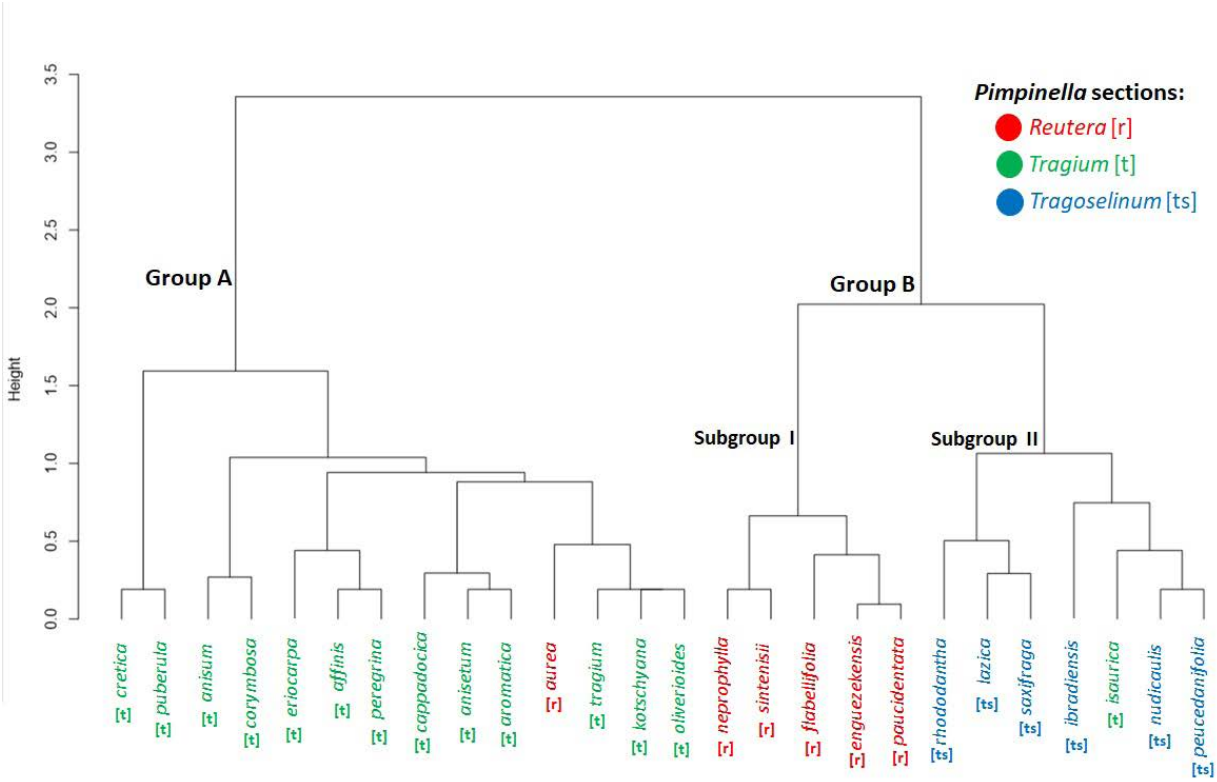

Fig. 5. The cluster dendrogram of the Turkish taxa of *Pimpinella* L.

Type 2, rugose: irregularly colliculate with interconnected foldings. It occurs on *P. nephrophylla* Rech.f. & Riedl, *P. peregrina,* and *P. puberula* (DC.) Boiss. (fig. 2).

Type 3, rugulose: colliculate-tuberculate surface pattern, but with very few tubercules—*P. anisetum* Boiss. & Balansa, *P. aromatica* M.Bieb., and *P. eriocarpa* Banks & Sol.—(fig. 2).

Type 4, rugose-striate: with uneven, short and incomplete folds bearing secondary striate parallel furrows—*P. anisum, P. corymbosa* Boiss., *P. nudicaulis,* and *P. peucedanifolia* Fisch. ex Ledeb.—(figs. 2–3).

Type 5, rugose-reticulate: with nerve-like elevations that come from a reticular surface—*P. affinis, P. lazica, P. saxifraga* L., and *P. sintenisii* H.Wolff—(fig. 3).

Type 6, reticulate-striate: striate with longitudinal folds—*P. rhodantha* Boiss. and *P. enguezekensis*—(fig. 3).

Type 7, striate-ruminate: densely striate with irregular folds—*P. isaurica* subsp. *isaurica*—(fig. 3I, i).

Type 8, striate: irregularly colliculate and with folding-like elevations—*P. aurea* DC., *P. cappadocica* Boiss. & Balansa, *P. flabellifolia, P. kotschyana* Boiss., *P. olivierioides* Boiss. & Hausskn. ex Boiss., *P. tragium* var. *pseudotragium* (DC.) V.A.Matthews—(fig. 4).

Type 9, ribbed-striate: parallel longitudinal striations with distinct ribbed—*P. ibradiensis*—(fig. 4G, g).

Cluster analysis of fruit and whole plant morphology

The results of the cluster analysis of 26 taxa based on fruit morphology as well as whole plant morphology clusters Turkish *Pimpinella* into 2 groups. Group A contains white-flowered species with the single exception of *P. aurea* (fig. 5). Group B contains both yellow-flowered and white-flowered species, mostly with glabrous fruits but also a few species with hairy fuits. This group is more heterogeneous than A and includes two differentiated subgroups. Subgroup I contains yellow-flowered species with sparsely hairy fruits whereas subgroup II contains white-flowered species with glabrous fruits, except for *P. isaurica* V.A.Matthews (fig. 5).

DISCUSSION

The first carpological study of Turkish *Pimpinella*—c. 80% of the taxa—using both SEM and light microscopy has found considerable variation affecting shape and surface. However, variation in shape and surface characters is not correlated and species with similar fruit shapes do not necessarily have similar surface ornamentation. Fruit morphological patterns of variation here analyzed are not fully compatible with classification by Wolff (1927).

Phylogenetic relationships are not fully compatible with Wolff's classification either since the three sections come out as polyphyletic (Magee & al. 2010). However, the combination of fruit and whole plant morphological characters in the cluster analysis provides a partly congruent picture with the classification of Wolff (1927). For instance, our group A includes species from *P.* sect. *Tragium,* except for *P. aurea,* which belongs to *P.* sect. *Reutera.* Our subgroup I of group B includes species from *P.* sect. *Reutera* whereas subgroup II includes species from *P.* sect. *Tragoselinum* except for *P. isaurica,* which belongs to *P.* sect. *Tragium* (fig. 5).

A detailed comparison of Wollf's classification with the available phylogenetic studies (Tabanca & al. 2005; Magee & al. 2010; Fereidounfar & al. 2016) is hampered by the limited sampling in those studies. However, there is some consistency in the phylogenetic position of the Turkish species across those three studies although with some exceptions—e.gr., *P. aurea*—. In addition, our micro and macromorpohological study is not fully consistent with the previous anatomical study (Akalın & al. 2016) but a number of associations occurs that is worth commenting, most of which are wholly or partly consistent with the phylogenetic studies. For instance, most species with oblong-ovoid fruits are in the first anatomical group in Akalın & al. (2016). *Pimpinella affinis, P. peregrina,* and *P. eriocarpa* all have elliptic fruits and the first two species are in the second anatomical group of Akalın & al. (2016). Our cluster analysis grouped the three species together (fig. 5) and *P. peregrina* and *P. eriocarpa* are sister species in the three available phylogenetic studies (Tabanca & al. 2005; Magee & al. 2010; Fereidounfar & al. 2016).

Pimpinella cretica var. *cretica* and *P. puberula* share ovoid-globose fruits and other morphological characters (Akalın & al. 2016). Therefore, they come out together in our cluster analysis (fig. 5) and are sister species in Fereidounfar & al. (2016). However, these two species have very different fruit surfaces (fig. 2).

Pimpinella cappadocica, P. anisetum, and *P. aromatica* share ovoid fruits and are grouped together in the cluster analysis (fig. 5). However, *P. anisetum* and *P. aromatica* have rugulose fruit surface whereas that of *P. cappadocica* is striate. Two of the phylogenetic studies support the closeness of *P. cappadocica* var. *cappadocica* and *P. anisetum* (Tabanca & al. 2005; Magee & al. 2010); the third one does not.

Pimpinella lazica, P. saxifraga, P. enguezekensis, and *P. rhodantha* all have oblong-ovoid glabrous fruits and came out within group B in the cluster analysis (fig. 5). However, these four species do not share the micromorphological structure of the mericarps since *P. lazica* and *P. saxifraga* have rugose-reticulate surface whereas *P. enguezekensis* and *P. rhodantha* have it reticulate striate (fig. 3). In contrast, *P. saxifraga* and *P. rhodantha* are sister species both in Tabanca & al. (2005) and in Magee & al. (2010); two species that can be distinguished by their flower color as well as their basal and cauline leaves.

Pimpinella nephrophylla, P. sintenisii H.Wolff, and *P. paucidentata* V.A.Matthews all have oblong fruits, fall within the same cluster—subgroup I of B; fig. 5—and belong to the fourth anatomical group in Akalın & al. (2016), but *P. nephrophylla* and *P. sintenisii* differ in their fruit surface (figs. 2, 3). In two of the phylogenetic studies, *P. sintenisii* and *P. paucidentata* are closely related (Tabanca & al. 2005; Magee & al. 2010).

Another contrast between morphological and molecular phylogenetic data concerns *P. corymbosa* and *P. kotschyana,* which have both ovoid-subglobose fruits but differ in fruit surface and other morphogical characters of the whole plant and yet are closely related in the phylogenetic trees (Tabanca & al. 2005; Magee & al. 2010; Fereidounfar & al. 2016).

The relationships of *P. aurea* are also controversial. It has a distinct fruit shape (fig. 1) with a striate ornamentation that is similar to *P. cappadocica, P. flabellifolia, P. kotschyana, P. oliverioides,* and *P. tragium* var. *pseudotragium* and falls in the cluster analysis together with *P. kotschyana, P. oliverioides,* and *P. tragium* var. *pseudotragium.* Yet, the phylogenetic position of *P. aurea* in the two studies in which it was sampled (Tabanca & al. 2005; Magee & al. 2010) differs although in the latter work *P. aurea* species fell in the same clade as *P. cappadocica* and *P. oliverioides,* and the three of them are also closely related to *P. kotschyana.*

The newly described species *P. ibradiensis,* which has not been yet included in any phylogenetic study, has been suggested to belong to *P.* sect. *Reutera* and to be closely related to *P. nephrophylla, P. sintenisii, P. paucidentata,* and *P. flabellifolia* by its authors (Çingilbel & al. 2015). However, our SEM study has found significant differences in micromorphology of fruits (fig. 4) and, in addition, *P. ibradiensis* can be distinguished from these species by its white petals, serrulate basal leaves, larger fruits, and the presence of bracts and bracteoles. Besides, our cluster analysis placed it together with species of *P.* sect. *Tragoselinum* specifically close to *P. nudicaulis* and *P. peucedanifolia.*

Our carpological study provides useful previously undetected characters for distinguishing species and, to a lesser degree, for aiding in infrageneric classification of *Pimpinella.* However, the patterns of variation in fruit micromorphological structures here reported are only partly consistent with our previous anatomical study (Akalın & al. 2016) and with morphological characters of

other parts of the plant that are normally used in taxonomy of this genus. This suggests that some of these macro and micromorphological characters may have been acquired independently and thus the information they contain for supporting infrageneric taxonomy of *Pimpinella* should be ideally confronted to a strongly supported phylogenetic backbone for this genus, which is not yet available.

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