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Karyological analysis of twelve *Euphorbia* species from Turkey

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Abstract. Karyotypes of 12 *Euphorbia* species were studied and described for the first time; *Euphorbia cheiradenia, E. pannonica, E. pestalozzae, E. petrophila, E. pisidica, E. thessala* and *E. yildirimli*. Karyological analyses indicate relationships among the species with respect to their asymmetry indices. Most of the investigated taxa are diploids with 2n = 2x = 18. *E. macroclada* and *E. smirnovii* showed tetraploid cytotypes 2n = 4x = 36. All karyotypes are symmetrical, consisting of metacentric and submetacentric chromosomes. The chromosomes range in size from 0.79 µm to 2.20 µm. The total haploid chromosome length (THL) ranges from 8.75 µm (*E. terracina*) to 16.78 µm (*E. petrophila*). Principal Coordinate Analysis with five uncorrelated parameters was performed to determine the karyological relationships among the taxa.

Keywords: chromosome number, karyotype asymmetry, *Pithyusa*, *Esula*, tetraploid, karyosystematics.

INTRODUCTION

Genus *Euphorbia* (Euphorbiaceae), with over 2000 species, is the second-largest genus of flowering plants in the world (Bruyns *et al.* 2006; Horn *et al.* 2012). The use of *Euphorbia* species as medicine and poison are of greatest biocultural importance and the most-valued medicinal use of *Euphorbia* species is in the treatment of digestive and respiratory complaints, inflammation and injuries (Ernst *et al.* 2015). Additionally, *Euphorbia* taxa have been used in Turkish folk medicine to treat rheumatism, swelling and especially as a wart remover (Baytop 1984). There are also various biological effects as antioxidant, antimicrobial and wound healing activities (Barla *et al.* 2006, 2007; Özbilgin *et al.* 2018, 2019).

The genus is represented in Turkey by two subgenera, E. subg. *Chamaesyce* Raf. and E. subg. *Esula* Pers., with a total of 120 taxa (Öztekin 2012; Genç and Kültür 2016). *Euphorbia* subg. *Esula* comprises about 480 species, most of which are annual or perennial herbs (Riina *et al.* 2013). According to the recent classification of Riina *et al.* (2013), the subgenus is represented by 14 sections in the Turkey (Genç and Kültür 2018).

The chromosome numbers in these two subgenera are very variable. Various chromosome numbers have been reported for the subg. *Esula* (2n = 10,

12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 36, 40, 42, 44, 56, 60, 64, 72) and sect. *Pithyusa* (2n = 16, 18, 28, 36, 40, 72) worldwide (Riina *et al.* 2013).

The chromosome numbers of 64 taxa, which are distributed in Turkey naturally have not been determined yet. Up to now, the chromosome numbers of 12 *Euphorbia* taxa were reported from Turkey (Radcliffe-Smith 1976; Strid 1987; Kesercioglu *et al.* 1990; Iyer 1991; Vicens *et al.* 1991; Rice *et al.* 2015; Genç and Kültür 2017). Considering that the number of chromosomes of the vast majority of these species is not yet clear, there is a remarkable gap in this area.

This study aimed to determine the chromosome numbers and karyomorphology of 11 perennial *Euphorbia* sect. *Pithyusa* species distributed in Turkey. In addition, *E. terracina* (sect. *Pachycladae*) was also included in the present study as an external group.

MATERIALS AND METHODS

Twelve *Euphorbia* species were analysed in this study. Seeds of 12 species were collected from mature capsules in their natural habitats as given in Table 1. Voucher specimens are deposited in İstanbul University, Faculty of Pharmacy Herbarium (ISTE). For each population, 9-27 mature seeds were selected from at least 5 individuals (inflorescence). The seeds germinated on wet filter paper in petri dishes at room temperature.

The root tips meristems were excised at 9-9.30 am. Mitotic chromosomes were prepared from root tips and pre-treated with 8-Hydroxyquinoline at +4 °C for 24 h. Roots were fixed for a minimum of 2 h in absolute ethanol:glacial acetic acid, (3:1,v/v), hydrolysed at 60 °C in 1 N HCl for 15 min and stained with Feulgen reagent. Finally, root tips were squashed in a drop of 1% aceto-orcein. Permanent microscopic slides were prepared with Entellan (Rapid Mounting Medium / Merck Darmstadt Germany). Microphotographs of good quality metaphase plates were taken using an Olympus BX53 (Tokyo, Japan) microscope equipped with a high-resolution digital camera. Metaphase observations and chromosome measures were made using the image analysis systems Kameram (Argenit Microsystems, İstanbul, Turkey). The chromosome number and karyotype details were studied in five to fourteen well-spread metaphase plates from different individuals; mean values were used for the analysis.

Chromosome pairs were identified and arranged on the basis of chromosome/chromatid length and any other evident karyomorphological features. The nomenclature used for describing karyotype followed Levan *et*

Table 1. Localities and voucher data of *Euphorbia* taxa examined in the present study.

Species	Voucher specimens	Previous counts (Rice *et al.* 2014)
E. cheiradenia Boiss. & Hohen.	Şanlıurfa, Tektek Mountain National Park, 460m, 18.6.2015, İ.Genç *2398,* Ş.Kültür.	--
E. macroclada Boiss.	Eskişehir-Antalya road, roadsides, 10.8.2015, İ.Genç *2472.*	18
E. niciciana Borbás ex Novák	Karabük, Bolkuş village, 220 m, 03.vi.2015, İ.Genç *2290, G. Ecevit Genç.*	18, 18+1
E. pannonica Host	İstanbul, Dağyenice-Kalfaköy, roadsides, 110m, 18.vii.2016, İ.Genç *2477, G. Ecevit Genç.*	--
**E. pestalozzae* Boiss.	Antalya, Saklıkent, 1780m, 30.vii.2016, İ.Genç *2499.*	--
E. petrophila C.A.Meyer	Kastamonu, Kastamonu-Sinop roadsides, 720m, 14.vii.2015, İ.Genç *2419, G. Ecevit Genç.*	--
**E. pisidica* Hub.-Mor. & M.S.Khan	Burdur, Altınyayla-Gölhisar road, Dirmil pass, 1585m, 23.vi.2014, İ.Genç *2114, G. Ecevit Genç.*	--
E. seguieriana Necker	Ağrı, between Doğubeyazıt-Çaldıran, roadsides, 2600m, 24.vii.2015, İ.Genç *2453.*	16, 18, 40
E. smirnovii Geltman	Erzincan, Ergan mountain, 1510m, 25.vii.2015, İ.Genç *2468, A. Kandemir.*	18 (Genç & Kültür 2017)
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, İ.Genç *2267, G. Ecevit Genç.*	--
E. terracina L.	Bursa, Mudanya, Söğütlüpınar village, s.l., 7 vıı 2014, İ.Genç *2192, S. Yüzbaşıoğlu*	18, 20, 36

* The species endemic to Turkey.

Table 2. PCoA graphic symbols of some important taxonomic characteristics for *Euphorbia*.

	Seed surface smooth	Seed surface not smooth
Cyathial glands horned	●	■
Cyathial glands hornless	●	■

al. (1964). To determine the karyological relationships among taxa, we performed Principal Coordinate Analysis (PCoA) with five uncorrelated parameters as suggested by Peruzzi and Altınordu (2014). These parameters are basic chromosome number (x), total haploid length (THL), mean centromeric asymmetry (M_{CA}) it is calculated as the mean (L-S)/(L+S)×100 where, for each chromosome, L is the length of long arm and S is the length of short arm; coefficient of variation of chromosome length (CV_{CL}=(L+S)×100) and coefficient of variation of centromeric index (CV_{CI} =S/(L+S) ×100) (Paszko 2006; Peruzzi *et al.* 2009; Zuo and Yuan 2011; Peruzzi and Eroğlu 2013). The software Past 3.03 (Hammer *et al.* 2001; Hammer 2018) was used to perform this analysis.

Seed and gland morphology in the classification of the genus *Euphorbia* is one of the most important taxonomic characters. Therefore, in order to evaluate the resulting PCoA graphic in terms of the seed and gland morphological characters, some symbols are given to the studied species (Table 2).

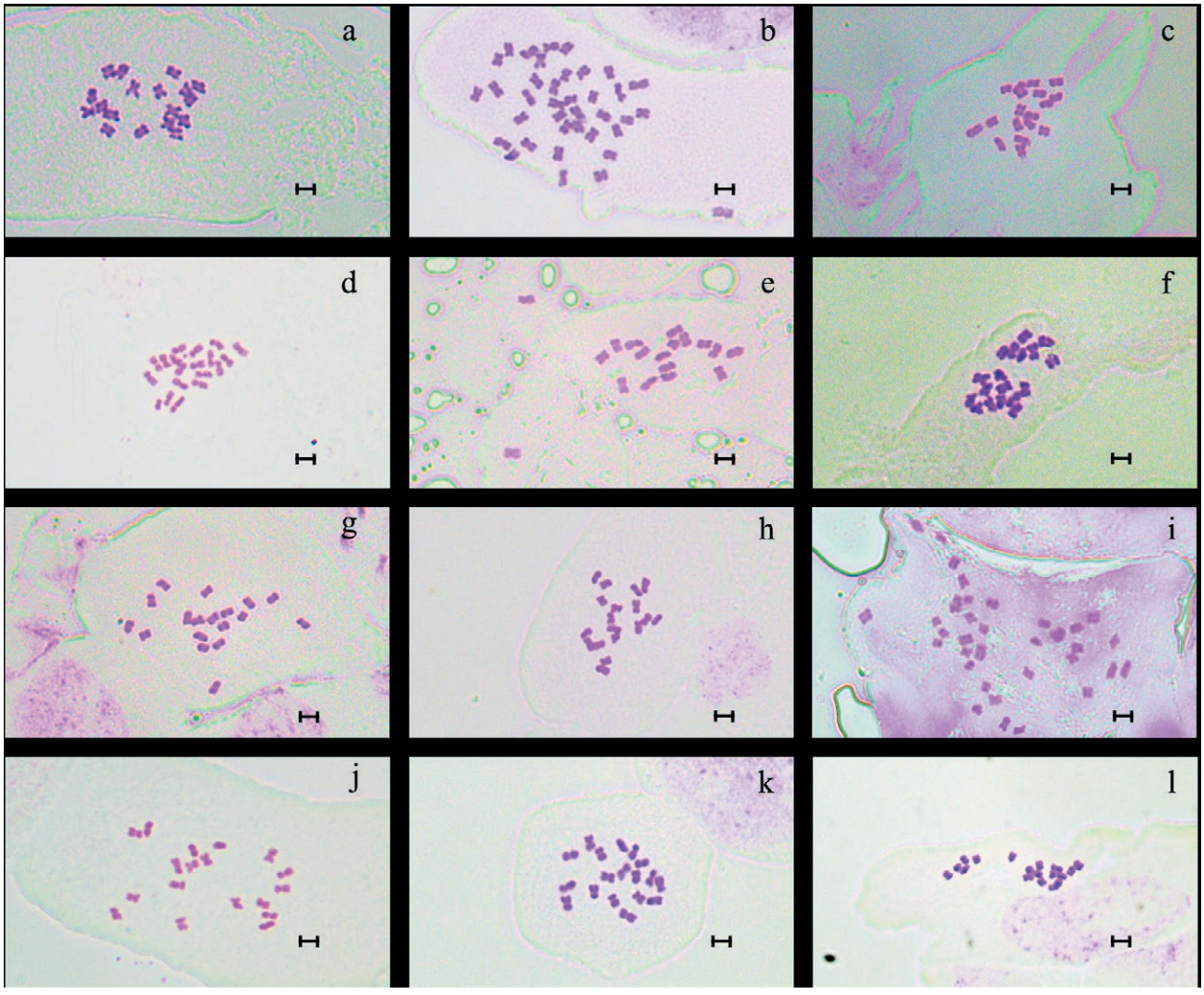

Figure 1. Somatic chromosomes of the studied taxa. (a) *E. cheiradenia*; (b) *E. macroclada*; (c) *E. niciciana*; (d) *E. pannonica*; (e) *E. pestalozzae*; (f) *E. petrophila*; (g) *E. pisidica*; (h) *E. seguieriana*; (i) *E. smirnovii*; (j) *E. thessala*; (k) *E. yildirimli*; **(l)** *E. terracina.* (Scale bars 2 *μm*).

Mitotic metaphase chromosomes are given in Figure 1. Ideograms of these taxa are arranged in order of centromere position and then decreasing the length of homologue chromosome pairs (Figure 2).

RESULTS

Karyomorphological details (shortest chromosome length; longest chromosome length; mean long arm length; mean short arm length; karyotype formula) of 12 species of *Euphorbia* are listed in Table 3. The chromosome numbers, total haploid length, mean centromeric asymmetry, coefficient of variation of chromosome length and the coefficient of variation of centromeric index of the species are also summarized in Table 4. Metaphase plates and their related ideograms of the studied species are presented in Figures 1 and 2.

Karyotype analysis revealed the basic chromosome number x=9 for all species. Most of the species showed diploid cytotypes 2n = 18 but *E. macroclada* and *E. smirnovii* showed tetraploid cytotypes 2n = 4x = 36 (Figure 1).

The chromosomes were mainly metacentric with centromeres localized in the median position. The karyotypes of four species (*E. cheiradenia, E. macroclada, E. smirnovii, E. yildirimli*) included one submetacentric chromosome. Only karyotype of *E. petrophila* included two submetacentric chromosomes.

The ratio of the shortest and the longest chromosome lengths ranged from 0.79 µm (*E. smirnovii*) to 2.20 µm (*E. petrophila*). The ratio of the shortest and the longest total haploid chromosome length (THL) ranged from 8.75 µm to 16.78 µm in *E. terracina* and *E. petrophila,* respectively. The smallest mean short arm length (q) was observed in *E. terracina* (0.44 µm) and *E. seguieriana* (0.51 µm), the largest mean long arm length (p) was observed in *E. petrophila* (1.07 µm) (Table 3).

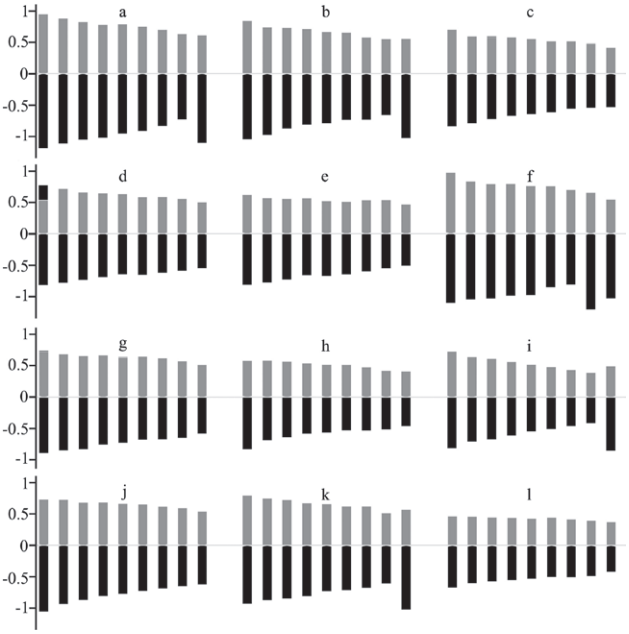

Figure 2. Haploid ideograms of the studied taxa. (a) *E. cheiradenia*; (b) *E. macroclada*; (c) *E. niciciana*; (d) *E. pannonica*; (e) *E. pestalozzae*; (f) *E. petrophila*; (g) *E. pisidica*; (h) *E. seguieriana*; (i) *E. smirnovii*; (j) *E. thessala*; (k) *E. yildirimli*; (l) *E. terracina.*

Table 3. Karyotype analysis of the investigated *Euphorbia* taxa.

	SC–LC	q (µm) Mean (±SD)	p (µm) Mean (±SD)	p+q Mean (±SD)	Karyotype formula
E. cheiradenia	1.35–2.12	0.76(±0.10)	0.98(±0.14)	1.75(±0.22)	16 m + 2 sm
E. macroclada	1.21–1.89	0.67(±0.09)	0.85(±0.13)	1.53(±0.20)	32 m + 4 sm
E. niciciana	0.94–1.53	0.55(±0.08)	0.65(±0.10)	1.20(±0.17)	18 m
E. pannonica	1.05–1.60	0.63(±0.08)	0.68(±0.08)	1.31(±0.16)	18 m
E. pestalozzae	0.98–1.44	0.54(±0.04)	0.67(±0.09)	1.21(±0.13)	18 m
E. petrophila	1.59–2.20	0.79(±0.12)	1.07(±0.12)	1.86(±0.18)	14 m + 4 sm
E. pisidica	1.10–1.64	0.63(±0.06)	0.75(±0.10)	1.38(±0.16)	18 m
E. seguieriana	0.88–1.42	0.51(±0.06)	0.61(±0.11)	1.11(±0.16)	18 m
E. smirnovii	0.79–1.52	0.52(±0.10)	0.62(±0.14)	1.14(±0.22)	32m + 4 sm
E. thessala	1.15–1.78	0.65(±0.06)	0.79(±0.13)	1.44(±0.19)	18 m
E. yildirimli	1.13–1.74	0.66(±0.08)	0.80(±0.13)	1.47(±0.18)	16 m + 2 sm
E. terracina	0.80–1.14	0.44(±0.03)	0.54(±0.07)	0.97(±0.09)	18 m

Abbreviations:**SC**: the shortest chromosome length; **LC**: the longest chromosome length; **p**: mean long arm length; **q**: mean short arm length; **SD**: standard deviation; m: metacentric; sm: submetacentric.

Table 4. Chromosome numbers and karyo-morphometric parameters, symmetry indices for investigated taxa.

	2n	x	THL	M_{CA}	CV_{CL}	CV_{CI}
E. cheiradenia	18	9	15.71	12.56	12.51	6.82
E. macroclada	36	9	13.73	11.37	12.89	7.73
E. niciciana	18	9	10.78	8.43	14.61	3.34
E. pannonica	18	9	11.77	4.17	12.40	1.53
E. pestalozzae	18	9	10.86	10.52	10.87	5.16
E. petrophila	18	9	16.78	14.90	9.49	10.44
E. pisidica	18	9	12.41	8.06	11.25	2.99
E. seguieriana	18	9	10.01	8.49	14.33	4.73
E. smirnovii	36	9	10.30	7.71	19.65	7.96
E. thessala	18	9	12.93	9.56	13.16	4.64
E. yildirimli	18	9	13.21	9.37	12.35	7.54
E. terracina	18	9	8.75	9.85	9.76	3.93

Abbreviations: **THL**: total haploid length; **M_{CA}**: mean centromeric asymmetry; **CV_{CL}**: coefficient of variation of chromosome length; **CV_{CI}**: coefficient of variation of centromeric index.

Euphorbia petrophila, with relatively high intrachromosomal (M_{CA}= 14.90) and *E. pannonica* (4.17) with low intrachromosomal asymmetry, also *E. smirnovii* with high interchromosomal (CV_{CL}=19.65) and *E. petrophila* (9.49) low interchromosomal asymmetry were observed. Intrachromosomally, seed surface smooth and cyathial glands horned species are more asymmetrical than seed surface smooth and cyathial glands hornless species (Table 4).

Twelve *Euphorbia* species were analysed by PCoA (cumulative variance explained by the first two axes: 88.21%). And, the species with the same seed and cyathial gland morphology tend to cluster together except *E. smirnovii* (Figure 3). The most important characters in recognizing these groups as distinct resulted THL, M_{CA} and CV_{CL}.

DISCUSSION

The number, size, and asymmetry of chromosomes are important characteristics that help explain the phylogenetic relationships of species (Eroğlu *et al.*, 2013).

Karyotype data for seven taxa (3 endemic) are reported for the first time in the present study.

Various chromosome numbers have been reported for the sect. *Pithyusa* (2n=16, 18, 28, 36, 40, 72) (Riina et al 2013). However, most of the investigated taxa in this study have the same chromosome number (2n=18), only *E. macroclada* and *E. smirnovii* showed tetraploid cytotypes 2n = 4*x* = 36. According to Riina *et al.* (2013) chromosome number of *E. terracina* is 18 and our results supported this report. However, even though *E. terracina* has 18 chromosomes, it differs from the other investigated taxa in smaller chromosomes (THL=8.75).

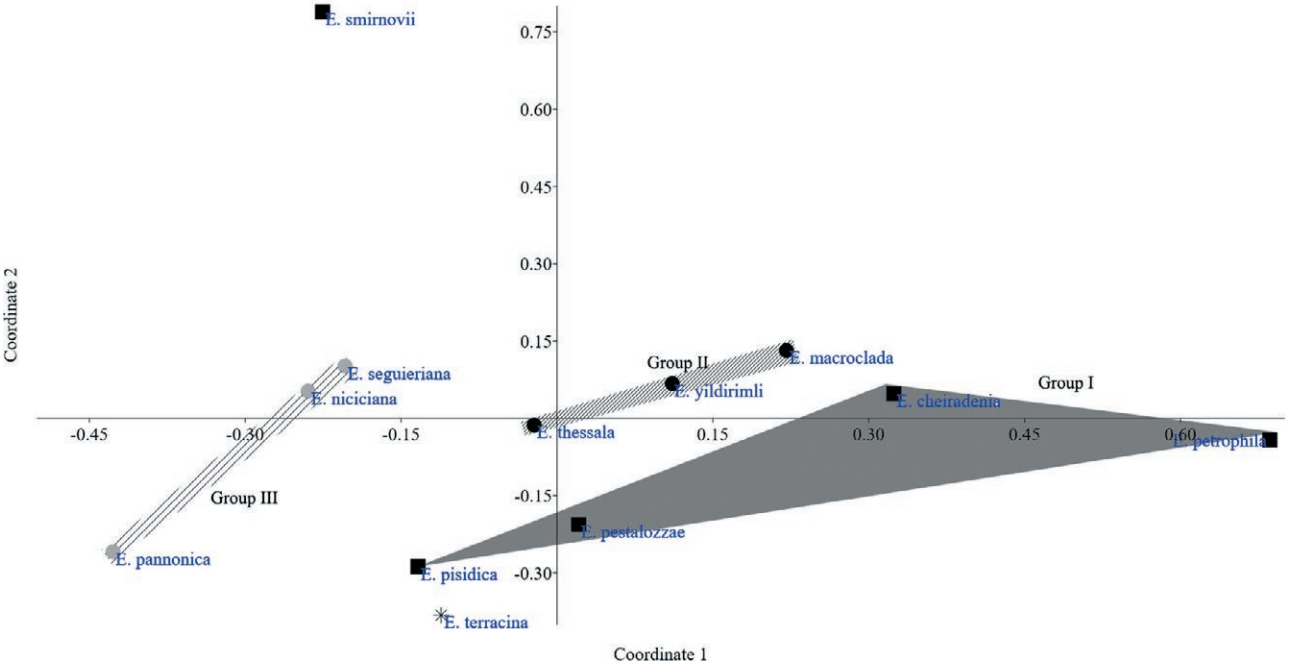

Figure 3. PCoA analysis based on five quantitative karyological parameters of investigated taxa.

The chromosome number of *E. macroclada* was reported 2n = *2x* = 18 by Lessani and Chariat-panahi (1979) and Chariat-Panahi *et al.* (1982). Tetraploid cytotype is reported for the first time for this species. Different chromosome numbers were reported for *E. seguieriana* subsp. *seguieriana* (2n= 16, 18, 40) (Rice *et al.* 2015). In this study, the chromosome number of this species is reported to be 2n = 2*x* = 18.

Euphorbia terracina is a bit separated from other taxa according to the PCoA analysis. So, our results support the position of *E. terracina* in a distinct section (E. sect. *Pachycladae*), as suggested by Riina *et al.* (2013).

Three groups are examined in PCoA graph as **group I** (taxa with seed surface not smooth and cyathial glands horned), **group II** (taxa with seed surface smooth and cyathial glands horned) and **group III** (taxa with seed surface smooth and cyathial glands hornless). But *E. smirnovii* is also partially out of these groups (Figure 3).

As a conclusion, further studies on the chromosome morphology of *Euphorbia* taxa should be performed. Thus, it can be seen more clearly how species are grouped according to the chromosome morphology. In our opinion, studies on the chromosome morphology of *Euphorbia* will contribute to the taxonomy of the genus.

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