Research Article

CAPSULE, LEAF AND CYATHIAL GLAND MORPHOLOGY OF TURKISH PERENNIAL TAXA OF *Euphorbia* L. SECTION *Pithyusa* (Raf.) Lázaro

İlker GENÇ*, Şükran KÜLTÜR, Gülay ECEVİT-GENÇ

İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany, İstanbul, TURKEY *Corresponding author: e-mail: <u>ilkgenc@istanbul.edu.tr</u>

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Abstract: Macro and micromorphology of cyathial glands, capsules and cauline leaf surfaces of 13 perennial species of *Euphorbia* L. subg. *Esula* Pers. sect. *Pithyusa* (Raf.) Lázaro in Turkey were studied. Cyathial gland structure, based on cyathial gland shapes, colour and appendages was examined from live material by direct field observations and investigations. Cauline leaves and capsule surface features were analysed with scanning electron microscopy (SEM). Capsule sizes, capsule shapes and capsule and cauline leaf surface ornamentations were also studied. The capsule shape was found to vary from subglobose to conical. In most species, cyathial gland colour ranged from greenish-yellow to yellowish-green but some species were distinguished by their reddish or purplish cyathial glands. Although the number of cyathial glands in all species was four, five cyathial glands were determined to exist in some specimens of *E. pestalozzae* Boiss. Three different cyathial gland shapes (elliptic, trapezoid-elliptic or elliptic-reniform) were observed. Capsule surface of more than half of the studied species (7 taxa) is covered with nipple-like projections (papillate-mammillate) and the rest are covered with tiny rounded or hillock-like ornamentation (colliculate). The cauline leaf surface is papillate-mammillate in the majority of the studied species, although, colliculate surface is also observed in two species (*E. seguieriana* Necker and *E. thessala* (Form.) Degen & Dörf.). In conlusion, macro and micromorphological structures of cyathial gland and capsule of *Euphorbia* sect. *Pithyusa* taxa appeared to be useful diagnostic characters for species identifications.

Key words: Leafy spurge, micromorphology, leaf surface, subg. Esula, Euphorbiaceae.

Özet: Bu çalışma *Euphorbia* L. subg. *Esula* Pers. sect. *Pithyusa* (Raf.) Lázaro çok yıllık türlerinin siatyum gland şekilleri, kapsula ve gövde yaprak yüzey mikromorfolojilerine dayanmaktadır. Siatyum gland yapılarının incelenmesi doğrudan arazi çalışmaları sırasında canlı örnekler üzerinden yapılmıştır. Gövde yaprağı ve kapsulanın yüzey özellikleri taramalı elektron mikroskobu (SEM) ile incelenmiştir. Ayrıca kapsula boyutu, kapsula şekli, kapsula ve gövde yaprak yüzey özellikleri de incelenmiştir. Çalışılan türlerin kapsula şekilleri hemen hemen küresel'den koniğe değişir. Araştırılan türlerden çoğunun gland rengi parlak sarı-yeşil veya yeşilimsi sarıdır. Bazı türlerde ise glandlar kırmızı veya mor renkli olabilir. Tüm türlerde gland sayıları *E. pestalozzae* Boiss. haricinde dörttür. Bu türün bazı bireylerinin 5 glandlı olduğu saptanmıştır. Üç farklı gland şekli (eliptik, trapezoid-eliptik veya böbreksi) gözlenmiştir. kapsula yüzeyleri, incelenen türlerin yarısından fazlasında (7 takson) meme başı benzeri çıkıntılarla (papillat-mammillat) kaplı iken, diğer türlerde küçük yuvarlak veya tepecik benzeri yükseltilerle (kollikulat) kaplıdır. İncelenen türlerin çoğunluğunda gövde yaprak yüzeyleri papillat-mammillatır. Kollikulat yüzeyler de iki türde görülmüştür (*E. seguieriana* Necker ve *E. thessala* (Form.) Degen & Dörf.) Sonuç olarak, *Euphorbia* sect. *Pithyusa* türlerine ait gland ve kapsula morfolojik özelliklerinin türlerin ayrımında ayırt edici karakterler olarak kullanılabileceği gösterilmiştir.

Introduction

Euphorbia L. is one of the largest genera of flowering plants with more than 2000 species distributed throughout the world. Life forms within the genus vary from tiny annuals to perennial herbs and trees (Bruyns *et al.* 2006, Horn *et al.* 012). Previous phylogenetic studies on *Euphorbia* yielded results used for separation of the genus into four main clades corresponding four subgenera as *Athymalus* Neck. ex. Rchb., *Chamaesyce* Raf., *Esula* Pers. and *Euphorbia* (Steinmann & Porter 2002, Bruyns *et al.* 2006, Zimmerman *et al.* 2010, Horn *et al.* 2012, Yang *et al.* 2012, Dorsey *et al.* 2013, Peirson *et al.* 2013, Riina *et al.* 2014, Dorsey *et al.* 2015, Dorsey *et al.* 2015, Dorsey *et al.* 2015, Dorsey *et al.* 2015, Dorsey *et al.* 2015, Dorsey *et al.* 2015, Dorsey *et al.* 2015, Dorsey *et al.* 2016, Dorsey *et al.* 2013, Peirson *et al.* 2013, Riina *et al.* 2015, Dorsey

al. 2013). Genus *Euphorbia* is represented in Turkey by two subgenera *Chamaesyce* and *Esula* with a total of 120 taxa (Öztekin 2012, Genç & Kültür 2016).

Subgenus *Esula* comprises about 490 species most of which are annual or perennial herbs mostly distributed in the temperate regions of the northern hemisphere in the Old World (Geltman 2015, Pahlevani *et al.* 2017). Temperate Eurasia, particularly the Mediterranean and the Irano-Turanian regions are two most important diversity centres of *Euphorbia* subgenus *Esula* (Riina *et al.* 2013).

Turkish subgenus *Esula* has recently been embedded and classified, based on recent molecular studies, into 14 sections [*Arvales* (Geltman) Geltman, *Chylogala* (Fourr.) Prokh., *Esula* (Pers.) Dumort., *Exiguae* (Geltman) Riina & Molero, *Helioscopia* Dumort., *Lagascae* Lázaro, *Lathyris* Dumort., *Myrsiniteae* (Boiss.) Lojac., *Pachycladae* (Boiss.) Tutin, *Paralias* Dumort., *Patellares* (Prokh.) Frajman, *Pithyusa* (Raf.) Lázaro, *Szovitsiae* Geltman and *Tithymalus* (Gaertn.) Roep.] (Riina *et al.* 2013).

17 species of the 60 reported species of *Euphorbia* sect. *Pithyusa* occur in Turkey. Turkey is the second most species-rich country after Iran both in the number of species and endemics in sect. *Pithyusa* (Pahlevani 2017). The members of the section are mostly characterized by semisucculent and glaucous leaves, palmate leaf venation, papillose indumentum, usually entire leaf margin, conical or nearly so rounded capsules and elliptic or trapezoidal cyathial glands (Riina *et al.* 2013).

The only comprehensive study on the genus *Euphorbia* in Turkey was performed by Radcliffe-Smith (1982) and the last morphological study concerning the genus was made by Can & Küçüker (2015).

Seed, capsule and cyathial gland morphologies are useful parameters used in distinguishing taxa in *Euphorbia* (Pahlevani & Akhani 2011, Salmaki *et al.* 2011, Riina *et al.* 2013, Pahlevani *et al.* 2015). However, detailed studies are missing especially for most species distributed in Turkey, raising the need of comprehensive studies on seed, capsule and cyathial gland morphologies of Turkish *Euphorbia*. We, therefore, aimed in the present study to provide a detailed description and importance of capsula and cyathial gland morphology of perennial Turkish *Euphorbia* sect. *Pithyusa.* We also investigated the micromorphology of the cauline leaf surfaces after determining some variations on leaf surfaces of herbarium specimens included in the study.

Materials and Methods

<u>Plant Material</u>

The material included in the study was collected by the authors during field trips carried out between 2014 and 2017 in different parts of Turkey. Herbarium specimens are deposited in the Herbarium of the Faculty of Pharmacy of İstanbul University (ISTE). A list of the taxa included in the study was given in Table 1.

Table 1. List of voucher specimens of Euphorbia sect. Pithyusa studied in the study (* Endemic).

Species	Voucher specimens (ISTE)			
E. cheiradenia Boiss. & Hohen.	Şanlıurfa, Tektek Mountain National Park, 460m, 18.vi.2015, İ.Genç 2398, Ş.Kültür.			
E. erythrodon Boiss. & Heldr.	Erzincan, Sipikor mountain pass, 1400m, 25.vii.2015, <i>İ.Genç 2463, A. Kandemir.</i> Antalya, Bozburun mountain, 1600m, 27.vii.2017, <i>İ.Genç 2564, İ.G. Deniz, G. Ecevit Genç.</i>			
<i>E. glareosa</i> Pall. ex Bieb.	Kastamonu, Kastamonu-Karabük, roadsides, 600m, 14.vii.2015, İ.Genç 2418, G. Ecevit Genç.			
E. macroclada Boiss.	Isparta , Isparta-Eğirdir, roadside, 990m, 9.vii.2014, <i>İ.Genç 2210, S. Yüzbaşıoğlu</i> . Sivas , Tokat-Sivas road, near Kızıliniş, roadsides, 700m, 22.vii.2015, <i>İ.Genç 2445</i> . Erzincan -Erzurum road, roadsides, 1510m, 23.vii.2015, <i>İ.Genç 2448</i> . Şanlıurfa , Tektek Mountain National Park, 410m, 18.vi.2015, <i>İ.Genç 2392, Ş.Kültür</i> . Van -Erciş road, near Van Lake, 1750m, 24.vii.2015, <i>İ.Genç 2454</i> .			
E. niciciana Borbás ex Novák	Edirne, Edirne-Uzunköprü, roadside, 70m, 6.vi.2015, <i>İ.Genç 2310, G. Ecevit Genç.</i> Kırklareli, Mahya Mountain, 720m, 17.viii.2014, <i>İ.Genç 2251, G. Ecevit Genç.</i> Adapazarı, Serindere valley, 760m, 11.vii.2014, <i>İ.Genç 2235, S. Yüzbaşıoğlu.</i>			
E. pannonica Host	Kırklareli, Kırklareli-Edirne, roadside, 280m, 5.vi.2015, <i>İ.Genç 2306, G. Ecevit Genç.</i> İstanbul, Dağyenice-Kalfaköy, roadsides, 110m, 18.vii.2016, <i>İ.Genç 2477, G. Ecevit Genç.</i>			
*E. pestalozzae Boiss.	Antalya , Kuhu mountain, Çığlıkara, 1650m, 27.vi.2014, <i>İ.Genç 2168, İ.G.Deniz</i> . Kızlarsivrisi, 1650m, 26.vi.2014, <i>İ.Genç 2161, İ.G.Deniz</i> . Saklıkent, 1780m, 30.vii.2016, <i>İ.Genç 2499</i> .			
E. petrophila C.A.Meyer	Kastamonu, Kastamonu-Sinop, roadsides, 720m, 14.vii.2015, <i>İ.Genç 2419, G. Ecevit Genç.</i> Çankırı, Ilgaz Mountain National Park, 1900m, 14.vii.2015, <i>İ.Genç 2435, G. Ecevit Genç.</i>			
*E. pisidica HubMor.&M.S.Khan	Burdur, Altınyayla-Gölhisar road, Dirmil pass, 1585m, 23.vi.2014, İ. Genç 2114, G. Ecevit Genç.			
E. seguieriana Necker	Iğdır-Doğubeyazıt, roadsides, 1500m, 23.vii.2015, <i>İ.Genç 2451</i> . Ağrı, between Doğubeyazıt-Çaldıran, roadsides, 2600m, 24.vii.2015, <i>İ.Genç 2453</i> . Van, Erciş-Van roadsides, 1650m, 24.vii.2015, <i>İ.Genç 2456</i> .			
E. smirnovii Geltman	Erzincan, Ergan mountain, 1510m, 25.vii.2015, İ.Genç 2468, A. Kandemir.			
E. thessala (Form.) Degen & Dörf.	Kırklareli, Kırklareli-Pınarhisar, roadside, 190m, 5.vi.2015, İ.Genç 2304, G. Ecevit Genç.			
*E. yildirimlii Dinç	Eskişehir, Sivrihisar, Aşağıkepen village, gypsum slopes, 900m, 26.viii.2014, <i>İ.Genç 2267, G. Ecevit Genc.</i>			

The classification based on the recent molecular phylogenetic study was used (Riina *et al.* 2013).

Morphological Investigations

Capsule, cauline leaf and cyathial gland morphologies were investigated as described below. The structures of the cyathial glands were examined using their photographs taken in the natural habitats of the plants or from herbarium materials when needed. Cyathial gland shapes and cyathial gland appendages were regarded as diagnostic characters for each taxon. To ensure stability and limit the range of variation in cyathial gland characters within taxa, several samples were investigated and photographed for each taxa (if available, because some of the studied samples were low in number).

The quantitative capsule characters were measured using different numbers of capsules depending on the material used. The number of capsules ranged from three in one population to a maximum of 24 in four populations because of the limited distribution of some taxa (*E. pisidica, E. smirnovii, E. thessala* and *E. yildirimlii*). For species with wider distributional ranges (e.g. *E. macroclada* and *E. niciciana*), populations from different parts of Turkey were selected. Only mature capsules were used for measurement. The capsules were examined in terms of size (length×width), shape, color and surface structures. Widths of the capsules were measured from the widest point. All measurement data of capsules were obtained under a stereomicroscope (Leica S8APO) with a camera attachment (Leica DFC295).

Sculpturing characteristics of capsule and cauline leaf surfaces were based on investigations of capsules and leaves mounted directly on stubs, attached with double adhesive tape and coated with a gold layer. Morphological observations concerning sculpturing were carried out with a FEI Quanta 450 FEG-EDS scanning electron microscope (SEM). All leaves were scanned from both surfaces. The terminology of macro- and micromorphology were based on Harris & Harris (1994) and Stearn (2004).

Results

The investigations of cyathial glands showed that they vary in shape and colour (see Fig. 1 for all cyathial gland visuals and Table 2 for cyathial gland shapes and the appendages). The majority of the cyathial glands are yellow to yellowish-green in colour while cyathial glands of *E. erythrodon, E. petrophila* and *E. smirnovii* are red to purplish (Figs. 1b, i, 1). Reddish cyathial glands were also seen in *E. cheiradenia* which have normally yellowish cyathial glands (Fig. 1a).

There was no significant difference in cyathial gland size between the taxa. The cyathial gland number of all investigated species except *E. pestalozzae* was found to be four but some *E. pestalozzae* specimens with five cyathial glands were also determined (Fig. 1h). Cyathial gland shapes are elliptic, trapezoid-elliptic or reniform. Some species are hornless but truncate (*E. glareosa, E. niciciana, E. pannonica* and *E. seguieriana*) (Figs, 1c, f, g, k). Most species are characterized by their horned appendages. The horns are either short, as in *E. erythrodon, E. petrophila* and *E. smirnovii* (Figs. 1b, i, 1), long as in *E. pisidica* (Fig. 1j) or lobate and denticulate as in *E. cheiradenia, E. macroclada* and *E. thessala* (Figs. 1a, d, m). *E. thessala* (Fig. 1m) has the longest horns among the studied species. Although it varied for some species, the number of cyathial gland horns is often two. Polymorphism has also been observed in some species, i.e. *E. cheiradenia* (Fig. 1a) and *E. thessala* (Fig. 1m) with two horns or denticulate ones.

The morphological characters related with capsules and cauline leaf surfaces of the studies species are presented in Table 2. Stereomicroscope images of the capsules are given in Fig. 2 and SEM micrographs of the studied species are provided in Figs. 3-4.

According to the morphometric measurements, the longest capsule (c. 6.7mm long) occurred in *E. macroclada* (Fig. 2d) followed by *E. cheiradenia* (Fig. 2a) and *E. pisidica* (Fig. 2i) with c. 5 and 4.75mm, respectively). The shortest capsule (c. 1.9mm long) was determined to be in *E. niciciana* (Fig. 2e). The widest capsule (c. 4.45-5.5mm wide) was seen in *E. macroclada* (Fig. 2d). The narrowest capsule (c. 2.25mm wide) was in *E. petrophila* (Fig. 2h), followed by *E. niciciana* and *E. seguieriana* with capsules about 2.4mm width (Figs. 2e, 2j).

The capsule shapes of the studied species vary from subglobose (*E. glareosa* and *E. niciciana*) to conical (Figs. 1, 2). Capsules of all taxa are trilobate, but the significance of the lobes varies from shallow to deep (Fig. 1, 2).

The micromorphological features of capsule surfaces are summarized in Table 2. The capsule surface is mostly covered with nipple-like projections (papillate-mammillate) (7 taxa) and others are covered with little rounded or hillock-like elevations (colliculate). The capsules of *E. erythrodon* (Fig. 3b), *E. glareosa* (Fig. 3c) and *E. macroclada* (Fig. 3d) are covered with long and nearly acute projections (papillate), while both projection types were observed in *E. pannonica, E. petrophila, E. smirnovii* and *E. yildirimlii*. The type of capsule indumentum varied from glabrous to villous (Fig. 1, 2).

The investigation of leaves showed that dorsal and ventral cauline leaf surface sculptures are correspondent, so only dorsal cauline leaf surface is discussed. Cauline leaf dorsal surface properties are summarized in Table 2. The cauline leaf surfaces are papillate-mammillate in most of the studied species. The colliculate surfaces are also observed in two species, *E. seguieriana* (Fig. 4j) and *E. thessala* (Fig. 4l).

Table 2. The morphological features of capsules, cauline leaf surfaces and cyathial glands of the examined Euphorbia species (w: width; l: length).

Taxa	Capsule size (w×l)	Capsule shape	Capsule surface	Cauline Leaf dorsal surface	Cyathial gland shape
E. cheiradenia	3-3.6×4.8-5	Conical, shallowly trilobate	Colliculate, glabrous	Colliculate- mammillate	Elliptic, two- or multi-horned
E. erythrodon	2.7-3.05×3.25-3.75	Ovoid-conical, trilobate	Papillate, glabrous to sparingly villose	Papillate	Trapezoid-elliptic, hornless or two short horned
E. glareosa	2.5-3.3×2.4-3.2	Subglobose-ovoid, shallowly trilobate	Papillate, glabrous to sparingly villose	Mammillate	Trapezoid-elliptic, hornless
E. macroclada	4.45-5.5×4.2-6.7	Subglobose-ovoid, shallowly trilobate	Papillate, glabrous to villose	Papillate	Elliptic, two lobate or simple horned to denticulate
E. niciciana	2.4-3×1.95-2.5	Subglobose-ovoid, deeply trilobate	Colliculate, glabrous	Mammillate	Trapezoid-elliptic, hornless
E. pannonica	2.6-3.35×2.95-3.75	Ovoid-conical, trilobate	Papillate- mammillate, glabrous to villose	Mammillate	Trapezoid, hornless
E. pestalozzae	3.55-3.65×4.25-4.4	Conical, shallowly trilobate	Colliculate, glabrous	Mammillate	Elliptic-truncate, two horned
E. petrophila	2.25-2.6×2.5-3	Ovoid-conical, shallowly trilobate	Papillate- mammillate, glabrous	Papillate	Truncate-reniform, two horned
E. pisidica	2.5-3.3×3.3-4.75	Conical, shallowly trilobate	Colliculate, glabrous	Mammillate	Elliptic, two- or multi- long horned
E. seguierana	2.4-3.2×2.6-3.2	Subglobose-ovoid, deeply trilobate	Colliculate, glabrous	Colliculate	Trapezoid-elliptic, hornless
E. smirnovii	2.6-3.7×3.6-3.75	Ovoid-conical, shallowly trilobate	Papillate- mammillate, glabrous	Papillate- mammillate	Trapezoid, two short horned
E. thessala	2.95-3.5×3.75-4.1	Conical, shallowly trilobate	Colliculate, glabrous	Colliculate	Trapezoid-elliptic, two horned
E. yildirimlii	3.05-4.1×2.3-4.2	Subglobose-ovoid, shallowly trilobate	Papillate- mammillate, glabrous to sparingly villose	Papillate- mammillate	Elliptic, two short horned

The density of the projections of cauline leaf surfaces decreased by aging of the plants. For example, the immature individuals of E. cheiradenia are covered with dense mammillate projections, whereas the mature ones are covered with colliculate ornamentations. With the exception of E. pisidica with pubescent leaves, the remained taxa are glabrous (Fig. 4i). Capsule and cauline leaf surfaces of all investigated taxa are covered with epicuticular wax secretion forming fine platelets. As the plant matures, surface secretions may be eroded (Figs. 3n, 4n).



Fig. 1. Photographs of cyathial glands of studied taxa: a. E. cheiradenia, b. E. erythrodon, c. E. glareosa, d. & e. E. macroclada, f. E. niciciana, g. E. pannonica, h. E. pestalozzae, i. E. petrophila, j. E. pisidica, k. E. seguierana, l. E. smirnovii, m. E. thessala, n. E. yildirimlii.



Fig. 2. Stereomicroscopic micrographs of capsules of studied taxa: a. E. cheiradenia, b. E. erythrodon, c. E. glareosa, d. E. macroclada, e. E. niciciana, f. E. pannonica, g. E. pestalozzae, h. E. petrophila, i. E. pisidica, j. E. seguierana, k. E. smirnovii, l. E. thessala, m. E. yildirimlii.



Fig. 3. Scanning electron micrographs of capsule surface of studied taxa: **a.** *E. cheiradenia*, **b.** *E. erythrodon*, **c.** *E. glareosa*, **d.** *E. macroclada*, **e.** *E. niciciana*, **f.** *E. pannonica*, **g.** *E. pestalozzae*, **h.** *E. petrophila*, **i.** *E. pisidica*, **j.** *E. seguierana*, **k.** *E. smirnovii*, **l.** *E. thessala*, **m.** *E. yildirimlii*, **n.** epicuticular wax.

Fig. 4. Scanning electron micrographs of cauline leaf surface of studied taxa: a. E. cheiradenia, b. E. erythrodon, c. E. glareosa, d. E. macroclada, e. E. niciciana, f. E. pannonica, g. E. pestalozzae, h. E. petrophila, i. E. pisidica, j. E. seguierana, k. E. smirnovii, l. E. thessala, m. E. yildirimlii, n. epicuticular wax.

Discussion

Based on capsule micromorphology, two groups are recognized, one including species with colliculate capsule surface (*E. cheiradenia, E. niciciana, E. pestalozzae, E. pisidica, E. seguierana* and *E. thessala*) and the other with species with papillate-mammillate capsule surface. According to the result of this study, micromorphology of capsule surfaces is more useful as an identification character compared to the cauline leaf surfaces because the cauline leaf surface properties vary as the plant matures.

Members of section *Pithyusa* in Iran are characterized by a conical capsule shape (Pahlevani *et al.* 2015), however subglobose and ovoid capsule shapes are recorded in our present study.

Our results related to capsule sizes correspond to the results of Pahlevani *et al.* (2015) about the species with wider distribution ranges which also occur in Iran (*E. cheiradenia, E. glareosa, E. macroclada* and *E. seguieriana*). However, the capsule shape of *E. glareosa* and *E. seguieriana* differs from Iranian members. According to Pahlevani *et al.* (2015) these two species have conical shallowly trilobate capsules but the specimens in Turkey have subglobose clearly trilobate capsules.

The results show that capsule size may be used to determine some species morphologically close to each other. For example, *E. smirnovii* can be distinguished from *E. petrophila* with larger capsules as in the case of *E. macroclada* and *E. glareosa*. Morphology of cyathial glands shapes, their colour and appendages have been

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useful tools for identifications of some species. The cyathial gland shapes are elliptic or trapezoid-elliptic for all investigated taxa, except *E. petrophila*. The gland shape is truncate-reniform of this species (Fig. 1i).

In terms of cyathial gland appendage characteristics, intraspecific variation was most commonly seen in *E. macroclada*. The appendages of this species vary from not very distinct horns to lobate-multifid horns. No similar variation is observed in other species.

The number of cyathial glands of sect. *Pithyusa* is reported as four by Riina *et al.* (2013). On the other hand, it has been determined that some specimens of *E. pestalozzae* have five cyathial glands in this study.

The variation in the number of horns of *E. cheiradenia* cyathial glands has been previously reported by Salmaki *et al.* (2011). But no colour variation was reported. Reddish cyathial glands, in addition to yellow cyathial glands were also observed in some specimens of *E. cheiradenia* included in our present study.

The observed morphological characters of capsule and cyathial glands can be used as effective diagnostic characters to separate some close species (eg. *E. macroclada-E. yildirimlii; E. petrophila-E. smirnovii; E. pannonica-E. glareosa*) in the sect. *Pithyusa* of the genus *Euphorbia*.

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