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MORPHOLOGICAL CHARACTERIZATION OF *Cyclamen* sp. GROWN NATURALLY IN TURKEY: PART II

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Abstract. The morphology of 279 accessions of *Cyclamen* sp. growing naturally in Turkey, namely *C. alpinum* (syn. *C. trochopteranthum*), *C. graecum*, *C. hederifolium* (syn. *C. neapolitanum*) and *C. mirabile*, was characterized. Plants with intact tubers were collected from locations in Antalya, Isparta, Aydın, Muğla, İzmir and Denizli, determined by GPS, where they grow naturally in spring and autumn. The morphology of the four *Cyclamen* species was characterized using one year old regenerated plants based on 27 morphological traits (13 flower, 11 leaf, 2 plant, 1 tuber). There were distinct differences among these accessions related to petal colour, pedicel length, leaf length and width, leaf shape, and tuber diameter. Even though principle component analysis confirmed the grouping of characters into species-specific clusters, a wider range of morphological data as well as molecular data are needed for more reliable conclusions to be drawn about the classification of these *Cyclamen* species.

Key words: *Cyclamen*, genetic resources, morphological characterization

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INTRODUCTION

The horticulturally important genus *Cyclamen* L. was formerly classified in the family Primulaceae but was recently reclassified into the family Myrsinaceae [Kallersjö et al. 2000, Yesson and Culham 2011, Jalali et al. 2012]. The *Cyclamen* genus consists of more than 20 species of perennial plants [Grey-Wilson 2002, Compton et al. 2004, Yesson and Culham 2006] native to the Mediterranean Basin, 10 of which grow naturally in Turkey, and five of which are endemic. *Cyclamen* plants have a tuber and nodding flowers [Takamura 2006]. The flowering season varies with the species. For example, *C. alpinum* (syn. *C. trochopteranthum* Schwarz) flowers in spring while *C. hederifolium* Aiton., *C. graecum* Link., and *C. mirabile* Hildebr. flower in autumn, even when different species are cultivated in the same place [Takamura 2006]. The leaves, which have long petioles that arise 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 colour can be white, pale rose, pale to deep pink, carmine, purplish, red, or crimson and they may have marked spots at their base. Depending on the species, the roots are mainly produced on the upper part of the tuber or at the base [Le Nard and De Hertogh 1993]. Chromosome numbers of *Cyclamen* species vary, for example *C. hederifolium* 2n = 34, 68 or 102, *C. mirabile* and *C. trochopteranthum* 2n = 30, *C. graecum* 2n = 84 [Cyclamen Society 2015]. Tubers of *Cyclamen* species enter dormancy throughout the period of physical drought in summer [Lazarević and Lazarević 2010].

C. mirabile and other rare *Cyclamen* species with beautiful flowers and leaves will always be attractive for bulb markets [Karagoz et al. 2010]. Therefore, *Cyclamen* species are under extinction due to destruction of natural habitats, unconscientious use of agricultural areas and removal of tubers from nature for export. For these reasons, conservation studies and research on *Cyclamen*, which is an important ornamental source of genetic resources, are needed and efficient propagation methods must be developed to diminish the pressure imposed by legal or illegal harvesting of natural populations of rare and attractive species. Yamaner and Erdag [2008] developed an efficient *in vitro* propagation method for *C. mirabile*, Muftuoglu and Altay [2006] determined the best sowing time to grow *C. hederifolium* tubers that did not need to be collected from nature and that could be grown easily from seeds, while Muftuoglu and Altay [2009] determined the effects of different mineral elements on the production of *C. hederifolium*, in order to reduce the time needed for tubers to reach exportable quality (i.e., 10 cm in diameter) when produced by growers, and to prevent its environmental destruction. Halevy [2005] and Burun and Sahin [2009] could propagate *C. persicum* and *C. mirabile*, respectively from seeds. Thus, there are currently a range of viable methods to propagate wild *Cyclamen* species.

Genetic diversity in the *Cyclamen* genus has been evaluated via morphological, molecular and biochemical markers by various researchers [Anderberg et al. 2000, Chahal and Gosal 2002, Halevy 2005, Mih et al. 2008, Saad and Mahy 2009, Solmaz and Sari 2009, Bozokalfa et al. 2010, Coskun et al. 2010, Kandemir 2010, Parmaksiz and Ozcan 2011, Taskin et al. 2012, Okon et al. 2013]. Molecular marker analyses can affirm conventional classifications such as morphological and physiological features, assign genet-

ic affinity within and among species, define the relationship between populations and detect whether there is genetic drift or migration between them [Dolezalova et al. 2003, Alhajjar et al. 2011]. The combination of morphological, chemical, and molecular marker analyses will result in a better classification of the genus *Cyclamen*. The objective of this study was to characterize the morphological traits of four Turkish *Cyclamen* species (*C. mirabile*, *C. graecum*, *C. hederifolium* and *C. alpinum*) growing in natural conditions and to analyze their relationships using cladistic analyses.

MATERIALS AND METHODS

Plant material. *Cyclamen* plants were collected with intact tubers from locations where they grow naturally, in spring and autumn, when they were in flower: *C. graecum* (fig. 1) from Kemer (Antalya), *C. mirabile* (fig. 2) from Barla (Isparta) and Karacasu (Antalya), *C. hederifolium* (fig. 3) from Germencik and Kosk (Aydin) and Selcuk (Izmir), and *C. alpinum* (syn. *C. trochopterantum*) (fig. 4) from Koycegiz and Turgut (Mugla) and Cukurkoy (Denizli) (tab. 1). The altitute of each location was determined with a GPS instrument (Magellan SporTrak Color, 980616-20, San Dimas, CA, USA). Plants were identified according to the Flora of Turkey [Davis 1978, Davis et al. 1988, Guner et al. 2000]. *Cyclamen* populations were surveyed in each province and 50 plants were collected from each location. Plants were sprayed with water to retain high relative moisture in a plastic bag packed into a cardboard box used for transporting the material to an unheated greenhouse in Adana where they were propagated. For propagation, plants were sprayed with an insecticide (Viaduct, water-soluble granules; 5% Emamectin benzoate (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 a net providing 60% shade, irrigated weekly with 300 ml of tap water per pot and fertilized every two months with 2 g/pot of NPK 20-20-20 + ME fertilizer (Nutri-Leaf EC Fertilizer, Miller Chemical & Fertilizer Corp., Hannover, PA, USA) [Curuk et al. 2015]. Plants were transferred to a cooler place during summer and were not watered. When leaves started to emerge in the next season, pots were transferred them to a greenhouse where plants were once again watered and fertilized. *Cyclamen* species were characterized morphologically one year later in regenerated plants (not all plants regenerated and the number of regenerated plants for each species were 56 accessions out of 100 in *C. mirabile*, 154 accessions out of 205 in *C. hederifolium*, 10 accessions out of 100 in *C. graecum* and 59 accessions out of 209 in *C. alpinum*).

Morphological characterization. There is no description list in UPOV and IPGRI for the *Cyclamen* genus. Thus, the properties of the four *Cyclamen* species studied were determined with a description list (tab. 2) modified from Debussche and Thompson [2002] containing important leaf-, flower- and tuber-related features of *Cyclamen*. A total of 27 phenotypic characters (13 flower, 11 leaf, 2 plant, 1 tuber) including 13 quantitative (7 flower, 5 leaf, 1 tuber) presented in Table 3 and 14 qualitative traits (6 flower, 6 leave, 2 plant) were evaluated in most of the accessions. A few genotypes were excluded because values were missing, because of the use of an insufficient num-

ber of morphological characters or mistiming of measurements for parameters resulting in a failure to characterise them with confidence. 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), lamina length/lamina width ratio, petiole length (cm) and tuber diameter (mm) were examined. Length was determined by a ruler or a digital compass (Mitutoyo CD-15D, Kawasaki, Japan).

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

Variety	Collection site	Sampling date	Coordinates	Elevation (m)	Vegetation
Cyclamen hederifolium	Meze Köyü, Köşk/Aydın	7.12.2010	37°54′24″N 28°03′32″E	289	*Pinus brutia*, *Quercus ilex* forest and evergreen shrubland
	Ilıdağ Köyü, Köşk/Aydın	20.12.2010	37°56′45″N 28°02′37″E	541	
	Dağkaraağaç Köyü, Germencik/ Aydın	22.12.2010	37°54′00″N 27°36′27″E	159	
	Meryemana Yolu, Selçuk/İzmir	22.12.2010	37°55′24″N 27°20′53″E	197	
Cyclamen graecum	Kemer /Antalya	5.12.2010	36°51′04″N 30°31′12″E	610	Woodland and shrubland dominated by *Platanus orientalis* and *Pinus brutia*
	Beycik Köyü, Kemer/Antalya	6.12.2010	36°29′45″N 30°26′02″E	713	
Cyclamen mirabile	Barla/Isparta	16.12.2010	38°01′00″N 30°47′00″E	1085	Woodland and shrubland dominated by *Platanus orientalis* and *Pinus brutia*
	Yılanlı Dağ/Muğla	3.12.2010	37°12′52″N 28°27′57″E	1158	
	Bölükçam Mevkii, Turgut/Muğla	4.12.2010	37°22′13″N 28°02′05″E	503	
	Yeşilyurt Köyü, Karacasu/Aydın	21.12.2010	37°40′22″N 28°40′57″E	575	
Cyclamen alpinum syn. *C. rochopteranthum*	Eski Tavas Köy Yolu/Denizli	13.04.2011	37°14′49″N 29°19′45″E	636	Woodland and shrubland dominated by *Pinus brutia*, *Liquidambar orientalis*, *Laurus nobilis* and *Ceratonia siliqua*
	Cankurtaran Mev. Çukurköy/Denizli	14.04.2011	37°41′37″N 29°12′30″E	560	
	Yayla Köyü, Köyceğiz/Muğla	15.04.2011	37°01′51″N 28°44′53″E	762	
	Sakarca Mevkii, Kızılkaya Köyü, Köyceğiz/Muğla	15.04.2011	36°51′53″N 28°51′43″E	570	

Statistical analysis. All phenotypic characters and quantitative morphological traits were measured in triplicate for each accession. 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]. Data for the 13 quantitative characters was analyzed to determine means, standart deviations, minimum and maximum values of each species using SPSS.14.0 [2006] (IL, USA). Data were then subjected to Principle Component Analysis (PCA) using SAS.

Table 2. Descriptors 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	Class	Number of accessions (%)									
					C. mirabile		*C. hederifolium*		*C. graecum*		*C. alpinum*		Mean	
FLOWERS	1. Petal length (mm)	short	≤ 15	1	5	8.9	10	6.5	–	–	59	100	74	26.5
		medium	15 < a < 25	3	51	91.1	135	87.7	10	100	–	–	196	70.2
		long	≥ 25	5	–	–	9	5.8	–	–	–	–	9	3.3
	2. Petal width (mm)	small	≤ 3	1	–	–	–	–	–	–	–	–	–	–
		medium	3 < a < 10	3	56	100	115	74.7	10	100	46	78	227	81.4
		large	≥ 10	5	–	–	39	25.3	–	–	13	22	52	18.6
	3. Petal colour	white	–	1	27	48.2	12	7.8	1	10	–	–	49	17
		diffuse-pink	–	3	17	30.4	40	26	4	40.4	10	16.9	71	24.7
		pale-pink	–	5	12	21.4	102	66.2	5	50	28	47.5	147	51
		deep-pink	–	7	–	–	–	–	–	–	21	35.6	21	7.3
		carmine	–	9	–	–	–	–	–	–	–	–	–	–
	4. Basal corolla ring colour	white	–	1	–	–	–	–	–	–	8	13.6	8	2.9
		diffuse-pink	–	3	–	–	–	–	–	–	–	–	–	–
		pale-pink	–	5	2	3.6	–	–	–	–	9	15.3	11	4
		deep-pink	–	7	38	67.9	151	98.1	10	100	6	10.2	205	73.5
		carmine	–	9	16	28.6	3	1.9	–	–	36	61	55	19.6
	5. Colour of upper corolla	white	–	1	–	–	–	–	–	–	–	–	–	–
		diffuse-pink	–	3	–	–	–	–	–	–	–	–	–	–
		pale-pink	–	5	–	–	–	–	–	–	–	–	–	–
		deep-pink	–	7	40	71.4	151	98.1	10	100	6	10.2	207	74.2
		carmine	–	9	16	28.6	3	1.9	–	–	53	89.9	72	25.8
	6. Colour of lower corolla	white	–	1	–	–	–	–	–	–	–	–	–	–
		diffuse-pink	–	3	–	–	–	–	–	–	–	–	–	–
		pale-pink	–	5	–	–	–	–	–	–	–	–	–	–
		deep-pink	–	7	40	71.4	151	98.1	10	100	6	10.2	207	74.2
		carmine	–	9	16	28.6	3	1.9	–	–	53	89.9	72	25.8
	7. Basal corolla ring diameter (mm)	narrow	≤ 2	1	–	–	–	–	–	–	–	–	–	–
		medium	2 < a < 5	3	56	100	16	10.4	3	30	59	100	134	49.8
		broad	≥ 5	5	–	–	138	89.6	7	70	–	–	135	50.2
	8. Pistil length (mm)	short	≤ 3	1	–	–	–	–	–	–	–	–	–	–
		medium	3 < a < 6	3	41	73.2	10	6.5	4	40	55	93.2	110	39.4
		long	≥ 6	5	15	26.8	144	93.5	6	60	4	6.8	169	60.6
	9. Stamen length (mm)	short	≤ 2	1	–	–	–	–	–	–	–	–	–	–
		medium	2 < a < 5	3	43	76.8	47	30.5	8	80	36	61	134	48
		long	≥ 5	5	13	23.2	107	69.5	2	20	23	39	145	52
	10. Flower number/plant	few	≤ 5	1	33	58.9	74	48.1	8	80	51	86.4	166	59.5
		medium	5 < a < 15	3	17	30.4	59	38.3	2	20	8	13.6	86	30.8
		most	≥ 15	5	6	10.7	21	13.6	–	–	–	–	27	9.7
	11. Pedicel length (cm)	short	≤ 5	1	1	1.8	3	1.9	1	10	4	6.8	9	3.2
		medium	5 < a < 10	3	24	42.9	41	26.6	6	60	40	67.8	111	39.8
		long	≥ 10	5	31	55.4	110	71.4	3	30	15	25.4	159	57
	12. Darker petal margin	absent	–	1	56	100	154	100	10	100	52	88.1	272	97.5
		present	–	9	–	–	–	–	–	–	7	11.9	7	2.5
	13. Stigma position	non-exserted	–	1	37	66.1	19	12.3	–	–	51	86.4	107	38.6
		exserted	–	9	17	30.4	135	87.7	10	100	8	13.6	170	61.4

LEAF	14. Leaf number/ plant	few	≤ 5	1	2	3.6	17	11	2	20	9	15.3	30	10.8
		medium	5 < a < 10	3	13	23.2	39	25.3	2	20	23	39	77	27.6
		most	≥ 10	5	41	73.2	98	63.6	6	60	27	45.8	172	61.6
	15. Lamina length (cm)	short	≤ 3	1	28	50	12	7.8	–	–	41	69.5	81	29
		medium	3 < a < 6	3	28	50	99	64.3	8	80	18	30.5	153	54.9
		long	≥ 6	5	–	–	43	27.9	2	20	–	–	45	16.1
	16. Lamina width (cm)	narrow	≤ 3	1	33	58.9	15	9.7	1	10	34	57.6	83	29.7
		medium	3 < a < 6	3	23	41.1	113	73.4	9	90	25	42.4	170	61
		broad	≥ 6	5	–	–	26	16.9	–	–	–	–	26	9.3
	17. Lamina length/ width ratio	low	< 1	1	12	21.4	8	5.2	1	10	32	54.2	53	19
		medium	= 1	3	4	7.1	6	3.9	–	–	6	10.2	16	5.7
		high	> 1	5	40	71.4	140	90.9	9	90	21	35.6	210	75.3
	18. Lamina (intensity of main colour)	light	–	1	–	–	6	3.9	–	–	–	–	6	2.1
		medium	–	3	–	–	11	7.1	–	–	8	13.6	19	6.8
		dark	–	5	56	100	137	89	10	100	51	86.4	254	91.1
	19. Lamina (degree of lobing)	weak	–	1	54	96.4	16	10.4	–	–	58	98.3	128	46.2
		medium	–	3	–	–	29	18.8	10	100	1	1.7	40	14.4
		strong	–	5	2	3.6	107	69.5	–	–	–	–	109	39.4
	20. Leaf pattern	absent	–	1	–	–	–	–	–	–	2	3.4	2	0.7
		present	–	9	56	100	154	100	10	100	57	96.6	277	99.3
	21. Leaf pattern colour	white	–	1	–	–	–	–	–	–	–	–	–	–
		silver	–	3	53	94.6	75	48.7	8	80	33	55.9	169	60.6
		yellow green	–	5	–	–	35	22.7	1	10	1	1.7	37	13.2
		green	–	7	3	5.4	44	28.6	1	10	25	42.4	73	26.2
	22. Petiole length (cm)	short	≤ 5	1	6	10.7	11	7.1	–	–	14	23.7	31	11.1
		medium	5 < a < 10	3	34	60.7	85	55.2	5	50	23	39	147	52.7
		long	≥ 10	5	16	28.6	58	37.7	5	50	22	37.3	101	36.2
	23. Leaf abaxial colour	green	–	1	–	–	30	19.5	6	60	–	–	36	12.9
		mixed	–	3	1	1.8	106	68.8	2	20	–	–	109	39.1
		purple	–	5	55	98.2	18	11.7	2	20	59	100	134	48
TUBER	24. Diameter (mm)	small	≤ 30	1	–	–	–	–	–	–	–	–	–	–
		medium	30 < a < 50	3	8	80	5	25	7	70	–	–	20	50
		broad	≥ 50	5	2	20	15	75	3	30	–	–	20	50
	25. Plant growth habit	weak	–	1	2	3.6	15	9.7	2	20	9	15.3	28	10
		medium	–	3	33	58.9	78	50.6	6	60	42	71.2	159	57
		strong	–	5	21	37.5	61	39.6	2	20	8	13.6	92	33
	26. Pedicel coiling	absent	–	1	–	–	–	–	–	–	–	–	–	–
		present	–	9	56	100	154	100	10	100	59	100	279	100
	27. Flowering	before leaf	–	1	56	100	149	96.8	10	100	59	100	274	98.2
		after leaf	–	9	–	–	5	3.2	–	–	–	–	5	1.8

C. mirabile (n = 56), *C. hederifolium* (n = 154), *C. graecum* (n = 10), *C. alpinum* syn. *C. trochopterantum* (n = 59)

RESULTS AND DISCUSSION

Six provinces in Turkey were visited and over 600 *Cyclamen* accessions were collected between 2010 and 2012. Morphological and cladistic characterization of current natural and endemic Turkish *Cyclamen* resources will allow for conservation efforts to be better coordinated, while optimizing biotechnology programmes involving *in vitro* conservation and cryopreservation aimed at preserving rare or endangered *Cyclamen* germplasm [Aka Kacar et al. 2013]. Yamaner and Erdag [2008] developed an *in vitro* propagation protocol for *C. mirabile* in which microtubers developed shoots. Prange et al [2008] also established an *in vitro* propagation protocol for *C. mirabile*, *C. coum* Mill., *C. graecum*, *C. libanoticum* Hildebr. and *C. hederifolium*).

Fig. 1. *C. graecum* (Antalya)

Flower characteristics. Petal length was classified as medium in 196 (70.2%), short in 74 (26.5%), and long in 9 (3.3%) accessions (tab. 2). Petal width was medium in 227 (81.4%) and large in 52 (18.6%) accessions. Petals were white in 49 (17%), diffuse-pink in 71 (24.7%), pale pink in 147 (51%) and deep pink in 21 (7.3%) accessions (fig. 1, 2, 3, 4, 5, 6). Variation in petal colour within a species is a common feature in *Cyclamen*, similar to that observed for *Iris* [Saad and Mahy 2009]. In nature, deep-pink coloured petals are usually seen in *C. alpinum* (fig. 6). Basal corolla ring colours were white in 8 (2.9%), pale pink in 11 (4%), deep pink in 205 (73.5%) and carmine in 55 (19.6%) accessions. Upper and lower corollas were deep-pink in 207 (74.2%), and carmine in 72 (25.8%) accessions. Basal corolla ring diameter was medium in 134 (49.8%), and broad in 135 (50.2%) accessions (fig. 6). Pistil length was medium in 110 (39.4%), and long in 169 (60.6%) accessions. Stamen length was medium in 134 (48%), and long in 145 (52%) accessions. Flower number/plant were few in 166 (59.5%), medium in 86 (30.8%), and many in 27 (9.7%) accessions. Pedicel length was short in 9 (3.2%), medium in 111 (39.8%), and long in 159 (57%) accessions. Darker petal margin was absent in 272 (97.5%) and present in 7 (2.5%) accessions. All accessions with a darker petal margin or spottiness were observed exclusively in

C. alpinum (fig. 6). In part I of our examination of wild Turkish *Cyclamen* accessions [Curuk et al. 2015], accessions with a darker petal margin or spottiness belonged exclusively to *C. persicum* (59.1%). 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.

Fig. 2. *C. mirabile* (Isparta)

The position of the stigma was non-exserted in 107 (38.6%) and exserted in 170 (61.4%) accessions. Debussche and Thompson [2002] stated that in *C. repandum*, *C. balearicum* and *C. creticum*, stigma position is significantly associated with flower colour, with the highest proportion of inserted stigmas occurring in the white flowers of *C. balearicum* (97.3%) and *C. creticum* (37.8%) and fewest (0%) in the pink flowers of *C. repandum*, *C. rhodense*, *C. peloponnesiacum* and *C. vividum*. Unlike their results, our study showed that *C. alpinum* (fig. 4) had a higher incidence of inserted stigmas (86.4%) than other species. Incidentally, *C. alpinum* accessions (37.6%) have deep-pink flowers. The percentage of exserted stigmas was high in *C. graecum* (100%) and *C. hederifolium* (87.7%) accessions (tab. 2).

In terms of quantitative traits, specifically petal number, different values were observed in *C. hederifolium* (fig. 5) accessions. This may be because the temperature of the cultivation site was higher (average temp. 19.1°C) than their natural location (average temp. 17.9°C in Izmir, and 17.6°C in Aydin) [MGM 2015].

Fig. 3. *C. hederifolium* (Selçuk/Izmir)

Leaf characteristics. A high level of variation in leaf characteristics was observed among the four *Cylamen* species (fig. 7). There were few leaves/plant in 30 (10.8%), medium number in 77 (27.6%), and a high number in 172 (61.6%) accessions. The lamina was short in 81 (29%), medium in 153 (54.9%), and long in 45 (16.1%) accessions. The lamina width was narrow in 83 (29.7%), medium in 170 (61%), and broad in 26 (9.3%) accessions. The lamina length/lamina width ratio was low (< 1) in 53 (19%), medium (= 1) in 16 (5.7%), and high (> 1) in 210 (75.3%) accessions. The intensity of the main colour of the lamina was light in 6 (2.1%), medium in 19 (6.8%), and dark in 254 (91.1%) accessions. Leaf pattern was absent in two accessions of *C. alpinum* (0.7%) but present in 277 (99.3%) accessions. Leaf pattern colours were silver in 169 (60.6%), yellow-green in 37 (13.2%), and green in 73 (26.2.9%) accessions. The degree of lamina lobing was weak in 128 (46.2%), medium in 40 (14.4%), and strong in 109 (39.4%) accessions. The petiole was short in 31 (11.1%), medium in 147 (52.7%), and long in 101 (36.2%) accessions. The leaf abaxial surface colour was green in 36 (12.9%), a mixture of green and purple in 109 (39.1%), and purple in 134 (48%) accessions (fig. 7).

Fig. 4. *C. alpinum* (Denizli)

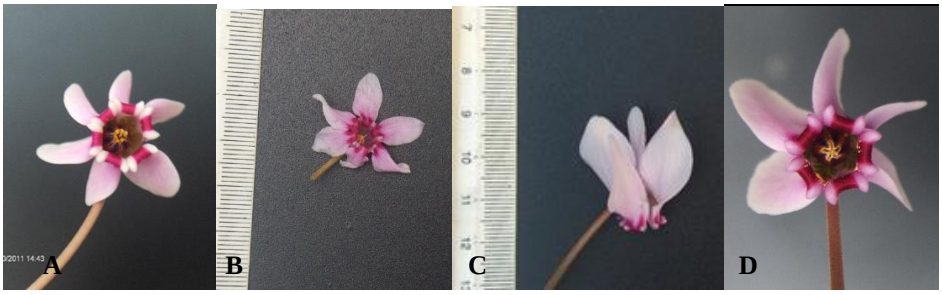

Fig. 5. Flowers of *C. hederifolium* with 6 petals (A, B) and 5 petals (C, D)

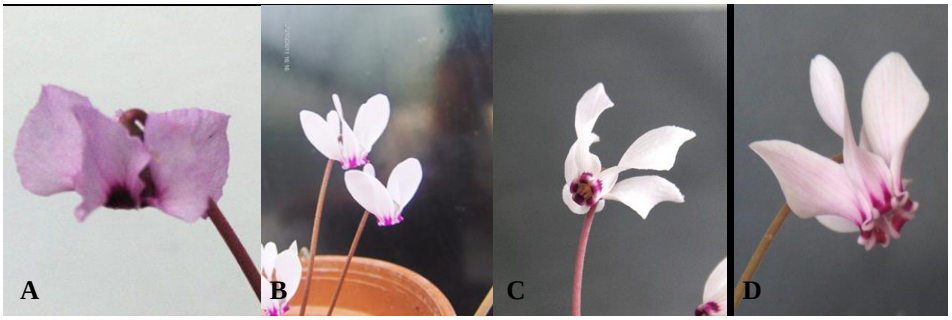

Fig. 6. Flowers of four Turkish *Cyclamen* species: (A) *C. alpinum*, (B) *C. graecum*, (C) *C. mirabile*, (D) *C. hederifolium*

Tuber characteristics. Tuber diameter was medium in 20 (50.0%), and broad in 20 (50.0%) accessions (fig. 9). Tubers were larger in *C. graecum* and *C. hederifolium*. *Cyclamen* species with more than 30 chromosomes, such as *C. hederifolium* (2n = 34) and *C. graecum* (2n = 84), 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]. It can also be associated with the age of the accessions since *Cyclamen* can not produce sister tubers but enlarge with ageing [Le Nard and De Hertogh 1993]. Plant growth habit was weak in 28 (10%), medium in 159 (57%), and strong in 92 (33%) accessions. Flowering preced-

ed leaf development in 274 (98.2%), or followed leaf development in 5 (1.8%) accessions. The fruit is a capsule, often drawn down to the soil level by the twisting of the floral stalk [Davis 1978, Le Nard and De Hertogh 1993, Debussche and Thompson 2002]. Pedicel coiling was present in all accessions (279, 100%) of all four species (fig. 8). These findings were adjusted with Clennett's cladistic data matrix [Clennett 2002]. These species share several characteristic features that diagnose them as a monophyletic group, such as a well-developed tuberous subterranean bulb formed by swelling of the hypocotyl, conspicuously reflexed corolla lobes, and coiled fruiting pedicels. In all *Cyclamen* species except for four, the pedicel coils down to the soil surface when the fruit ripens, usually coiling from the apex downwards, but in *C. rohlfsianum* the pedicel coils from the base towards the apex, and in *C. graecum* it coils two ways from the middle (fig. 8B). In two other species, *C. persicum* and *C. somalense*, the pedicel does not coil, but becomes curved at anthesis [Anderberg et al. 2000]. All *Cyclamen* species have the same broad growth aspect, but differ from each other in characters such as size and structure of the tubers, denticulation of the leaf margin, width of corolla mouth, presence or absence of auricles at the corolla mouth, chromosome number, and the time of year in which the flowers develop [Anderberg et al. 2000, Mammadov et al. 2016]. This range of phenomena was also confirmed for the four species examined in this study, and in another four species examined in our previous study [Curuk et al. 2015].

Fig. 7. Leaves of four Turkish *Cyclamen* species: (A) *C. alpinum*, (B) *C. graecum*, (C) *C. mirabile*, (D) *C. hederifolium*

The standard deviations, as well as minimum and maximum values were compared (tab. 3). Morphological diversity does not correspond to geographic region but rather to species. Among the species sampled, *C. hederifolium* contained the highest mean values for flower number/plant (7.2), petal length (20.6 mm), pedicel length (11.4 cm), lamina length (5.3 cm) and tuber diameter (5.4 cm). *C. alpinum* and *C. graecum* shared the highest mean values for petiole length (10.45 cm). The highest mean values for leaf number/plant were 15.6 in *C. mirabile* and followed by 13.3 in *C. hederifolium*. Tuber

diameter (fig. 9) was greatest in *C. graecum* and *C. hederifolium* (45.13 and 53.89 mm, respectively). These results are broadly compatible with the description of the Flora of Turkey [Guner et al. 2000]. Also, in Sahin and Burun's [2010] study, morphological measurements and observations were performed on *C. alpinum* plants collected from two different locations (Marmarlı and Gökbel) in Dalyan in the border of Ortaca town in Muğla showing an average diameter of tubers 4.04 cm, tuber height 2.17 cm, tuber weight 21.25 g, leaf width-length 4.28–3.45 cm, number of leaves/plant 9.05, and flower number/plant 5.25. Some morphological measurements mean values of *C. mirabile* were, tuber diameter 3.83 cm, height of plant 12.71 cm, flower number/plant 5 [Mammadov et al. 2016].

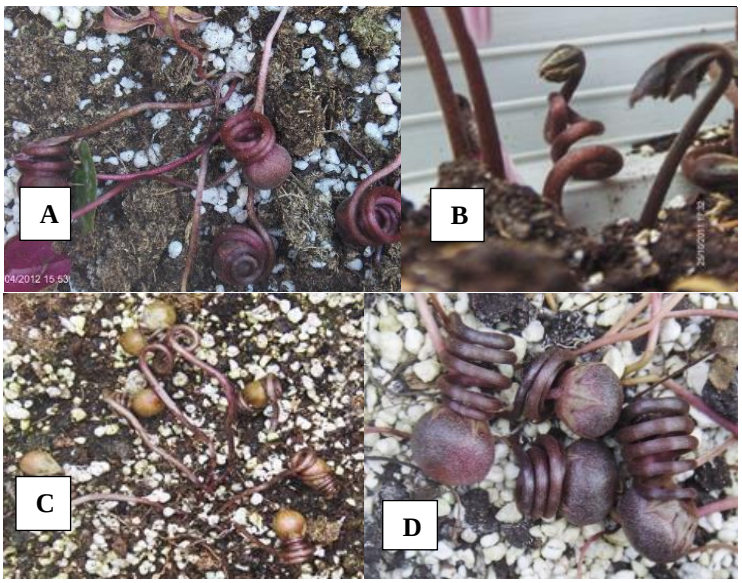

Fig. 8. Fruits of four Turkish *Cyclamen* species: (A) *C. alpinum*, (B) *C. graecum*, (C) *C. mirabile*, (D) *C. hederifolium*

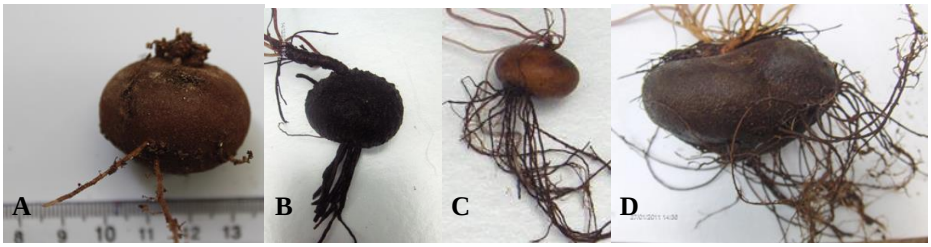

Fig. 9. Tubers of four Turkish *Cyclamen* species: (A) *C. alpinum*, (B) *C. graecum*, (C) *C. mirabile*, (D) *C. hederifolium*

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

Traits	*C. hederifolium*			*C. mirabile*			*C. alpinum*			*C. graecum*			Overall		
	mean	min.-max.	SD	mean	min.-max.	SD	mean	min.-max.	SD	mean	min.-max.	SD	mean	min.-max.	SD
Petal length (mm)	**20.61 ±0.6**	7.61–27.73	3.498	18.26 ±0.3	13.48–23.27	2.133	10.88 ±0.2	8.00–15.00	1.684	19.12 ±0.5	16.55–20.84	1.476	18.17	7.61–38.98	4.787
Petal width (mm)	**9.09 ±0.1**	4.43–13.12	1.545	6.74 ±0.1	5.03–9.08	1.009	9.05 ±0.1	7.00–12.00	1.114	8.31 ±0.4	5.84–9.40	1.196	8.49	3.47–15.00	1.643
Flower number/plant	**7.18 ±0.4**	1.00–29.00	5.266	6.75 ±0.8	1.00–28.00	6.046	3.53 ±0.3	1.00–14.00	2.602	2.70 ±0.9	1.00–9.00	2.869	6.54	1.00–47.00	5.168
Pedicel lenght (cm)	**11.36 ±0.2**	3.00–17.70	3.034	10.40 ±0.4	3.00–20.00	2.793	8.70 ±0.4	4.00–17.00	3.085	9.33 ±1.2	5.00–18.00	3.676	10.73	3.00–28.00	3.191
Basal corolla ring diameter **(**mm)	**5.97 ±0.1**	3.31–8.45	0.928	3.04 ±0.1	2.12–4.90	0.589	3.33 ±0.1	2.00–5.00	0.398	5.18 ±0.2	4.27–5.79	0.645	4.01	1.44–8.45	1.562
Pistil length (mm)	**7.26 ±0.1**	4.00–9.8	0.864	5.35 ±0.1	3.85–8.03	1.000	5.02 ±0.1	4.00–7.00	0.632	6.22 ±0.2	5.30–7.40	0.670	6.14	2.58–10.00	1.321
Stamen length (mm)	**5.24 ±0.1**	2.15–7.53	0.757	4.55 ±0.1	3.10–5.72	0.563	4.82 ±0.1	3.00–7.00	0.690	4.60 ±0.2	3.56–5.63	0.665	4.99	2.15–9.94	0.760
Leaf number/plant	13.32 ±0.6	3.00–35.00	6.984	**15.60 ±1.0**	3.00–37.00	7.480	9.61 ±0.5	3.00–19.00	4.017	10.60 ±1.3	5.00–18.00	4.006	13.88	1.00–70.00	6.771
Petiol length (cm)	9.36 ±0.3	2.30–20.20	3.605	8.63 ±0.4	3.70–17.00	2.804	**10.45 ±0.9**	3.00–26.00	7.128	**10.45 ±1.0**	6.67–17.33	3.105	9.29	1.50–26.00	4.473
Lamina length (cm)	**5.25 ±0.1**	2.00–9.00	1.371	3.11 ±0.1	1.70–4.60	0.592	2.90 ±0.1	2.00–5.00	0.626	4.99 ±0.2	3.90–6.17	0.740	4.03	1.30–9.00	1.556
Lamina width (cm)	**4.70 ±0.1**	1.60–8.00	1.270	2.96 ±0.1	1.60–4.30	0.561	2.99 ±0.1	2.00–5.00	0.704	4.33 ±0.3	2.87–5.63	0.875	3.77	1.10–8.00	1.334
Lamina: length/width ratio	1.13 ±0.0	0.90–2.28	0.142	1.05 ±0.0	0.89–1.20	0.067	0.98 ±0.0	1.00–1.00	0.108	**1.17 ±0.0**	0.99–1.56	0.157	1.07	0.66–2.28	0.138
Diameter of tuber (mm)	**53.89 ±2.5**	35.73–66.25	7.596	40.4 ±4.0	30.98–69.82	12.416	N/A	N/A	N/A	45.13 ±3.1	30.50–58.30	9.778	44.21	16.75–110.99	

According to the list (tab. 2) of morphological characterization criteria (27 traits), leaf pattern was observed as present in > 90%, the intensity of the leaf blade's main colour was dark in > 90%, and the formation of an inflorescence prior to leafing (hysteranthous) was > 95%, while coiling petioles were observed in 100% of accessions of all species. The presence of a darker petal margin was only observed in *C. alpinum* (present in 11.9% of 59 accessions) but not in the remaining three species (*C. graecum*, *C. hederifolium* and *C. mirabile*) (fig. 6). A wide range of variation was observed in the evaluated agronomic traits (22 traits out of 27; in 5 out of 27 traits, over 85% of features were similar).

Table 4. Results of principle component analysis (PCA) (for the first three PCs) of four *Cyclamen* species and 279 accessions collected from Turkey for 12 agro-morphological traits

Traits	PC1	PC2	PC3
Petal length (mm)	0.35	0.01	-0.21
Petal width (mm)	0.24	-0.27	0.23
Flower number/plant	0.16	0.62	-0.06
Pedicel length (cm)	0.25	0.08	0.29
Basal corolla ring diameter (mm)	0.39	-0.07	-0.13
Pistil length (mm)	0.38	-0.18	-0.23
Stamen length (mm)	0.26	-0.26	0.02
Leaf number/plant	0.12	0.64	-0.12
Petiole length (cm)	0.08	0.12	0.67
Lamina length (cm)	0.41	0.03	0.10
Lamina width (cm)	0.38	0.04	0.25
Lamina length/width ratio	0.19	-0.03	-0.45
Eigenvalue	4.44	1.58	1.41
Proportion	0.37	0.13	0.12

Based on a cluster analysis (data not shown) performed using morphological data, accessions were clustered into the same groups based on species but not on geographic origin. For example, *C. coum* and *C. persicum* from part I [Curuk et al. 2015] and *C. hederifolium* accessions collected from different locations flowered in the same season, and had the same colour of the abaxial leaf surface and the same petal colour (tab. 2). Clustering according to plant species has also been reported in *Oncocyclus* irises [Saad and Mahy 2009] and chamomile (*Matricaria chamomilla*) [Solouki et al. 2008].

Principle component analysis. The results of PCA are presented in Table 4. The first PC explained 37% (eigenvalue of 4.44), while the second and third PCs contributed 13 and 12%, respectively to total variance (eigenvalues of 1.58 and 1.41, respectively). PCA explained 62% of total morphological variation. Lamina length (0.41), lamina width (0.38), basal corolla ring diameter (0.39), pistil length (0.38) and petal length (0.35) were important variables defining PC1. Leaf and flower number/plant (eigenvalues of 0.64 and 0.62, respectively) were the two traits that were highly correlated with PC2. Petiole length (eigenvalue of 0.67) was the most important trait for PC3.

From all characters, petiole length was found to be the most discriminative parameter differentiating accessions. Similar trends were observed in *C. persicum*, *C. cilicium*, *C. pseudibericum* and *C. coum* growing naturally in Turkey [Curuk et al. 2015]. Mih et al. [2008] evaluated the morphology of four selections of *Vernonia hymenolepis* A. Rich. based on PCs, reporting a high level (80%) of morphological variation.

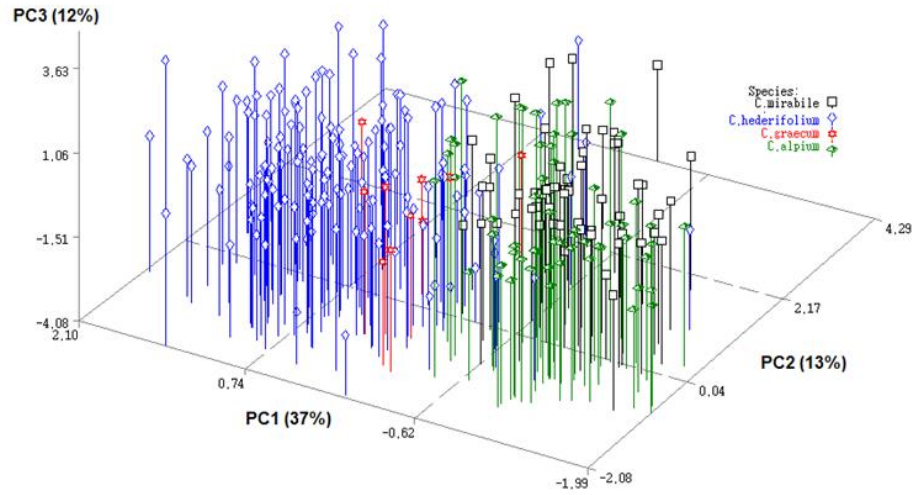

Fig. 10. Plot of four *Cyclamen* (*C. alpinum*, *C. graecum*, *C. mirabile*, *C. hederifolium*) accessions collected from Turkey on the first three principle components (PCs) obtained from the analysis of 12 quantitative agro-morphological traits using PCA

According to PCA using qualitative and quantitative data obtained from the observations of 27 morphological characters in these four Turkish *Cyclamen* species, accessions could not be separated into different groups (fig. 11). While PC1 explained 30% of total morphological variation, PC2 explained 8% and PC3 8% of total variation, with a total of 46% variation accounted for. Aalaei et al. [2007] found that effective traits related to the colour of flowers, petioles, young leaves on the adaxial site, and flower shelf life could be categorized into six groups that covered a total of 86.89% of total variation. In this study, PCA results showed that *Cyclamen* species that originated from a similar geographic area or the same species that originated from different geographical areas were grouped separately (fig. 10). Zhao et al. [2007] found that the lack of conformity between genetic and geographic 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 topographic and climatic variation. However, the appearance of different accessions is related to basic evolutionary elements (selection, adaptation, migration, self-pollination and genetic drift), which are related to environmental and

anthropogenic activities over time [Martins et al. 2006]. In the present study, during our survey, we came across *C. hederifolium* which grows naturally in the western part of Turkey, in one location of Urun village, Osmaniye located in the southern part of Turkey. This might be because of human interference, introduction of new accessions or exchange of genetic material.

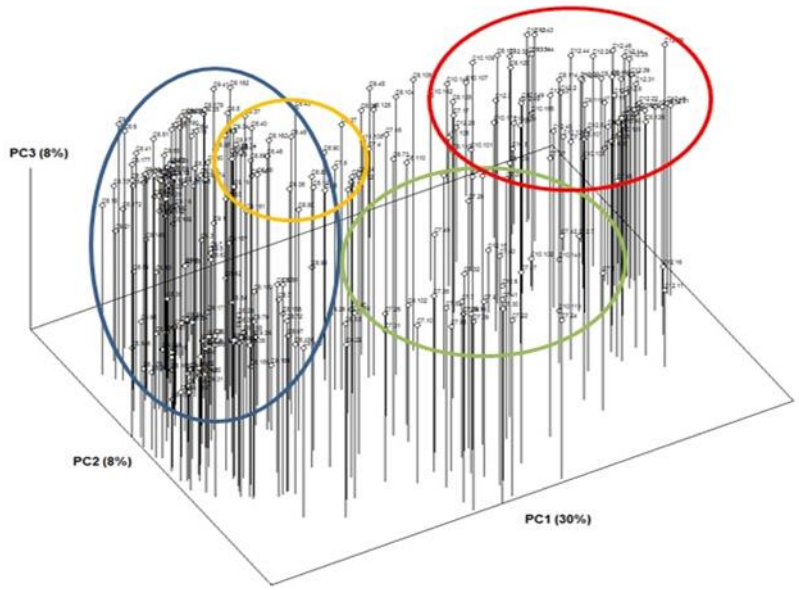

Fig. 11. Plot of cyclamen accessions collected from Turkey on the first three principle components (PC) obtained from analysis of 27 agro-morphological traits using PC analysis

CONCLUSIONS

The results obtained in this study allowed for the accurate identification and discrimination of four *Cyclamen* species (*C. hederifolium*, *C. alpinum* (syn. *C. trochopterantum*), *C. graecum*, *C. mirabile*) growing wild in Turkey, which boasts over 270 accessions. This set of results complements our investigation of *C. persicum*, *C. cilicium*, *C. pseudibericum* and *C. coum*, also growing naturally in Turkey [Curuk et al. 2015]. Tuber diameter, leaf width, petal length, petal colour, leaf shape, and the presence of auriculate characters were the most acutely different morphological characteristics. Such a detailed morphological characterization is useful for identifying superior accessions that are hardy to cold, have attractive leaves, or flower fragrance, which could be taken into consideration in future hybridization programs of *Cyclamen*. In such a programme, a wide range of variability, heritability, genetic advances and positive correlation coefficients among traits can be excellent tools to improve or select accessions of interest [Akbar et al. 2003]. There were distinct morphological differences among all four spe-

cies which allowed them to be clearly distinguished based on morphological characteristics alone, as well as on PCA. There is a need to compare *Cyclamen* species from other Mediterranean countries where they grow naturally, and to compare them with species from Turkey. Additionally, other DNA loci as well as isozyme data need to be analyzed to clarify the taxonomic name and endemic status of populations distributed naturally in Turkey. 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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MORFOLOGICZNA CHARAKTERYSTYKA *Cyclamen* sp. ROSNĄCYCH W TURCJI W WARUNKACH NATURALNYCH: CZĘŚĆ II

Streszczenie. Scharakteryzowano morfologię 279 obiektów *Cyclamen* sp. rosnących w warunkach naturalnych w Turcji, mianowicie *C. alpinum* (syn. *C. trochopteranthum*), *C. graecum, C. hederifolium* (syn. *C. neapolitanum*) i *C. mirabile*. Rośliny z nienaruszonymi bulwami zebrano ze stanowisk w Antylii, Isparcie, Aydın, Muğla, İzmir oraz Denizli, według wskazań GPS, gdzie rosną naturalnie wiosną i jesienią. Scharakteryzowano morfologię czterech gatunków *Cyclamen* przy użyciu jednorocznych zregenerowanych roślin w oparciu o 27 cech morfologicznych (13 dotyczyło kwiatów, 11 liści, 2 roślin, 1 bulw). Między badanymi obiektami stwierdzono znaczne różnice dotyczące barwy płatków, długości szypułki, długości i szerokości liścia, kształtu liścia oraz średnicy bulwy. Chociaż analiza składników potwierdziła zgrupowanie cech w grupy specyficzne dla gatunku, to potrzeba więcej danych morfologicznych oraz molekularnych, aby wyciągnąć rzetelne wnioski na temat klasyfikacji tych gatunków *Cyclamen*.

Słowa kluczowe: *Cyclamen*, zasoby genetyczne, charakterystyka morfologiczna

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