



Morphological characterization of *Cyclamen* sp. grown naturally in Turkey: Part I



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ABSTRACT

Twenty cyclamen taxa belonging to the *Primulaceae* originate from the Mediterranean region and grow under trees and bushes. There are 10 cyclamen species growing naturally in Turkey, five of which are endemic. In this study, intact cyclamen plants were collected from nature with their tubers intact. Collection took place in spring and autumn in Adana, Osmaniye, Kahramanmaraş, İzmir and Eskişehir provinces, 50 samples per location. Sampled plants were propagated and cultivated in Adana. The morphology of four *Cyclamen* species (*C. persicum* Mill., *C. cilicium* Boiss.e.Heldr., *C. pseudibericum* Hildebr. and *C. coum* Mill.) was characterized using 1-year-old regenerated plants. A total of 27 phenotypic characters (13 flower, 11 leaf, 2 plant, 1 tuber) were evaluated based on a detailed descriptor's list. In addition to these morphological observations, 13 quantitative traits (7 flower, 5 leaf, 1 tuber) were measured. The measurements related to morphological characteristics had a wide range of variation, including in tuber diameter, leaf length and width, petal length, petal color, and leaf shape, indicating the vast morphological differences among these four cyclamen species. In most cases, the use of principal component analysis confirmed the grouping of characters into species-specific clusters although one or two clusters could not differentiate species, indicating that morphological and cluster analyses alone are not enough for characterizing this complex *Cyclamen* germplasm and that molecular techniques may reveal more intricate and useful relationships.

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1. Introduction

Turkey has an exceptionally diverse topographical structure, resulting in differing climatic features. Turkey is also at the junction point of three great floristic regions (Euro-Siberian, Mediterranean and Irano-Turanian) and is one of the world's richest countries in terms of plant species diversity (Atalay, 1994; Güner et al., 2000; Atalay, 2002; Yilmaz et al., 2003). From 12,476 wild plant species in Turkey, 4080 are endemic (i.e., 32.7%) (Karagöz et al., 2010).

The genus *Cyclamen* is traditionally classified into the *Primulaceae* family, but in recent years it has been reclassified in the *Myrsinaceae* family and cultivated throughout temperate zones (Jalali et al., 2012). Containing only 22 species (Grey-Wilson, 2003), or 21 species according to

Compton et al. (2004) and Yesson and Culham (2006), the genus is relatively small, but is an attractive one. The horticulturally important genus *Cyclamen* has been the focus of an unusual amount of taxonomic activity over the last 85 years. Nine different classifications have been reported for this genus (Schwarz, 1938, 1955, 1964; Grey-Wilson, 1988; Anderberg, 1994; Anderberg et al., 2000; Clennett, 2002; Grey-Wilson, 2003).

Cyclamen species are perennial plants with a tuberous rootstock, nodding flowers and corolla lobes that have a contorted bud whose petals reflex upon anthesis (Grey-Wilson, 1988; Takamura, 2006). They undergo an annual cycle of growth and rest, usually with the plant dying down to the ground level for a part of the year (Lazarević and Lazarević, 2010). Most cyclamen species have distinct climatic niches, with the exception of several wide-ranging, geographically expansive species (Yesson and Culham, 2006). Their distribution in the wild is focused in the Mediterranean (Lebanon, Syria, Turkey, Cyprus, Greece, Israel, and Iran) and North Africa (Dole and Wilkins, 1999; Aalaey et al., 2007; Schwartz-Tzachor et al., 2008), from sea level to 2400 m (Grey-Wilson, 1988).

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Table 1Locations, co-ordinates, elevation and vegetation of *Cyclamen* accessions collected from Turkey.

<i>Cyclamen</i> species	Collection site	Sampling date	Coordinates	Elevation (m)	Vegetation
<i>C. cilicium</i> var. <i>cilicium</i>	Pozantı/ADANA	26.11.2010	37°28'34"N 034°54'16"E	1058	Woodland and shrubland dominated by <i>Pinus brutia</i> and <i>Cistus</i> sp.
	Aladağ/ADANA	26.01.2011	37°32'47"N 035°24'10"E	782	
<i>C. persicum</i>	Ç.Ü. Kampüsü/ADANA Yumurtalık/ADANA	20.01.2010	37°03'11"N 035°20'36"E	100	Woodland and shrubland dominated by <i>Pinus brutia</i> , <i>Quercus macrolepis</i> , <i>Erica</i> spp. and <i>Olea europea</i>
	Çeşme/İZMİR	23.02.2011	36°49'06"N 035°34'08"E	92	
		05.04.2011	38°19'47"N 026°18'53"E	78	
<i>C. pseudibericum</i>	Aladağ /ADANA	26.01.2011	37°35'33"N 035°16'44"E	917	Woodland and shrubland dominated by <i>Platanus orientalis</i> , <i>Juniperus drupacea</i> , <i>Quercus coccifera</i> , <i>Styrax officinalis</i>
	Feke/ADANA	16.03.2011	37°46'15"N 035°54'19"E	825	
	Andırın Başkonuş/KAHRAMANMARAŞ	29.05.2011	37°33'0"N 036°35'0"E	1320	
<i>C. coum</i> var. <i>coum</i>	Ürün Köyü/OSMANIYE	03.02.2011	37°01'25"N 036°16'07" E	705	Woodland and shrubland dominated by <i>Platanus orientalis</i> and <i>Pinus brutia</i>
	İnönü/ESKİŞEHİR	04.04.2011	39°47'0"N 030°31'0" E	811	

Ten cyclamen species grow naturally in Turkey and six of them are endemic (*C. pseudibericum* Hildebr., *C. trochopteranthum* O. Schwarz, *C. parviflorum* Pobed., *C. cilicium* Boiss.e.Heldr., *C. cilicium* var. *intaminatum* Miekle Grey-Wilson, and *C. mirabile* Hildebr.). Flowering season varies with the species: for example, *C. persicum*, *C. pseudibericum*, *C. coum*, *C. trochopteranthum* and *C. parviflorum* flower in spring while *C. hederifolium* Aiton., *C. graecum*, *C. cilicium*, *C. cilicium* var. *intaminatum* and *C. mirabile* flower in autumn. The leaves, which have long petioles arising from the crown, are elliptic or orbiculate in shape, and leaf margins are toothed to different degrees. Flowers are solitary on leafless scapes, pedicels are long (5–20 cm), they have five reflexed petals, their color can be white, pale rose, pale to deep pink, carmine, purplish, red, and crimson and they can show marked spots at their base. The fruit is a capsule, often drawn down to the soil level by the twisting of the floral stalk. According to the species, the roots are mainly produced on the upper part of the tuber or at the base. Chromosome numbers vary across species, for example *C. persicum* ($2n = 48$), *C. hederifolium* Aiton ($2n = 2x = 34$, $4x = 68$ or $6x = 102$), *C. graecum* ($2n = 84$), *C. coum*, *C. pseudibericum*, *C. cilicium*, *C. mirabile*, *C. parviflorum* ($2n = 30$), or *C. repandum* ($2n = 20$) (Cyclamen Society, 2008).

The evaluation of genetic diversity can be performed by morphological, molecular and biochemical markers (Chahal and Gosal, 2002). Molecular methods support classic methods such as the analysis of morphological and physiological traits (Dolezalova et al., 2003). The objective of this study was to characterize the morphological traits of Turkish *Cyclamen* species growing in natural conditions and to analyze their relatedness using cladistic analyses.

2. Materials and methods

2.1. Plant material

Whole cyclamen plants were collected with their tubers intact from locations where they were known to grow naturally in winter, spring and autumn in Adana (Cukurova University campus area, Feke, Aladağ, Yumurtalık and Pozantı towns) and Osmaniye (Ürün and Çiftmazı village), Kahramanmaraş (Andırın-Başkonuş plateau), İzmir (Selçuk), and Eskişehir (Table 1, Fig. 1). The altitudes of collecting sites were determined with a GPS device (Magellan SporTrak Color, 980616–20, San Dimas, CA, USA). Plants were identified using the 'Flora of Turkey' (Davis, 1965–1985; Davis et al., 1988; Güner et al., 2000). A total of 50



Fig. 1. Four endemic Turkish *Cyclamen* species collected and examined in this study. (A) *C. pseudibericum* (Aladağ, Adana); (B) *C. persicum* (Ç.Ü. campus, Adana); (C) *C. coum* (Ürün village, Osmaniye); (D) *C. cilicium* (Pozantı, Adana).

Table 2

Descriptor used for morphological characterization in *Cyclamen* sp. Each accession is represented by two values: absolute number of accessions (left column) and percentage (right column).

Traits	Description	Ranges	Values	<i>C. pseudibericum</i>	<i>C. cilicium</i>	<i>C. persicum</i>	<i>C. coum</i>	Mean					
<i>Flowers</i>													
1. Petal length (mm)	Short	≤15	1	2	1.5	13	13.3	–	–	51	100	66	15.0
	Medium	15 < a < 25	3	120	88.2	82	83.7	65	42.2	–	–	267	60.8
	Long	≥25	5	14	10.3	3	3.1	89	57.8	–	–	106	24.2
2. Petal width (mm)	Small	≤3	1	–	–	–	–	–	–	–	–	–299	–
	Medium	3 < a < 10	3	32	23.5	98	100	121	78.6	48	94.1	140	68.1
	Large	≥10	5	104	76.5	–	–	33	21.7	3	5.9	–299	31.9
3. Petal color	White		1	–	–	3	3.1	96	62.3	–	–	99	22.6
	Diffuse-pink		3	2	1.5	61	62.2	29	18.8	4	7.8	96	21.9
	Pale-pink		5	11	8.1	34	34.7	29	18.8	10	19.6	84	19.1
4. Basal corolla ring color	Deep-pink		7	123	90.4	–	–	–	–	37	72.5	160	36.4
	Carmine		9	–	–	–	–	–	–	–	–	–	–
	White		1	135	99.3	10	10.2	–	–	44	86.3	188	44.0
5. Color of upper corolla	Diffuse-pink		3	–	–	–	–	1	0.6	–	–	1	0.2
	Pale-pink		5	–	–	7	7.1	5	3.2	5	9.8	17	4.0
	Deep-pink		7	1	0.7	70	71.4	148	96.1	–	–	219	51.3
6. Color of lower corolla	Carmine		9	–	–	–	–	–	–	2	3.9	2	0.5
	White		1	3	2.2	–	–	–	–	–	–	3	0.7
	Diffuse-pink		3	–	–	–	–	–	–	–	–	–	–
7. Basal corolla ring diameter (mm)	Pale-pink		5	2	1.5	–	–	1	0.6	–	–	3	0.7
	Deep-pink		7	6	4.4	83	84.7	5	3.2	1	2.0	95	21.6
	Carmine		9	125	91.9	15	15.3	148	96.1	50	98.0	338	77.0
8. Pistil length (mm)	White		1	3	2.2	–	–	–	–	2	3.9	5	1.1
	Diffuse-pink		3	–	–	6	6.1	1	0.6	–	–	7	1.6
	Pale-pink		5	1	0.7	1	1.0	6	3.9	–	–	8	1.8
9. Stamen length (mm)	Deep-pink		7	6	4.4	76	77.6	147	95.5	1	2.0	230	52.4
	Carmine		9	126	92.6	15	15.3	–	–	48	94.1	189	43.1
	Narrow	≤2	1	–	–	12	12.2	–	–	2	3.9	14	3.2
10. Flower numbers/plant	Medium	2 < a < 5	3	122	89.7	86	87.8	59	38.3	48	94.1	315	71.8
	Broad	≥5	5	14	10.3	–	–	95	61.7	1	2.0	110	25.0
	Short	≤3	1	–	–	1	1.0	–	–	–	–	1	0.2
11. Pedicel lenght (cm)	Medium	3 < a < 6	3	7	5.1	84	85.7	23	14.9	46	90.2	160	37.3
	Long	≥6	5	129	94.9	13	13.3	131	85.1	5	9.8	268	62.5
	Short	≤2	1	–	–	–	–	–	–	–	–	–	–
12. Darker petal margin	Medium	2 < a < 5	3	13	9.6	73	74.5	67	43.5	37	72.5	190	42.3
	Long	≥5	5	123	90.4	25	25.5	87	56.5	14	27.5	249	56.7
	Few	≤5	1	67	49.3	31	31.6	62	40.3	41	80.4	201	45.8
13. Stigma position	Medium	5 < a < 15	3	56	41.2	46	46.9	67	43.5	10	19.6	179	40.8
	High	≥15	5	13	9.6	21	21.4	25	16.2	–	–	59	13.4
	Short	≤5	1	–	–	4	4.1	–	–	6	11.8	10	2.3
14. Leaf number/plant	Medium	5 < a < 10	3	31	22.8	52	53.1	17	11.0	36	70.6	136	31.0
	Long	≥10	5	105	77.2	42	42.9	137	89.0	9	17.6	293	66.7
	Absent		1	136	100	96	98	63	40.9	51	100	346	78.8
15. Lamina length (cm)	Present		9	–	–	2	2	91	59.1	–	–	93	21.2
	Non-exserted		1	41	30.1	33	33.7	–	–	32	62.7	106	24.1
	Exserted		9	95	69.9	65	66.3	154	100	19	37.3	333	75.9
<i>Leaves</i>													
16. Lamina width (cm)	Few	≤5	1	13	9.6	13	13.3	4	2.6	5	9.8	35	8.2
	Medium	5 < a < 10	3	31	22.8	14	14.3	30	19.5	29	56.9	104	24.3
	High	≥10	5	90	66.2	62	63.3	120	77.9	17	33.3	289	67.5
17. Lamina length/width ratio	Short	≤3	1	15	11.0	40	40.8	7	4.5	21	41.2	83	19.4
	Medium	3 < a < 6	3	98	72.1	24	24.5	106	68.8	30	58.8	258	60.3
	Long	≥6	5	20	14.7	26	26.5	41	26.6	–	–	87	20.3
18. Lamina (intensity of main color)	Narrow	≤3	1	14	10.3	44	44.9	19	12.3	21	41.2	98	23.0
	Medium	3 < a < 6	3	104	76.5	20	20.4	107	69.5	30	58.8	261	61.0
	Broad	≥6	5	15	11.0	26	26.5	28	18.2	–	–	69	16.0
19. Lamina (degree of lobing)	Low	< 1	1	47	34.6	15	15.3	7	4.5	22	43.1	91	21.2
	Medium	= 1	3	16	11.8	3	3.1	9	5.8	5	9.8	34	7.9
	High	> 1	5	70	51.5	72	73.5	138	89.6	24	47.2	304	70.9
20. Leaf pattern	Light		1	–	–	7	7.1	1	0.6	–	–	8	1.9
	Medium		3	3	2.2	8	8.2	19	12.3	–	–	30	6.9
	Dark		5	131	96.3	79	80.6	134	87.0	51	100	395	91.2
21. Leaf pattern color	Weak		1	117	85.7	98	100	50	32.5	51	100	316	72.0
	Medium		3	8	6.0	–	–	98	63.6	–	–	106	24.1
	Strong		5	11	8.3	–	–	6	3.9	–	–	17	3.9
22. Leaf pattern color	Absent		1	11	8.1	2	2	2	1.3	–	–	15	3.4
	Present		9	123	90.4	96	98	152	98.7	51	100	422	96.6
	White		1	–	–	–	–	1	0.6	–	–	1	0.2
23. Leaf pattern color	Silver		3	111	81.6	74	75.5	69	44.8	29	56.9	283	65.4
	Yellow-green		5	3	2.2	17	17.33.1	19	12.3	2	3.9	41	9.5
	Green		7	20	14.7	3	–	65	42.2	20	39.2	108	24.9

(continued on next page)

Table 2 (continued)

Traits	Description	Ranges	Values	<i>C. pseudibericum</i>		<i>C. cilicium</i>		<i>C. persicum</i>		<i>C. coum</i>		Mean	
<i>Leaves</i>													
22. Petiole length (cm)	Short	≤5	1	22	16.2	18	18.4	33	21.4	3	5.9	76	17.8
	Medium	5 < a < 10	3	55	40.4	36	36.7	71	46.1	44	86.3	206	48.1
	Long	≥10	5	56	41.2	36	36.7	50	32.5	4	7.8	146	34.1
23. Leaf : abaxial surface color	Green		1	–	–	–	–	63	40.9	–	–	63	14.5
	Mixed		3	1	0.7	3	3.1	57	37.0	5	9.8	66	15.2
	Purple		5	133	97.8	93	94.9	34	22.1	46	90.2	306	70.3
<i>Tuber</i>													
24. Diameter (mm)	Small	≤30	1	9	6.6	12	12.2	2	1.3	19	37.0	42	13.6
	Medium	30 < a < 50	3	41	32.4	4	3.7	40	26.0	32	63.0	117	37.7
	Broad	≥50	5	31	22.8	20	20.4	110	71.4	–	–	151	48.7
25. Plant growth habit	Weak		1	13	9.6	13	13.3	4	2.6	5	9.8	35	8.0
	Medium		3	74	54.4	37	37.8	66	42.9	38	74.5	215	49.0
	Strong		5	49	36.0	48	49.0	84	54.5	8	15.7	189	43.0
26. Pedicel coiling	Absent		1	–	–	–	–	154	100	–	–	154	35.1
	Present		9	136	100	98	100	–	–	51	100	285	64.9
27. Flowering	Before leaf		1	–	–	98	100	1	0.6	–	–	99	22.6
	After leaf		9	136	100	–	–	153	99.4	51	100	340	77.4

C. pseudibericum (n = 136); *C. cilicium* (n = 98); *C. persicum* (n = 154); *C. coum* (n = 51).

plants were sampled from each location after completing a population survey of each site. Collected plant samples were transported to an unheated greenhouse in Adana and propagated and cultivated. Plants were sprayed with an insecticide (Viaduct, water-soluble granules; emamectin benzoate 5% (Shenzhen Chuangye Industry Co. Ltd., China) then planted in 3-l plastic pots filled with peatmoss, sand, and perlite (1:1:1, v/v/v). Pots were placed on a raised bench in a greenhouse. The plants were shaded as needed using net providing 60% shade, irrigated weekly with 300 ml/pot tap water and fertilized every 2 months with 2 g/pot of NPK 20-20-20 + ME fertilizer (Nutri-Leaf EC Fertilizer, Miller Chemical & Fertilizer Corp., Hannover, PA, USA). The features of 1-year-old *Cyclamen* species were characterized morphologically, as detailed next.

2.2. Morphological characterization

There is no description list in UPOV and IPGRI for members of the *Cyclamen* genus. The properties of four *Cyclamen* species (*C. persicum*, *C. cilicium*, *C. pseudibericum* and *C. coum*) were determined with a descriptors list (Table 2) (modified from Debussche and Thompson, 2002) using the most important leaf-, flower- and tuber-related features of ornamental plants. A total of 27 phenotypic characters (13 flower, 11 leaf, 2 plant, 1 tuber) and 13 quantitative morphological traits (7 flower, 5 leaf, 1 tuber) were assessed (Table 2). The latter group included flower number/plant, pedicel length (cm), petal length (mm),

petal width (mm), basal corolla ring diameter (mm), pistil length (mm), stamen length (mm), leaf number/plant, lamina length (cm), lamina width (cm), growth rate (flower number/leaf number) and tuber diameter. All measurements were made with a ruler or a digital compass (Mitutoyo CD-15D, Kawasaki, Japan).

2.3. Statistical analysis

All phenotypic characters and quantitative morphological traits were measured in triplicate for each species. Mean values were recorded in a Microsoft Excel (2010) spreadsheet and raw data were then coded to allow analysis using Unistat 4.0 for Windows. Statistical analyses were conducted using SAS (SAS Institute Inc., NC, USA, 1990). The data for 13 quantitative characters was analyzed to determine means, minimum and maximum values of each species using SPSS 14.0 (Illinois, IL, USA; SPSS, 2006). Data were then subjected to principle component analysis (PCA) using the SAS procedure.

3. Results and discussion

Five different regions and five provinces were visited and over 400 *cyclamen* accessions were collected between 2010 and 2012. By characterizing their morphology and cladistically characterizing the current natural *Cyclamen* resources endemic to Turkey will allow for conservation efforts



Fig. 2. Petal colors of *Cyclamen* species. (A) *C. pseudibericum*; (B) *C. coum*; (C) *C. cilicium*; (D) *C. persicum*.



Fig. 3. Spottiness and darker petal margin in *C. persicum*.

to be better coordinated, while optimizing biotechnology programs aimed at preserving rare or endangered germplasm *in vitro*.

3.1. Flower characteristics

Petal length was classified as medium ($15 \text{ mm} < a < 25 \text{ mm}$) in 267 (60.8%), short ($\leq 15 \text{ mm}$) in 66 (15%), and long ($\geq 25 \text{ mm}$) in 106 (24.2%) accessions (Table 2). Petal width was medium ($3 \text{ mm} < a < 10 \text{ mm}$) in 299 (68.1%) and large ($\geq 10 \text{ mm}$) in 140 (31.9%) accessions. Petal colors were white in 99 (22.6%), diffuse-pink in 96 (21.9%), pale pink in 84 (19.1%) and deep pink in 160 (36.4%) accessions (Fig. 2). Variation in petal color within a species is a common feature in cyclamen, similar to that observed for *Iris* (Saad and Mahy, 2009). In nature, deep-pink colored petals are usually seen in *C. coum* and *C. pseudibericum*. Basal corolla ring colors were white in 188 (44%), diffuse-pink in 1 (0.2%), pale pink in 17 (4%), deep pink in 219 (51.3%) and carmine in 2 (0.5%) accessions. Upper corolla colors were white in 3 (0.7%), pale-pink in 3 (0.7%), deep-pink in 95 (21.6%), and carmine in 338 (77%) accessions. Lower corolla colors were white in 5 (1.1%), diffuse-pink in 7 (1.6%), pale-pink in 8 (1.8%), deep-pink in 230 (52.4%), and carmine in 189 (43.1%) accessions. Basal corolla ring diameter was narrow ($\leq 2 \text{ mm}$) in 14 (3.2%), medium ($2 \text{ mm} < a < 5 \text{ mm}$) in 315 (71.8%), and broad

($\geq 5 \text{ mm}$) in 110 (25%) accessions. Pistil length was short ($\leq 3 \text{ mm}$) in 1 (0.2%), medium ($3 \text{ mm} < a < 6 \text{ mm}$) in 160 (37.3%), and long ($\geq 6 \text{ mm}$) in 268 (62.5%) accessions. Stamen length was medium ($2 \text{ mm} < a < 5 \text{ mm}$) in 190 (42.3%), and long in 249 (56.7%) accessions. Flower numbers per plant were few (≤ 5) in 201 (45.8%), medium ($5 < a < 15$) in 179 (40.8%), and many (≥ 15) in 59 (13.4%) accessions. Pedicel length was short ($\leq 5 \text{ mm}$) in 10 (2.3%), medium ($5 \text{ mm} < a < 10 \text{ mm}$) in 136 (31%), and long ($\geq 10 \text{ mm}$) in 293 (66.7%) accessions. Darker petal margin was absent in 346 (78.8%) and present in 93 (21.2%) accessions. All accessions with a darker petal margin or spottiness belong to *C. persicum* (Fig. 3). Debussche and Thompson (2002) found a darker petal margin in *C. balearicum*, *C. peloponnesiacum*, *C. vividum* and *C. rhodense* accounting for 83%, 46.7%, 7% and 3.5% of all accessions, respectively.

The position of the stigma was non-exserted in 106 (24.1%) and exserted in 333 (75.9%) accessions. Debussche and Thompson (2002) stated that in *Cyclamen* (*C. repandum*, *C. balearicum* and *C. creticum*), stigma position is significantly associated with flower color, with the highest proportion of inserted stigmas occurring in white flowers of *C. balearicum* (97.3%) and *C. creticum* (37.8%) accessions and the lowest (0%) in pink flowers of *C. repandum*, *C. rhodense*, *C. peloponnesiacum* and *C. vividum* accessions. Unlike their results, the present study showed



Fig. 4. Number of petals/flowers. (A) 4 petals, (B) 5 petals, and (C) 6 petals in *C. pseudibericum*; (D) 5 petals, or (E–F) 6 petals in *C. cilicium*.

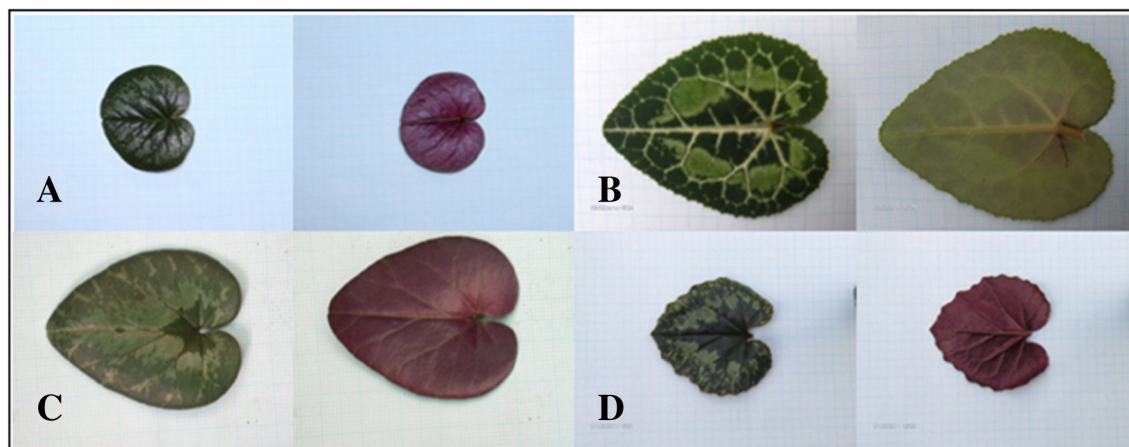


Fig. 5. Upper and lower side of cyclamen species leaves. (A) *C. coum*; (B) *C. persicum*; (C) *C. cilicium*; (D) *C. pseudibericum*.

that *C. coum* had a higher incidence of inserted stigmas (62.7%) than other species; incidentally, most *C. coum* accessions (72%) have deep-pink flowers. The percentage of exerted stigmas was high in *C. pseudibericum* and *C. cilicium* (69.9% and 66.3%, respectively), and 100% of *C. persicum* accessions had exerted stigmas (Table 2).

With regard to quantitative traits (data not shown), specifically petal number, different values were observed in *C. pseudibericum* and *C. cilicium* (Fig. 4) accessions. This may be because the altitude of the cultivation site (32 m) is much lower than the natural altitude where the species grow naturally (i.e., 900–1200 m); moreover, the temperature of the cultivation site was higher (average temperature 18.8 °C) than their natural location (average temperature 13.3 °C).

3.2. Leaf characteristics

A high level of variation in leaf characteristics was observed among the examined *Cyclamen* species (Fig. 5). There were few leaves/plant (≤ 5) in 35 (8.2%), medium number ($5 < a < 10$) in 104 (24.3%), and a high number (≥ 10) in 289 (67.5%) accessions. The lamina was short (≤ 3 cm) in 83 (19.4%), medium ($3 \text{ cm} < a < 6 \text{ cm}$) in 258 (60.3%), and long (≥ 6 cm) in 87 (20.3%) accessions. The lamina width was narrow (≤ 3 cm) in 98 (23%), medium ($3 \text{ cm} < a < 6 \text{ cm}$) in 261 (61%), and broad (≥ 6 cm) in 69 (16%) accessions. The lamina length/lamina width ratio was low (< 1) in 91 (21.2%), medium ($= 1$) in 34 (7.9%), and high (> 1) in 304 (70.9%) accessions. The intensity of the main color of the lamina was light in 8 (1.9%), medium in 30 (6.9%), and dark in 395 (91.2%) accessions. Leaf pattern was absent in 15 (3.4%) and present in 422 (96.6%) accessions. Leaf pattern colors were white in 1 (0.2%), silver in 283 (65.4%), yellow-green in 41 (9.5%), and green in 108 (24.9%) accessions. The degree of lamina lobing was weak in 316 (72.0%), medium in 106 (24.1%), and strong in 17 (3.9%) accessions. The petiole was short (≤ 5 cm) in 76 (17.8%), medium ($5 \text{ cm} < a < 10 \text{ cm}$) in 206 (48.1%), and long (≥ 10 cm) in 146 (34.1%) accessions. The leaf abaxial surface color

was green in 63 (14.5%), a mixture of green and purple in 66 (15.2%), and purple in 306 (70.3%) accessions.

3.3. Tuber characteristics

Tuber diameter was small (≤ 30 mm) in 42 (13.6%), medium ($30 \text{ mm} < a < 50 \text{ mm}$) in 117 (37.7%), and broad (≥ 50 mm) in 151 (48.7%) accessions. *Cyclamen* species with more than 30 chromosomes, such as *C. persicum* ($2n = 2x = 48$), can develop very large tubers (Clennett, 2002). Thus, variation among species in certain traits may be associated with differences in chromosome number associated with polyploidization (Debussche et al., 2004). Plant growth habit was weak in 35 (8%), medium in 215 (49%), and strong in 189 (43%) accessions. Flowering was before leaf development in 99 (22.6%), or after leaf development in 340 (77.4%) accessions. The fruit is a capsule, often drawn down to the soil level by the twisting of the floral stalk (pedicel). Pedicel coiling was absent in 154 (35.1%) but present in 285 (64.9%) accessions. Coiling pedicels were observed in 100% of three species (*C. pseudibericum*, *C. cilicium*, and *C. coum*) but not in *C. persicum* accessions (Fig. 6).

The standard deviations, as well as minimum and maximum values were compared (Table 3). Morphological diversity does not correspond to geographic region but rather to species. Among the species sampled, *C. persicum* contained the highest mean values for flower number/plant (9.3), petal length (26.6 mm), pedicel length (15.2 cm), leaf number/plant (21.0), lamina length (5.1 cm) and tuber diameter (6.3 cm). *C. pseudibericum* had highest petal width (11.3 mm), pistil length (7.9 mm) and stamen length (6.4 mm), *C. persicum* and *C. pseudibericum* shared the highest mean values for petal length (26.6 mm and 21.1 mm, respectively). Mean values of petiole length were highest in *C. cilicium*, *C. pseudibericum* and *C. persicum* (10.3 cm, 9.7 cm and 8.6 cm, respectively). These results

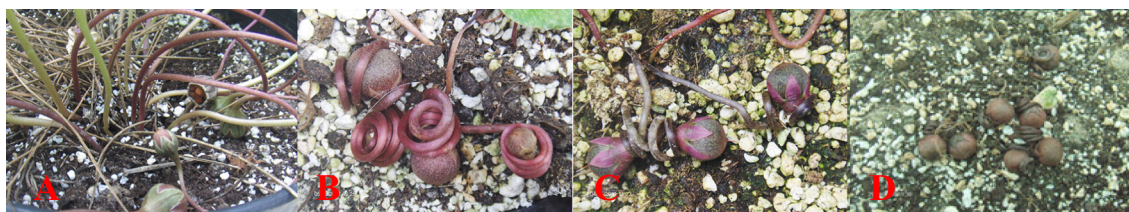


Fig. 6. Cyclamen fruits. (A) *C. persicum*; (B) *C. pseudibericum*; (C) *C. coum*; (D) *C. cilicium*.

Table 3
Means, standard deviations and minimum and maximum values of cyclamen accessions of four cyclamen species naturally grown in Turkey for 13 agro-morphological traits.

Traits	C. pseudibericum			C. cilicium			C. persicum			C. coum			Overall		
	Mean	Min.-Max.	SD	Mean	Min.-Max.	SD	Mean	Min.-Max.	SD	Mean	Min.-Max.	SD	Mean	Min.-Max.	SD
1. Petal length (mm)	21.1 ± 0.3	13.2–31.3	3.035	17.7 ± 2.5	8.8–22.3	2.631	26.6 ± 0.4	16.6–39.0	4.606	10.9 ± 0.3	5.6–14.6	1.920	21.0 ± 0.3	5.6–39.0	6.143
2. Petal width (mm)	11.3 ± 0.2	6.6–15.2	1.772	7.0 ± 1.0	3.5–9.5	1.067	8.8 ± 0.1	4.3–14.1	1.760	8.1 ± 0.2	4.4–10.7	1.332	9.1 ± 0.1	3.5–15.2	2.264
3. Flower number/plant	6.5 ± 0.4	1.0–30.0	5.020	10.2 ± 7.9	1.0–47.0	7.730	9.3 ± 0.6	1.0–40.0	7.454	4.2 ± 0.3	1.0–12.0	2.485	8.0 ± 0.3	1.0–47.0	6.719
4. Pedicel length (cm)	12.9 ± 0.3	5.8–23.0	3.775	9.7 ± 2.9	4.0–19.5	2.863	15.2 ± 0.4	5.3–37.0	5.068	7.7 ± 0.3	4.0–11.8	1.995	12.4 ± 0.2	4.0–37.0	4.781
5. Basal corolla ring diameter (mm)	4.2 ± 0.1	3.0–7.3	0.666	2.5 ± 0.4	1.4–4.1	0.451	5.3 ± 0.05	3.7–7.6	0.670	2.7 ± 0.1	1.8–5.4	0.556	4.0 ± 0.1	1.4–7.6	1.259
6. Pistil length (mm)	7.9 ± 0.1	4.5–10.3	0.994	5.4 ± 0.8	2.6–8.0	0.817	7.0 ± 0.1	4.0–9.0	1.092	5.3 ± 0.1	3.8–7.6	0.674	6.7 ± 0.1	2.6–10.3	1.423
7. Stamen length (mm)	6.4 ± 0.1	3.6–11.5	1.224	4.6 ± 0.9	2.6–9.9	0.946	5.3 ± 0.1	2.6–10.3	1.239	4.6 ± 0.1	3.4–7.1	0.676	5.3 ± 0.1	2.6–11.5	1.239
8. Leaf number/plant	13.2 ± 0.6	1.0–47.0	7.140	16.1 ± 11.2	2.0–55.0	11.233	21.0 ± 1.02	2.0–70.0	12.732	9.0 ± 0.4	3.0–16.0	3.010	16.1 ± 0.5	1.0–70.0	10.875
9. Petiole length (cm)	9.7 ± 0.4	2.3–21.3	4.363	10.3 ± 6.1	1.5–26.0	6.101	8.6 ± 0.3	2.0–20.3	3.997	7.0 ± 0.3	3.8–14.2	2.108	9.1 ± 0.2	1.5–26.0	4.573
10. Lamina length (cm)	4.6 ± 0.1	1.5–7.6	1.256	2.9 ± 0.7	1.3–4.6	0.712	5.1 ± 0.1	1.8–9.6	1.406	3.2 ± 0.1	1.7–5.3	0.849	4.3 ± 0.1	1.3–9.6	1.505
11. Lamina width (cm)	4.6 ± 0.1	2.2–7.1	1.103	2.7 ± 0.7	1.1–4.2	0.667	4.6 ± 0.1	1.6–8.0	1.316	3.1 ± 0.1	1.7–5.3	0.820	4.0 ± 0.1	1.1–8.0	1.365
12. Lamina: length/width ratio	1.0 ± 0.01	0.7–1.2	0.093	1.1 ± 0.1	0.7–1.4	1.324	1.1 ± 0.01	0.9–1.4	0.093	1.0 ± 0.01	0.9–1.4	0.090	1.1 ± 0.01	0.7–1.4	0.116
13. Tuber diameter (mm)	44.9 ± 1.4	22.0–67.0	12.665	44.2 ± 1.6	23.8–73.3	12.824	59.4 ± 1.4	25.8–111	16.535	23.9 ± 0.1	16.8–33.1	4.366	49.7 ± 1.0	16.8–111	17.644

C. pseudibericum (n = 136); C. cilicium (n = 98); C. persicum (n = 154); C. coum (n = 51).

are broadly compatible with the description of the Flora of Turkey (Güner et al., 2000).

According to Wendelbo (1965), morphology alone can be used to separate species within the *Cyclamen* subgenus *Gyrophoebe* O. Schwarz (*C. pseudibericum*, *C. coum*, *C. mirabile*, *C. cilicium*, *C. intaminatum*, *C. parviflorum*, *C. trochopteranthum* and *C. elegans*), although most of the taxa in this group have obvious affinities and appear to be closely related. In fact, all taxa of the subgenus *Gyrophoebe* are Turkish endemics, and geographic isolation within pockets of suitable habitats would appear to provide the best explanation of speciation within this part of the genus. The obvious exception here is *C. coum*, which has a wider distribution from the Black Sea region to around Eskişehir and the southern part of Turkey. *C. coum* accessions are also distributed in Ukraine, Georgia, Bulgaria, Lebanon, Syria, Israel and the south Caspian coast of Iran but nowhere else in the world. Field studies by the *Cyclamen* Society (Wood, 1990, 1991) and others (Brickell, 1979; Meikle, 1984, 1989; Clennett, 2002; Grey-Wilson, 2003) have shown a clinal pattern of variation in *C. coum*. Tuber diameter was greater in *C. graecum*, *C. persicum* and *C. hederifolium* (45.13, 59.60 and 61.27 mm, respectively). Pedicel coiling was observed in all accessions of three species (*C. pseudibericum*, *C. cilicium* and *C. coum*) but not in *C. persicum* accessions, which corresponds to our finding (Fig. 6).

According to the morphological description list, six out of 27 traits had similar values (over 85%) in all species: 1) leaf pattern was present (>90%); 2) the intensity of the lamina's main color was dark in >85% accessions of all species except for *C. cilicium* accessions; 3) the color of the lamina's abaxial surface was purple in >95% accessions of all species except for *C. persicum* accessions; 4) flowering in the absence of leaves (hysteranthous species) was >95% in *C. cilicium* accessions and flowering with fully developed foliage (synanthous species) was >99% in *C. persicum*, *C. pseudibericum* and *C. coum* accessions; 5) the presence of a darker petal margin was not observed in all species except for *C. persicum* (59.1% present); 6) coiling petioles were observed in 100% of all accessions except for *C. persicum*. Performance of the evaluated genotypically different individuals under similar environmental conditions on 21 wide ranges of variation observed in evaluated agronomic traits (petal length, petal width, petal color, basal corolla ring color, color of upper corolla, color of lower corolla, basal corolla ring diameter, pistil length, stamen length, flower numbers/plant, pedicel length, stigma position, leaf number/plant, lamina length, lamina width, lamina length/width ratio, lamina (degree of lobing), leaf pattern color, petiole length, tuber diameter, plant growth habit) and results allows, understanding the some accessions observed good potential over others. This great variation might be responsible for the different genetic characteristics and physiological development of plants.

According to a cluster analysis (data not shown) performed using morphological data, accessions were clustered into the same group based on species but not on geographic origin. For example, *C. coum*,

Table 4

Results of principle component analysis (PCA) (for the first three PCs) of cyclamen accessions collected from Turkey for 12 agro-morphological traits.

Trait	PC1	PC2	PC3
Petal length (mm)	0.37	0.24	−0.03
Petal width (mm)	0.31	−0.35	0.06
Flower number/plant	0.04	0.44	0.25
Pedicel length (cm)	0.33	0.15	0.3
Basal corolla ring diameter (mm)	0.36	0.17	−0.25
Pistil length (mm)	0.36	−0.2	−0.21
Stamen length (mm)	0.25	−0.29	−0.24
Leaf number/plant	0.08	0.52	0.06
Petiole length (cm)	0.15	−0.05	0.69
Lamina length (cm)	0.39	0.05	−0.05
Lamina width (cm)	0.39	−0.07	0.07
Lamina: length/width ratio	0.01	0.42	−0.44
Eigenvalue	4.47	1.93	1.42
Proportion	0.37	0.16	0.12

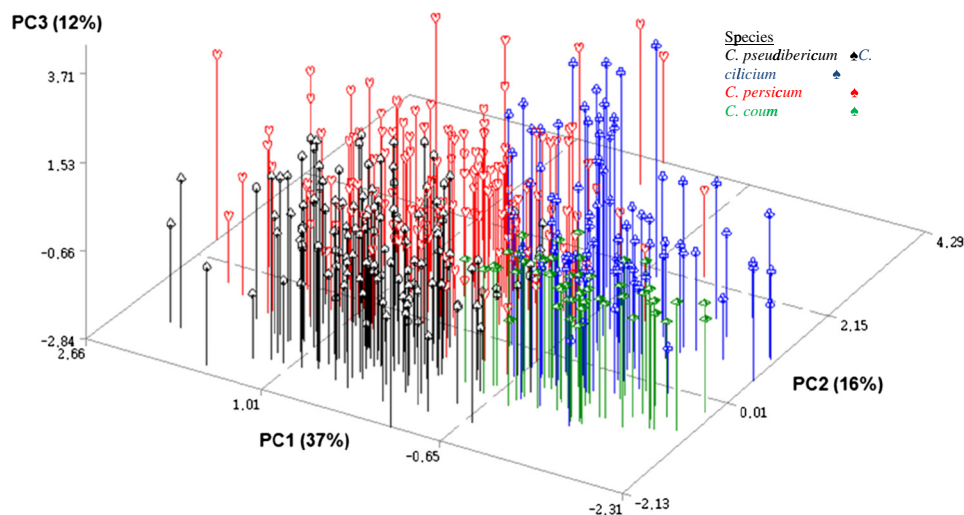


Fig. 7. Plot of four *Cyclamen* (*C. pseudibericum*, *C. cilicium*, *C. persicum*, *C. coum*) accessions collected from Turkey on the first three principle components (PCs) obtained from the analysis of 12 quantitative agro-morphological traits using PCA.

C. persicum and *C. hederifolium* accessions collected from different locations flowered in the same season, and had the same color of the abaxial leaf surface and same petal color (Table 2). Clustering according to plant species has also been reported in *onocyclus* irises (Saad and Mahy, 2009) and chamomile (*Matricaria chamomilla*) (Solouki et al., 2008).

3.4. Principle component analysis

The results of PCA are presented in Table 4. The first PC explained 37% (eigenvalue of 4.47), while the second and the third PCs contributed 16% and 12%, respectively to total variance (eigenvalues of 1.93 and 1.42, respectively). PCA explained 65% of total morphological variation. Lamina length (0.39), lamina width (0.39), petal length (0.37), petal width (0.31), basal corolla ring diameter (0.36) and pistil length (0.36) were important variables composing PC1. Flower and leaf

number/plant (eigenvalues of 0.44 and 0.52, respectively) were the two traits that were highly correlated with PC2. Petiole length (eigenvalue of 0.69) was the most important trait for PC3. From all characters, petiole length was found to be the most discriminative parameter differentiating accessions.

The four *Cyclamen* species accessions based on the three PCs in Table 4 were plotted (Fig. 7). The accessions were broadly grouped into their species. While *C. coum*, *C. pseudibericum* and *C. cilicium* accessions grouped close to each other, *C. persicum* accessions showed a distinct distribution.

According to PCA using qualitative data obtained from the observations of 27 morphological characters in the four Turkish *Cyclamen* species, accessions were separated into five different groups (Fig. 8). While PC1 explained 37% of total morphological variation, PC2 explained 17% and PC3 10% of total variation, with a total of 67% variation

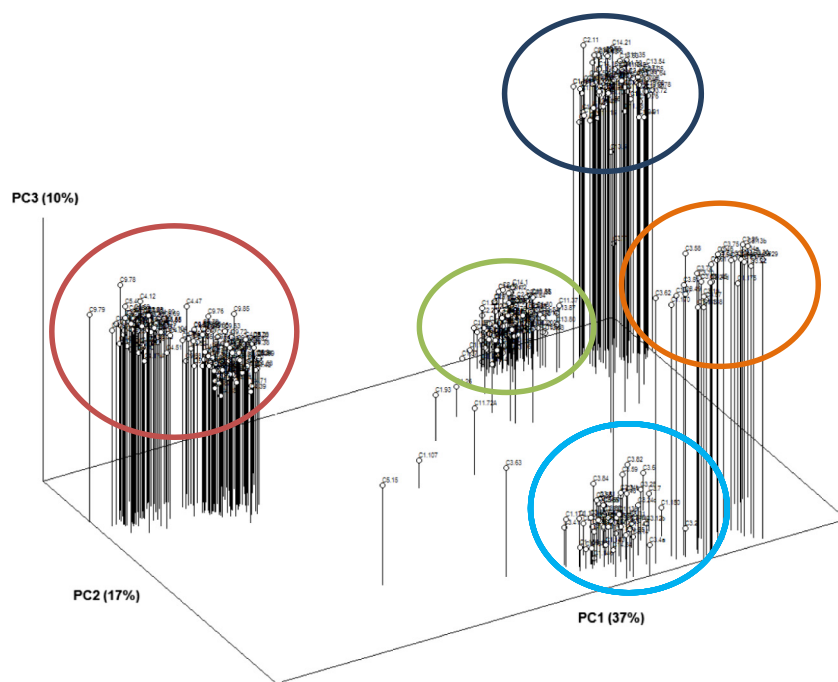


Fig. 8. Plot of cyclamen accessions collected from Turkey on the first three principle components (PC) obtained from analysis of 27 agro-morphological traits using PC analysis. Wine-colored circle: C4, C5 and C9 (*C. persicum*), green circle: C1, C2, C11 and C14 (*C. pseudibericum* and *C. coum*), navy blue circle: C1, C2, C13 and C14 (*C. pseudibericum* and *C. coum*), orange circle: C3 (*C. cilicium*), blue circle: C1 and C3 (*C. cilicium*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

accounted for. Aalaey et al. (2007) found that effective traits (related to the color of flowers, petioles, young leaves on the adaxial site, and flower shelf life) could be categorized into 6 groups that covered a total of 86.89% of total variance. Solmaz and Sari (2009) evaluated different morphological characters of Turkish watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) accessions. Based on PCA they reported that 50% of morphological variation was detected. In this study, the PCA result showed that different *Cyclamen* species that originated from a similar geographical area or the same species that originated from different geographical areas were placed into different groups (Fig. 8). Zhao et al. (2007) found that the lack of conformity between the genetic and geographical variation exists because of an exchange of genetic material, the introduction of new accessions, genetic drift and natural selection, or human interference. Plants are nearly always restricted to specific geographic and climatic zones, in areas with great topographical and climatic variation. However, the appearance of different accessions are concerned with basic evolutionary elements (selection, adaptation, migration, self-pollination and genetic drift), which are related to the environment and human activities, over time (Martins et al., 2006).

4. Conclusions

Knowledge of morphological and genetic variation and the association between these traits can provide support in a breeding program aimed at genetic improvement. This will assist plant breeders in choosing which agronomic traits should be used in their breeding program. In such a program, a wide range of variability, heritability, genetic advances and positive correlation coefficients among traits can be an excellent tool for improving or selecting accessions of interest (Akbar et al., 2003). This study provides a detailed morphological report on four *Cyclamen* species [*C. pseudibericum* ($n = 136$); *C. cilicium* ($n = 98$); *C. persicum* ($n = 154$); *C. coum* ($n = 51$)] growing wild in Turkey, which boasts over 400 accessions. The measurements related to morphological characteristics showed a wide range of variation. There were distinct morphological differences among all four species which allowed them to be clearly distinguished based on morphological characteristics alone, as well as on PCA. Tuber diameter, leaf width, petal length, petal color, leaf shape, and the presence of auriculate characters were the morphological characteristics with the most accentuated differences. These species are spread over a wide range of habitat types which could account for the variation observed. Such a detailed morphological characterization is useful for identifying superior accessions that are hardy to cold, have attractive leaves, or flower fragrance, that could be used in cross-breeding or inter-specific hybridization studies in the future. The use of wild genetic resources to expand the genetic base of *Cyclamen*, and their preservation *in vitro*, is still a relatively unexplored topic in *Cyclamen* biology (Kocak et al., 2014).

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