TÜBİTAK

Research Article

Turk J Bot
36 (2012) 9-19

doi:10.3906/bot-1011-19

Primula × *uzungolensis* (Primulaceae): a new natural hybrid from NE Anatolia

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Received: 10.11.2010
Accepted: 19.06.2011

Abstract: *Primula* × *uzungolensis* Terzioğlu & Coşkunçelebi, a natural hybrid from north-eastern Anatolia, is described and illustrated with its parents. Based on its morphology and molecular characteristics, we conclude that *P.* × *uzungolensis* is a new natural hybrid between *P. acaulis* (L.) Hill subsp. *rubra* (Sm.) Greuter & Burdet and *P. veris* L. subsp. *columnae* (Ten.) Maire & Petitmengin. Maximum parsimony (MP) analysis based on nuclear ribosomal DNA (nrDNA) and chloroplast DNA (cpDNA) shows that *P. veris* subsp. *columnae* is the maternal parent of the putative hybrid, and the hybridisation between *P. veris* subsp. *columnae* and *P. acaulis* subsp. *rubra* is unidirectional.

Key words: Natural hybrid, *Primula*, Primulaceae, taxonomy, Turkey

Primula × *uzungolensis* (Primulaceae): Kuzeydoğu Anadolu'dan yeni bir doğal hibrit

Özet: *Primula* × *uzungolensis* Terzioğlu & Coşkunçelebi Kuzeydoğu Anadolu'dan doğal bir hibrit olarak tanımlanmış, yeni taksonun ve ebeveynlerinin şekilleri verilmiştir. Morfolojik ve moleküler karakterlerine dayalı olarak *P.* × *uzungolensis*'in *P. acaulis* (L.) Hill subsp. *rubra* (Sm.) Greuter & Burdet ve *P. veris* L. subsp. *columnae* (Ten.) Maire & Petitmengin arasında yeni bir doğal hibrit olduğu sonucuna varılmıştır. nrDNA ve cpDNA verilerine dayalı olarak yapılan MP analizi, *P. veris* subsp. *columnae*' nın hibrid taksonun annesi olduğunu ve gen akışının *P. veris* subsp. *columnae* ve *P. acaulis* subsp. *rubra* arasında tek yönlü olarak gerçekleştiğini göstermiştir.

Anahtar sözcükler: Doğal hibrit, *Primula*, Primulaceae, taksonomi, Türkiye

Introduction

Natural hybridisation is a relatively common feature in vascular plants and it is known to be involved in a number of evolutionary and diversification processes in plants (Kirk et al., 2004). Although natural hybridisation events are assumed to be common in the genus *Primula* L. in many regions (Richards, 2002), including England (Clifford, 1958; Valentine, 1961) and China (Zhu et al., 2009), it has never been recorded in north-eastern Anatolia. A total of 12 taxa of *Primula* belonging to 8 species

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were recognised by Lamond (1978) in Turkey. Most of these taxa, with the exception of *P. davisii* W.W.Sm, are distributed in north-eastern Anatolia. During our field work for an ongoing project supported by the Turkish Ministry of Environment and Forestry, Environmental Protection Agency for Special Areas (EPASA), the authors found that *P. acaulis* (L.) Hill subsp. *rubra* (Sm.) Greuter & Burdet and *P. veris* L. subsp. *columnae* (Ten.) Maire & Petitmengin) coexisted in the protected area of Uzungöl (NE Turkey: A8 Trabzon). The most widespread taxon, *P. acaulis* subsp. *rubra*, presented in the habitat from 0 to 2200 m, whereas *P. veris* subsp. *columnae* is restricted to an area of 1200 to 2135 m in north-eastern Turkey. The 2 taxa also shared the same habitat; meadows and grassy banks above 1000 m. *P. acaulis* subsp. *rubra* has pink to mauve petals, while *P. veris* subsp. *columnae* has deep yellow petals. The leaves of *P. acaulis* subsp. *rubra* are obovate to oblanceolate and those of *P. veris* subsp. *columnae* are ovate-oblong to elliptic (Lamond, 1978). The author also noted that some specimens, which look like a rare form of *P. acaulis* at first glance, coexisted among *P. acaulis* subsp. *rubra* and *P. veris* subsp. *columnae*. However, after cautious morphological examination of this specimen based on the literature and many other examined materials, we decided that it was a new natural putative hybrid. Although many hybrids have intermediate morphological features between their parents (Rieseberg & Ellstrand, 1993), morphological intermediacy is not invariably associated with hybrids (Park et al., 2003). Therefore, a putative hybrid requires further investigation. In the present study the putative hybrid taxon is consequently described and illustrated together with its parents. Additionally, the nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) region from *P. acaulis* subsp. *rubra* and the putative hybrid taxon was sequenced to determine the status of this morphologically intermediate hybrid, and 1 coding region of chloroplast DNA (*mat*K) from 3 taxa was sequenced to determine the direction of hybridisation.

Materials and methods

Plant materials

Specimens of *P. acaulis* subsp. *rubra, P. veris* subsp. *columnae*, and the putative hybrid were collected from wild populations in Uzungöl (NE Turkey: A8 Trabzon). Leaves from at least 2 individuals from each taxon were collected in plastic bags with silica gel for DNA extraction. The details of the samples used in this study were listed in Table 1. Vouchers are deposited in the herbaria of both the Faculty of Forestry (KATO) and the Biology Department (KTUB) at Karadeniz Technical University.

Morphological analysis

The 9 populations listed in Table 1 were assessed to determine the phenetic similarity based on 19 morphological traits (Table 2). Phenetic measurement and observations were scored from at least 3 or 4 samples for all traits, and the average

Table 1. Locality information for the examined populations.

Pop. no.	Taxa	UTM		Altitude (m)	Date	Accession Number	
		x	y			ITS	matK
P80	*P.* × *uzungolensis*	611044	4496647	1133	02 iv 2009	HQ535974	HQ535970
P83	*P.* × *uzungolensis*	611042	4496703	1139	18 iv 2010	HQ535977	HQ535967
P85	*P.* × *uzungolensis*	608139	4497501	1272	18 iv 2010	HQ535982	HQ535972
P81	*P.* × *uzungolensis*	608061	4497466	1312	18 iv 2010	HQ535975	HQ535969
P86	*P. acaulis* subsp. *rubra*	608060	4497466	1313	18 iv 2010	HQ535979	HQ535965
P87	*P. acaulis* subsp. *rubra*	607879	4497600	1455	18 iv 2010	HQ535980	HQ535964
P79	*P. veris* subsp. *columnae*	607759	4497646	1491	18 iv 2010	HQ535981	HQ535971
P82	*P.veris* subsp. *columnae*	607702	4497657	1518	18 iv 2010	HQ535976	HQ535968
P84	*P. veris* subsp. *columnae*	607648	4497260	1542	18 iv 2010	HQ535978	HQ535966

Table 2. Characters used in phenetic analysis.

No.	Characters
X_1	Plant length (cm)
X_2	Leaf length (cm)
X_3	Leaf width (cm)
X_4	Leaf length/ leaf width
X_5	Pedicel length (cm)
X_6	Depth of calyx teeth (mm)
X_7	Calyx length/ depth of calyx teeth
X_8	Number of bracts
X_9	Number of flowers
X_{10}	The state of glands in the calyx thorax; 0: absence, 1: presence
X_{11}	The base shape of leaf; 0: truncate, 1: attenuate
X_{12}	The state of the gland on the pedicel; 0: absence, 1: presence
X_{13}	Inflorescence; 0: absence, 1: presence
X_{14}	The shape of the inflorescence; 0: absence, 1: second, 2: umbellate
X_{15}	The colour of flower; 0: pure yellow, 1: yellow to purple, 2: purple
X_{16}	The state of the bracts; 0: absence, 1: presence
X_{17}	The state of corolla tube length versus calyx; 0: shorter, 1: longer
X_{18}	Leaf shape; 0: elliptic, 1: obovate, 2: elliptic-obovate
X_{19}	Calyx shape; 0: obconical, 1: cylindrical

scores of all populations were combined to yield the basic raw data matrix. Principal component analysis (PCA) and cluster analysis (CA) were performed using SYN-TAX PC 5.0 (Podani, 1993). For the CA, a pairwise matrix of resemblance values was calculated from the raw data matrix using Gower's coefficient for mixed data sets (Sneath & Sokal, 1973). A dendrogram was generated by the unweighted pair-group method using arithmetic averages (UPGMA). For PCA, the standardised data were used to create a covariance matrix, and 2 eigenvectors were extracted, providing 2 axes onto which the standardised data were projected to give a 2-dimensional plot of the examined population (Table 1).

Molecular data analysis

Total genomic DNAs were extracted from silica-dried material following the modified CTAB extraction procedure of Doyle and Doyle (1987) according to Gültepe et al. (2010). The nrDNA ITS region and m*at*K were amplified by polymerase chain reaction (PCR) using a Biometra personal thermal cycler. The amplification reaction for ITS was performed using universal ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 primers (5'GGAAGTAAAAGTCGTAACAAGG-3') developed by White et al. (1990). The universal primers MG15 (5'-ATCTGGGTTGCTAACTCAATG-3') and MG1 (5' -CTACTGCAGAACTAGTCGGATGGAGTAGAT-3') designed by Liang and Hilu (1996) were used for *mat*K.

PCR product purification and DNA sequence analysis were performed by Macrogen Inc. (Seoul, South Korea). The sequencing process was conducted with BigDyeTM terminator cycling protocols (Applied Biosystems Inc., Foster City, CA, USA). PCR products were purified using ethanol precipitation and run on an automatic sequencer (ABI 3730 × 1) by a contract laboratory. Sequencing of 5' end of ITS region was carried out using the primer ITS4. Sequences with ambiguous sites were resequenced from the 3' end with the primer of ITS5.

For the sequencing of *mat*K gene, forward Mat2B (5'-TGAACGAACATAAAATGGCTTG-3') and reverse MatK1 (5'-CTTTCTCCGCAATCAATCTTC-3') primers from internal of *mat*K were designed. Sequencing of the 5' end of *mat*K region was carried out using the primer MatK1. Sequences with ambiguous sites were resequenced from the 3' end with the primer of Mat2B. Then these 2 sequences were combined. The sequences were automatically aligned using BioEdit v.7.0 software (Hall, 1999).

Phylogenetic analyses were conducted using the PAUP* 4.0 b10 (Swofford, 2002), with gaps treated as missing data. Maximum parsimony (MP) analyses were conducted using heuristic searches with 100 random addition replicates, with no more than 100 trees saved per replicate. Support for clades was estimated using 1000 bootstrap replicates—each with 100 random addition replicates, saving no more than 1500 trees per bootstrap replicate— and TBR branch swapping with the MulTrees option in effect. A Turkish endemic primrose, *Primula longipes* Freyn & Sint., was selected as outgroup (GenBank: EU643662) for MP analysis. All the sequence data were submitted to GenBank under the accession number given in Table 1.

Results

***Primula* × *uzungolensis* Terzioğlu & Coşkunçelebi** (*P. acaulis* (L.) Hill subsp. *rubra* (Sm.) Greuter & Burdet ≡ *Primula vulgaris* Huds. subsp. *rubra* (Sm.) Arcangeli ≡ *P. vulgaris* Huds. subsp. *sibthorpii* (Hoffmanns.) W.W. Sm. Forrest) × *Primula veris* L. subsp. *columnae* (Ten.) Maire & Petitmengin), **hybr. nov.**

Type: Turkey. NE Anatolia: A8 Trabzon, Uzungöl, 1139 m, meadows, 18 iv 2010 (Holotype: KATO 18794, Isotype: KTUB (P83), (Figures 1, 2).

Figure 1. Habits of *P. acaulis* subsp. *rubra* (♂) (a), *P.* × *uzungolensis* (b), and *P. veris* subsp. *columnae* (♀) (c); *P.* × *uzungolensis* with its parents in their habitat (d); fruiting calyx tubes: *P. acaulis* subsp. *rubra* (e), *P.* × *uzungolensis* (f), and *P. veris* subsp. *columnae* (g); determining global position of *P.* × *uzungolensis* with GPS (h).

Figure 2. *P. acaulis* subsp. *rubra* (♂) (a), *P.* × *uzungolensis* (b), and *P. veris* subsp. *columnae* (♀) (c).

Pubescens, scaposa. Folia petioli inclusi esque ad 5.8-10.2 × 2.5-3.22 cm. Lamina ovate-elliptica, Scapus 11.5-17 cm longus. Umbella 8-10 flora. Calyx 10-14 mm longus. Hybrida naturalis e *Primula acaulis* subsp. *rubra et Primula veris* subsp. *columnae.*

Acaulescent, densely pilose perennial herb, 11.5-17 cm with stout rhizome bearing numerous slender lateral roots. Leaves 5.8-10.2 × 2.5-3.22 cm, lamina ovate or elliptic, base truncate or truncate-cuneate, longer than the ± winged petiole, scarcely simple hairy above, often densely below, margin irregularly crenate. Scape with scarce to densely eglandular hairs. Inflorescence 8-10 flowered, umbellate. Pedicel 3-6 cm with several simple eglandular hairs. Bracts 7-9, lobes 7-12 mm, linear-lanceolate, long acute, scarcely with simple hairs. Calyx 10-14 mm, cylindrical, divided one-fourth to one-third into narrow-triangular acuminate teeth with several glandular hairs. Corolla tube 14-18 mm, equalling or longer than calyx; lobes 8-10 mm, emarginated purple to yellow. Capitula shorter than to equalling calyx.

Etymology: The specific epithet is derived from the type locality, Uzungöl.

Discussion

P. acaulis (*P. vulgaris*) is reported to hybridise with both *P. veris* and *P. elatior* (Jacquemyn et al., 2009), and several hybrids between primrose and cowslip were recorded in the literature. According to Jacquemyn et al. (2009), *P. acaulis* is represented by 5 subspecies in the world, and 2 of them are naturally distributed in north-eastern Anatolia (Lamond, 1978). *P. acaulis* subsp. *rubra* ≡ *P. vulgaris* subsp. *sibthorpii* is an Euxinian, and *P. veris* subsp. *columnae* is a Euro-Siberian element; no hybrid taxon has been reported between them from this phytogeographic region. However, hybrid taxon *P.* × *polyantha* Miller was recorded from Great Britain between *P. veris* subsp. *veris* and yellow flowered *P. acaulis* subsp. *rubra* (Brys & Jacquemyn, 2009). *P.* × *polyantha* hybrids are intermediate in leaf with the flower borne in an umbel on a scape as in *P.* × *uzungolensis*, but the maternal parents are different. Taylor and Woodell (2008) noted that these yellow flowered hybrids are sometimes confused with *P. elatior* (L.) Hill. *P. elatior* is represented by 3 subspecies in Turkey, but there is no population in the habitat of *P.* × *uzungolensis*. *P. murbeckii* Lindq. (*P. elatior* × *P. veris* × *P. acaulis*) is another reported hybrid taxa resulting from long-distance fertilisation of wild *P. elatior* by the pollen of the garden *P.* × *polyanthus* (Taylor & Woodell, 2008).

As seen in Table 3, several morphological traits of the putative hybrid are intermediate between those of its parents. Based on morphological comparisons, the unusual plants collected from Uzungöl were natural hybrids. As seen in Figure 3, all examined populations fall into 2 major clusters, with 1 cluster containing 2 of the 9 populations. The first group

Table 3. Comparison of *Primula* × *uzungolensis* with putative parents of *Primula acaulis* subsp. *rubra* and *P. veris* subsp. *columnae.*

Characters	***P. acaulis* subsp. *rubra***	***P.* × *uzungolensis***	***P. veris* subsp. *columnae***
Leaf shape	Elliptical	Elliptical to obovate	Obovate
Leaf base	Attenuate	Truncate	Truncate
The state of glands in the thorax of calyx	Presence	Presence	Absence
The state of the gland on the pedicel	Absence	Absence	Presence
Bracts	Absence	Presence	Presence
The colour of the flower	Purple	Yellow to purple	Yellow
The state of corolla tube length versus calyx	Longer	Shorter	Shorter
Calyx shape	Cylindrical	Cylindrical	Obconical
Depth of calyx teeth (cm)	0.5-0.6	0.5-0.7	0.2-0.3
Pedicel (cm)	-	3-6	0.8–1
Leaf width (cm)	3.5-4	2.5-2.86	2.2-2.96

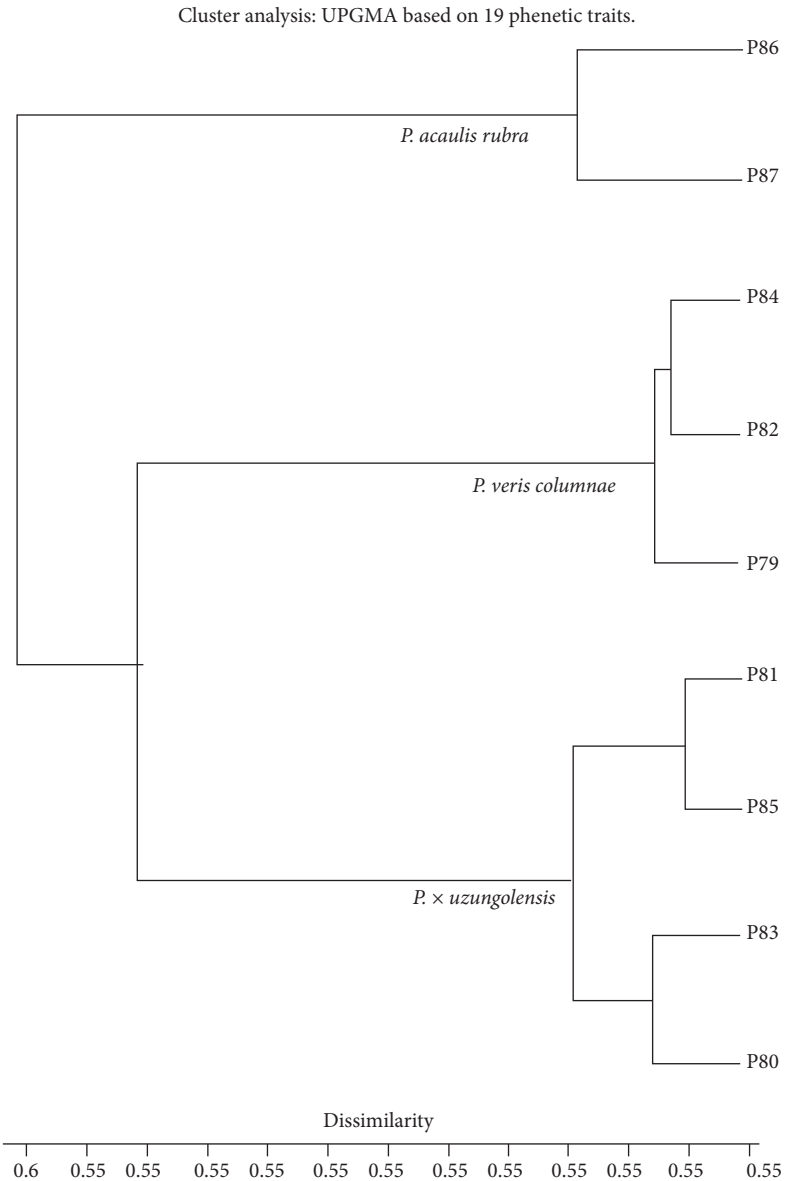

Figure 3. Dendrogram resulting from UPGMA. Population numbers are given in Table 1.

includes only representatives of *P. acaulis* subsp. *rubra*, which are linked to each other at a very high similarity level (0.15). The second larger group divides into 2 smaller clusters including the putative hybrid and *P. veris* subsp. *columnae* populations. Oyelana and Ogunwenmo (2009) indicated that morphological traits in the hybrids showed more maternal influence than paternal. In this group, representatives of putative hybrid taxa were nested in a tight subcluster at 0.1 dissimilarity level.

Results from UPGMA (Figure 3) and PCA (Figure 4) accord with those of morphological similarities listed in Table 3; results also demonstrate that, between its parents, the putative hybrid taxa is closer to *P. veris* subsp. *columnae* and the putative hybrid taxa crosses of *P. acaulis* subsp. *rubra* and *P. veris* subsp. *columnae*. According to our field observation in Uzungöl, there are at least 2 factors that contribute to this interspecific hybridisation in *Primula*. The first factor is that *Primula* taxa often have partially

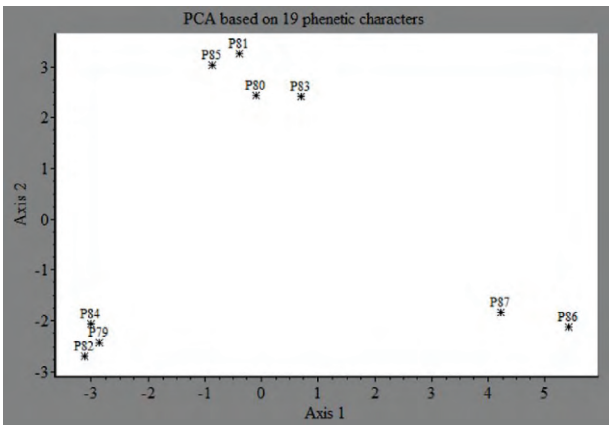

Figure 4. Scattergram resulting from PCA. Population numbers are given in Table 1.

overlapping geographic distribution and slightly overlapping habitats in the cited area. The second factor that may contribute to natural hybridisation is the long and partially overlapping flowering periods in this region.

Molecular techniques provide a powerful means of identifying hybrid genotypes (Rieseberg, 1998). For the identification of hybrid origin, nrDNA—especially the variable ITS region—is frequently employed (Rauscher et al., 2002; Guggisberg et al., 2006). The internal transcribed region is about 700 bp in angiosperms (Baldwin et al., 1995). This region ranges from 699 to 712 in the representatives of *P. acaulis* subsp. *rubra*, from 711 to 713 in the representatives of *P. veris* subsp. *columnae*, and from 712 to 713 in representatives of the putative hybrid (Table 4). In total, there were 12 nucleotide substitutions between *P. acaulis* subsp. *rubra* and *P. veris* subsp. *columnae*. On the other hand, some putative hybrid samples revealed intermediate sequences between *P. acaulis* subsp. *rubra* and *P. veris* subsp. *columnae*. It is well known that nuclear genes are biparentally inherited, and the hybrids should possess both divergent copies of their putative parents (Sang et al., 1995). However, there are also some base substitutions that are not similar to *P. acaulis* subsp. *rubra* or *P. veris* subsp. *columnae*, indicating that the original parents of *P.* × *uzungolensis* may come from a different population than the one sampled here. This situation is correlated with the previous study in which considerable base variations were recorded among the populations of *P. acaulis* subsp. *rubra* and *P. veris* subsp. *columnae* in north-eastern Anatolia (Gültepe et al., 2010). MP analysis also demonstrated that the putative hybrid presented intermediate sequences between those of its parents (Table 4, Figure 5). As seen in the MP tree, the putative samples denoted as P81 clustered with subspecies of *P. acaulis*, and the nucleotide substitutions also confirmed these relationships (Table 4). The remains of the putative hybrids denoted

Table 4. Variable sites of the nucleotide sequences of internal transcribed spacer (ITS).

Pop. no.	ITS (length: 699-713 bp)																					
P80	A_{144}	G_{159}	A_{298}	C_{450}	A_{506}	G_{546}	C_{553}	G_{557}	A_{573}	C_{576}	A_{582}	G_{614}	C_{617}	G_{625}	T_{646}	C_{651}	A_{654}	C_{661}	G_{664}	G_{669}	C_{678}	A_{298}	A_{703}
P83	"	A_{159}	"	"	G_{506}	T_{546}	A_{553}	C_{557}	G_{573}	G_{576}	"	T_{614}	A_{617}	A_{625}	"	G_{651}	G_{654}	G_{661}	C_{664}	C_{669}	A_{678}	"	G_{703}
P85	"	"	"	"	A_{506}	G_{546}	C_{553}	G_{557}	C_{573}	C_{576}	C_{582}	"	C_{617}	G_{625}	"	C_{651}	A_{654}	"	T_{664}	"	"	"	A_{703}
P81	G_{144}	G_{159}	"	"	G_{506}	"	A_{553}	C_{557}	"	"	"	"	A_{617}	A_{625}	"	G_{651}	G_{654}	"	"	A_{669}	"	"	G_{703}
P86	"	"	G_{298}	C_{450}	"	T_{546}	"	"	G_{573}	G_{576}	A_{582}	C_{614}	"	"	C_{646}	"	"	A_{661}	"	C_{669}	G_{298}	A_{298}	"
P87	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
P79	A_{144}	A_{159}	A_{298}	G_{450}	A_{506}	"	"	"	A_{573}	"	"	T_{614}	"	T_{625}	T_{646}	"	"	G_{661}	"	"	C_{678}	C_{298}	"
P82	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	A_{298}	"
P84	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"

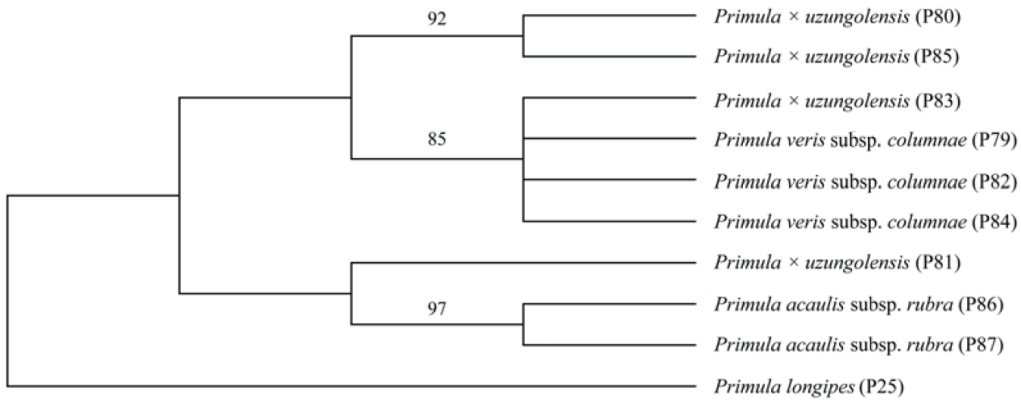

Figure 5. Dendrogram obtained from maximum parsimony analysis based on ITS sequences.

as P80, P83, and P85 grouped with the populations of *P. veris* subsp. *columnae* denoted as P79, P82, and P84. These results provide compelling evidence that the individuals with peculiar morphology in Uzungöl are hybrids between *P. acaulis* subsp. *rubra* and *P. veris* subsp. *columnae*.

Chloroplast DNA (cpDNA) is usually maternally transmitted in angiosperm (Morgensen, 1996) and sequencing can be used to determine hybrid origin (Ferris et al., 1997; Moody & Les, 2002). In the current study *mat*K was used in order to determine the maternal parents of *P.* × *uzungolensis*. This region exhibited limited variation in *P. veris* subsp. *columnae*, *P. acaulis* subsp. *rubra*, and *P.* × *uzungolensis* (Table 4). The length of the *mat*K gene of *P. veris* subsp. *columnae* is 1548 bp. It varies from 1547 to 1549 bp in *P. acaulis* subsp. *rubra* and from 1542 to 1548 bp in the putative hybrid. According to Table 5, the putative hybrid had the same base sequences as *P. veris* subsp. *columnae*. However, there were 7 nucleotide substitutions between the putative hybrid and *P. acaulis* subsp. *rubra*. MP analysis also supported and demonstrated that *P. veris* subsp. *columnae* is the maternal parent of *P.* × *uzungolensis* (Figure 6). However, the samples of putative hybrids and *P. veris* subsp. *columnae* formed 2 groups because SNP was in the 839th position in *mat*K sequences, as seen in Table 5. Additionally, there are several indels in the aligned data matrix of the examined populations, but they do not have parsimony informative character so they were not taken into consideration for the construction of the MP tree.

Table. 5. Variable sites of the chloroplast matK. *P.* × *uzungolensis* (P80, P81, P83, P85), *P. acaulis* subsp. *rubra* (P86, P87), and *P. veris* subsp. *columnae* (P79, P82, P84).

Pop. no.	**matK (length: 1542-1549 bp)**							
P80	A_{43}	T_{201}	C_{669}	G_{839}	T_{1058}	G_{1121}	C_{1559}	G_{1620}
P83	"	"	"	T_{839}	"	"	"	"
P85	"	"	"	G_{839}	"	"	"	"
P81	"	"	"	T_{839}	"	"	"	"
P86	C_{43}	C_{201}	T_{669}	"	C_{1058}	T_{1121}	T_{1559}	T_{1620}
P87	"	"	"	"	"	"	"	"
P79	A_{43}	T_{201}	C_{669}	G_{839}	T_{1058}	G_{1121}	C_{1559}	G_{1620}
P82	"	"	"	T_{839}	"	"	"	"
P84	"	"	"	"	"	"	"	"

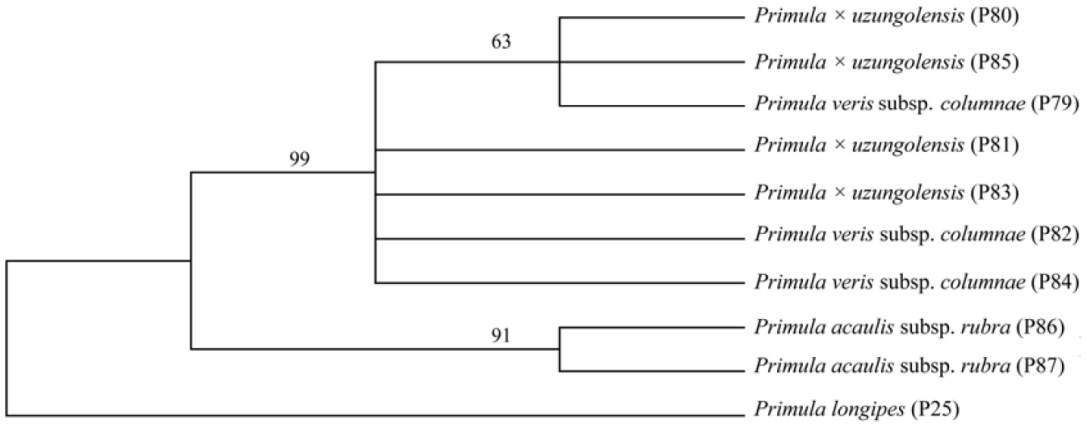

Figure 6. Dendrogram obtained from maximum parsimony analysis based on *mat*K sequences.

Hybridisation tends to be unidirectional at sites where 1 species is rare, because the pollens delivered to the rare species would consist mainly of pollen from the common species (Arnold, 1993; Rieseberg, 1995). In the habitat of our putative hybrids *P. veris* subsp. *columnae* is rare, and *P. acaulis* subsp. *rubra* is more abundant. Additionally, the putative hybrids share their habitat closely with *P. veris* subsp. *columnae*. Under these conditions the rare species is usually the maternal parent of the hybrids, as cited by Rieseberg (1995). Patterns of genetic variation in the nuclear and chloroplast regions lead us to conclude that *P. veris* subsp. *columnae* is the maternal and *P. acaulis* subsp. *rubra* is the paternal progenitor of *P.* × *uzungolensis*. All of these results together suggest that natural hybridisation between *P. veris* subsp. *columnae* and *P. acaulis* subsp. *rubra* is unidirectional; however, in the study by Zhu et al. (2009), in which a maternally inherited chloroplast DNA fragment was used to trace the maternal composition of hybrids, it was found that hybridisation between *P. secundiflora* and *P. poissonii* seems to be symmetrical rather than unidirectional.

Acknowledgements

The authors would like to express special thanks to the Turkish Ministry of Environment and Forestry, Environmental Protection Agency for Special Areas (EPASA) and 110T045 (TÜBİTAK) for financial support. We would also like to thank Murat Erdem Güzel, Zeynep Çolak, Osman Sinan, and Murat Fidan for their kind assistance and Dr. Yusuf Bektaş for performing the phylogenetic analysis.

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