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Contribution to infra-specific taxonomy of *Primula vulgaris* Huds. (Primulaceae) along an altitudinal gradient in Turkey

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ABSTRACT: This study was performed to explore the relationship between morphological characters and nrDNA ITS data belonging to two subspecies of *Primula vulgaris* Huds. (Primulaceae) along the altitudinal gradient over NE Anatolia. There were no significant molecular differences between *P. vulgaris* subsp. *vulgaris* and *P. vulgaris* subsp. *sibthorpii* (Hoffmanns) W.W.Sm. & Forrest related to the flower colour and altitudinal gradient. However, Maximum parsimony (MP) analysis showed that all the examined populations fall in two distinct clusters confirming that the analyzed sample corresponds to two subspecies. It was also determined that the presence or absence of leaf pubescence does not vary along the altitudinal gradient within each subspecies.

KEY WORDS: Altitudinal gradient, Anatolia, Flower colour, nrDNA ITS, Primrose, *Primula vulgaris*.

INTRODUCTION

The genus *Primula* L. (Primulaceae) includes about 430 species in the world belonging to 6 subgenera and 37 sections (Richards 2003). The genus is represented by 9 species including two subspecies of *P. vulgaris* Huds., three subspecies of *P. elatior* L. and two subspecies of *P. veris* L in Turkey (Coşkunçelebi, 2012). *Primula vulgaris* (=*P. acaulis* (L.) Hill) is a small, herbaceous, perennial plant distributed over the Mediterranean region, SW Ukraine, the Crimea, the Caucasus and the southern and western coasts of the Caspian Sea (Jacquemyn *et al.* 2009). Besides, it is quite abundant in woodland habitats and hazelnut plantation fields in the North Eastern Part of Turkey, characterized by very high precipitation and humidity rate (Coşkunçelebi, 2012).

P. vulgaris has been the subject of many researches due to style dimorphism (Li *et al.* 2011), hybridisation (Valentine, 1947; 1955) and floral colour polymorphism (Selander & Welander, 1984; Ünal *et al.* 2003; Shipunov *et al.* 2011, Marsden-Jones and Turrill, 1944; Valentine, 1947; 1948; 1955; Boyd *et al*. 1990; Karlsson, 2002). This species is also widely cultivated as ornamental plants (Mizuhiro *et al*. 2001) and used as medicinal plant in Anatolia and all over the world (Zeybek and Zeybek, 1994; Prajapati *et al.* 2003). Furthermore, it was included in many molecular studies focusing on phylogenetic relationships, character evolution and classification of the genus (Conti *et al.* 2000; Martins *et al.* 2003; Zhang and Kadereit, 2004; Kovtonyuk and Goncharov, 2009; Gültepe *et al.* 2010; Schmidt-Lebuhn *et al.* 2012; Terzioğlu *et al.* 2013). Volkova *et al.* (2013) investigated the relationship of the flower colour polymorphism based on molecular data in two subspecies of *P. vulgaris* distributed in Ponto-Caspian Region. The study reported that molecular differences were not correlated with colour polymorphism but with geographical distribution.

P. vulgaris is represented by *P. vulgaris* Huds. subsp. *vulgaris* and *P. vulgaris* Huds. subsp. *sibthorpii* (Hoffmanns) W.W.Sm. & Forrest including several flower colour morphs in Turkey (Coşkunçelebi, 2012). *P. vulgaris* subsp. *vulgaris* has white and yellow flowers. *P. vulgaris* subsp. *sibthorpii,* however, has dark purple to pink and white flowers. These two subspecies often occur at the same habitat along the eastern Black Sea coast of Turkey. Besides, Lamond (1978) reported that plants from North and South of Turkey at altitudes from 500 to 2100 m belong to *P. vulgaris* subsp. *vulgaris* and plants from North and South of Turkey at altitudes from 1 to 850 m belong to *P. vulgaris* subsp. s*ibthorpii.* This shows that two subspecies naturally grow together at the altitudes from 500 to 850 m and thus it's not always possible to distinguish them based solely on flower colour and distribution range.

In this paper, specimens belonging to two subspecies of *P. vulgaris* from 18 populations distributed along the altitudinal gradients in North Eastern Part of Turkey were compared to each other based on morphological and molecular data to address the following objectives: (1) identify nrDNA ITS polymorphism within and between *P. vulgaris* subsp. *vulgaris* and *P. vulgaris* subsp. *sibthorpii* and (2) explore relationships regarding the morphological characters and ITS profile along the altitudinal gradient in NE Anatolia.

MATERIALS AND METHODS

Plant materials

Plant materials used in this study were collected from

Table 1. Locality information of the examined populations of ***Primula vulgaris*** in North Eastern part of Anatolia

Pop No	Taxa/ Locality	Geographic Coordinates	Fl. Colour	Coll. Time	Voucher specimen
	P. vulgaris Huds. subsp. ***sibthorpii***				
PV34	A7 Trabzon: Araklı. Yalıboyu Village. 8 m.	40°57'20.6"; 40°00'31.2"	White	7 Apr. 2011	*Coşkunçelebi 1183*
PV36	A7 Trabzon: Araklı. Yalıboyu Village. 8 m.	40°57'20.6"; 40°00'31.2"	Purple	7 Apr. 2011	*Coşkunçelebi 1184*
PV23	A7 Trabzon: Esiroğlu. Gayretli Village. 211 m.	40°51'11.8"; 39°39'39.2"	White	20 Feb. 2011	*Coşkunçelebi 1174*
PV24	A7 Trabzon: Esiroğlu. Gayretli Village. 211 m.	40°51'10.7"; 39°39'39.4"	Purple	20 Feb. 2011	*Coşkunçelebi 1175*
PV39	A7 Trabzon: Maçka. Örnekalan Village. 404 m.	40°49'39.6"; 39°37'0.18"	Purple	10 Apr. 2011	*Coşkunçelebi 1186*
PV40	A7 Trabzon: Maçka. Örnekalan Village. 404 m.	40°49'39.6"; 39°37'0.18"	White	10 Apr. 2011	*Coşkunçelebi 1187*
PV42	A7 Trabzon: Maçka. Köprüyanı Village. 685 m.	40°46'10.1"; 39°34'18.5"	Purple	10 Apr. 2011	*Coşkunçelebi 1189*
	P. vulgaris *Huds.* subsp. ***vulgaris***				
PV25	A7 Trabzon: Esiroğlu. Gayretli Village. 211 m.	40°51'11.8"; 39°39'39.2"	Yellow	20 Feb. 2011	*Coşkunçelebi 1176*
PV41	A7 Trabzon: Maçka. Örnekalan Village. 404 m.	40°49'39.6"; 39°37'0.18"	Yellow	10 Apr. 2011	*Coşkunçelebi 1188*
PV28	A7 Trabzon: Maçka. Zigana road. 600 m.	40°44'35.5"; 39°33'15.7"	Yellow	13 Mar. 2011	*Coşkunçelebi1178*
PV29	A7 Trabzon: Maçka. Zigana road. 600 m.	40°44'35.5"; 39°33'15.7"	White	13 Mar. 2011	*Coşkunçelebi 1179*
PV43	A7 Trabzon: Maçka.Gürgenağaç Village. 923 m.	40°42'59.6"; 39°31'11.3"	Yellow	10 Apr. 2011	*Coşkunçelebi 1190*
PV44	A7 Trabzon: Maçka.Gürgenağaç Village. 923 m.	40°42'59.6"; 39°31'11.3"	White	10 Apr. 2011	*Coşkunçelebi 1191*
PV46	A7 Trabzon: Maçka. Hamsiköy. 1300 m.	40°41'32.3"; 39°28'35.6"	Yellow	29 Apr. 2011	*Coşkunçelebi 1192*
PV48	A7 Trabzon: Maçka. Hamsiköy. 1300 m.	40°41'32.3"; 39°28'35.6"	White	29 Apr. 2011	*Coşkunçelebi 1193*
PV49	A7 Trabzon: Maçka. Hamsiköy. Zitaş. 1686 m.	40°40'09.6"; 39°26'17.7"	Yellow	29 Apr. 2011	*Coşkunçelebi 1194*
PV50	A7 Trabzon: Maçka. Hamsiköy. Zitaş. 1686 m.	40°40'09.6"; 39°26'17.7"	White	29 Apr. 2011	*Coşkunçelebi 1195*
PV53	A7 Gümüşhane: Zigana Pass. 2120 m.	40°38'50.4"; 39°24'06.1"	Yellow	14 May 2011	*Coşkunçelebi 1196*

several localities during the field missions in Trabzon at different altitudes (0-2200 m). Locality information of the examined populations is given in Table 1. All samples were identified according to Flora of Turkey (Lamond, 1978) and Flora European (Valentine and Kress, 1972). Vouchers were stored in Herbarium of Biology at Karadeniz Technical University (KTUB) and used for further morphological examination and measurements. In order to explore the relationship depending on the flower colour along altitudinal gradient within and between two subspecies, we selected at least two populations including white, purple and yellow flowers due to unavailability of all colour forms across all elevation ranges.

Phenetic studies

All populations listed in Table 1 were assessed to determine the phenetic similarity based on 15 morphological traits that are among the most important taxonomical variable used in several local and national floras (Table 2). Phenetic measurement and observations were scored from at least 6 or 10 samples for all traits and combined to yield the basic raw data matrix. Cluster analysis (CA) was 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 and Sokal, 1973). A dendrogram was generated by the unweighted pair-group method using arithmetic averages (UPGMA).

Molecular studies

Due to lack of all colour form along the altitudinal range, only eight populations belonging to two subspecies of *P. vulgaris* were selected for molecular studies in the present study. Total genomic DNAs were extracted from silica-dried leaves or herbarium materials following the modified CTAB extraction procedure of Doyle and Doyle (1987) according to Gültepe *et al.* (2010). The isolated genomic DNAs were checked in a 1% agarose-TAE (Tris, Acetate and EDTA) gel containing 0.5 µg/L of ethidium bromide and examined under UV light.

The entire ITS regions (ITS1, 5.8S and ITS2) were amplified using universal ITS4 and ITS5 primers designed by White *et al.* (1990). The amplification process was performed in 50 µL of PCR reaction volume containing 10 mM of Taq polymerase reaction buffer and 2 mM of magnesium chloride (MgCl2). It was used 200 mM of dNTP, 1 µM (ITS4 and ITS5) each of the primer, 1-2 units of Taq DNA polymerase, 2-6 ng (1 µL of 2-6 ng/µL) of total template DNA and 14 µL of ddH2O. Reaction mixtures were sealed with 1 or 2 drops of mineral oil to prevent evaporation during thermal cycling. Thermal cycling amplification was performed with an initial denaturation step of 94 °C for 1 min, followed by 33 cycles of strand denaturation at 94 °C for 1 min, annealing at 56 °C for 30 s, primer extension at 72 °C for 40 s and a final elongation at 72 °C for 5 min.

PCR product purification and DNA sequence analysis were performed by Macrogen Inc. (Seoul. Korea). The nucleotide sequences were automatically aligned using BioEdit v.7.0 software (Hall, 1999). Maximum Parsimony (MP) tree was built with the Molecular Evolutionary Genetics Analysis (MEGA v 7.0) program (Kumar *et al.* 2016). All characters were unordered and equally weighted, and gaps were treated as missing data. The topology of the consensus tree was constructed and evaluated with 1000 bootstrap replications (Felsenstein, 1985) for the MP (Saitou and Nei, 1987) analysis. For the phylogenetic analysis of the

Table 2. Raw data matrix used for phenetic analysis.

Taxon Pop. No.	X_1 Plant length (cm)	X_2 Leaf length (cm)	X_3 Leaf width (cm)	X_4 Leaf beneath[a]	X_5 Bracts length (cm)	X_6 Pedicel length (cm)	X_7 Calyx length (cm)	X_8 Depth of calyx teeth (cm)	X_9 Length of corolla tube (cm)	X_{10} Length of corolla lobe (cm)	X_{11} Flower color[b]	X_{12} No. of basal leaf	X_{13} No. of flowers in inflorescence	X_{14} Stilus length[c] (mm)	X_{15} Leaf length/ leaf width (X_2/X_3)
P. vulgaris subsp. s*ibthorpii*															
PV34	13.8±1.3	12.1±1.82	3.22±0.68	0	2.08±0.26	9.5±1.94	1.46±0.11	0.5±0.07	1.54±0.27	0.96±0.18	0	16±3.65	40±5.9	14.6±1.98	3.75±1.23
PV36	12.5±1.27	11.91±1.74	3.3±0.58	0	1.73±0.49	8±2.28	1.41±0.1	0.6±0.09	1.58±0.28	1±0.06	2	9±2.39	29±17.4	15.33±1.26	3.60±0.53
PV23	9.53±0.91	13.3±1.91	3.91±1.36	0	1.76±0.11	15.3±1.3	1.76±0.11	0.7±0.12	1.42±0.13	1.08±0.08	0	15±1.76	16±7	10.2±1.04	3.40±0.93
PV24	8.84±0.79	14.82±1.31	3.22±0.57	0	1.84±0.17	6.84±0.55	1.72±0.25	0.62±0.13	1.34±0.35	1.06±0.23	2	14±3.87	21±2.79	10.93±0.9	4.60±0.71
PV39	7.6±2.3	10.5±3.16	2.4±0.65	0	1.32±0.23	5.8±1.82	1.32±0.31	1.14±0.5	1.46±0.15	0.88±0.13	2	10±2.92	14±6.06	10.5±0.5	4.37±0.73
PV40	8.64±2.63	10.8±2.47	3.44±0.47	0	1.28±0.13	7.1±2.93	1.32±0.13	0.68±0.15	1.74±0.19	1.18±0.23	0	10±0.84	15±4.44	11.50±0.71	3.13±0.56
PV42	11.92±1.87	11.24±2.94	2.5±1.15	0	1.5±0.19	8.9±2.56	1.32±0.31	0.54±0.09	1.7±0.27	1.08±0.08	2	9±1.82	13±3.35	12.1±0.74	4.49±3.22
P. vulgaris subsp. *vulgaris*															
PV25	8±2	10.58±3.53	3±1.1	1	1.42±0.33	3.73±0.95	1.3±0.19	0.46±0.11	1.42±0.33	0.88±0.13	1	16±4.39	17±7.43	10.3±0.76	3.52±0.79
PV41	7.8±1.36	9.06±2.73	2.64±0.95	1	1.18±0.33	5.32±1.52	1.34±0.22	0.38±0.08	1.32±0.29	0.84±0.11	1	10±2.28	11±3.42	10.5±0.71	3.43±0.85
PV28	8.55±1.33	8.33±0.61	2.5±0.71	1	1.93±0.66	9.56±8.45	1.53±0.1	0.56±0.09	1.61±0.17	0.95±0.15	1	9±3.1	15±9.77	10.66±0.58	3.33±0.89
PV29	7.9±1.14	9.63±1.86	3±0.77	1	1.8±0.21	5.66±1.08	1.55±0.08	0.52±0.08	1.75±0.36	0.83±0.08	0	12±5.94	16±6.69	10.5±0.71	3.21±0.54
PV43	8.02±0.64	7.56±1.76	2.06±0.56	1	1.14±0.19	5.62±0.7	1.36±0.11	0.48±0.08	1.64±0.15	0.9±0.16	1	11±2.28	20±9.76	9±1.41	3.66±1.06
PV44	7.66±1.6	8.92±0.8	2.66±0.41	1	1.55±0.6	6.88±1	1.46±0.17	0.9±0.06	1.7±0.31	1.16±0.13	0	8±1.94	6±1.14	12.33±0.58	3.35±0.45
PV46	8±0.71	7.06±0.59	2.14±0.19	1	1.7±0.27	5.6±0.82	1.6±0.14	0.5±0.07	1.48±0.04	1.12±0.11	1	11±2.39	20±9.61	19.66±3.06	3.29±0.49
PV48	9.1±0.89	6.4±2.19	2.2±0.57	1	1.38±0.13	6.3±1.04	1.42±0.08	0.56±0.09	1.72±0.15	1.2±0.19	0	10±3.05	14±5.37	17.1±0.74	2.90±0.69
PV49	9.39±2.69	8.4±3.36	2.68±0.92	1	1.74±0.51	4.9±0.42	1.66±0.32	0.68±0.08	1.4±0.16	1.2±0.19	1	12±1.48	19±3.51	14.23±2.44	3.13±0.36
PV50	6.82±0.77	5.4±0.55	1.6±0.55	1	1.3±0.27	4.5±0.87	1.36±0.09	0.5±0.07	1.52±0.11	1.1±0.12	0	11±1.3	11±4.39	15±0.4	3.37±1.20
PV53	7.42±0.41	6.44±1.39	1.9±0.26	1	0.84±0.09	4.18±0.75	1.2±0.12	0.66±0.21	1.4±0.16	0.96±0.15	1	11±2.77	7±3.51	8±0.71	3.38±0.81

a: Leaf beneath; 0: Pubescent. 1: Only veins
b: Flower color; 0: White. 1: Yellow. 2: Purple
c: in pin flowers

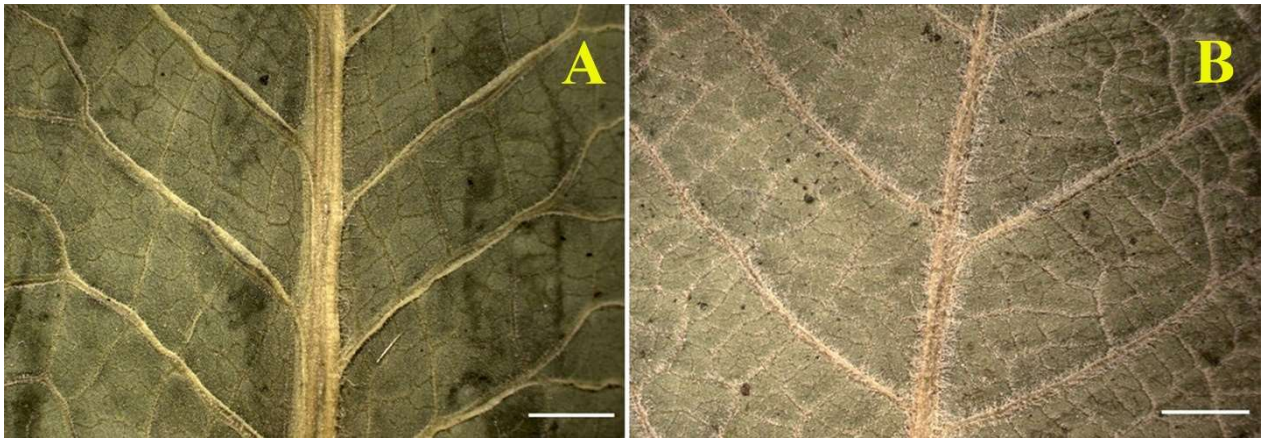

Fig. 1. Basal leaf beneath. **A**: ***P. vulgaris*** Huds. subsp. *Vulgaris*, **B**: ***P. vulgaris*** Huds. subsp. ***sibthorpii*** (Hoffmanns.) W.W. Sm. & Forrest, Scale bar: 2 mm.

ITS regions. *P. veris* L. subsp. *columnae* (Ten.) Lüdi (GenBank: EU643649). *P. longipes* Freyn & Sint. (GenBank: EU643662). *P. auriculata* Lam. (GenBank: EU643658). *P. algida* Adams (GenBank: EU643660). *P. davisii* W.W.Sm. (GenBank: EU643664) were selected as outgroups.

RESULTS

Average phenetic raw data matrix obtained from 95 individuals coming from 18 populations are presented in Table 2. As a result of observation and examinations, the following phenetic traits were reported here in detail for each subspecies of *P. vulgaris* in North East of Anatolia for the first time. *P. vulgaris* subsp. *vulgaris* is distinguished by the glabrous or weakly shortly pilose leaves; only on main veins (Fig. 1, A). However, *P. vulgaris* subsp. *sibthorpii* (Fig. 1, B) is distinguished by pilose leaf occasionally more densely on main and lateral veins beneath. Additionally, the leaves of *P. vulgaris* subsp. *vulgaris* are smaller in length (Table 2) with indistinct reticulate venation (Fig. 1, A). The leaves of *P. vulgaris subsp. sibthorpii* are, however, bigger (Table 1), more or less coriaceous with distinct reticulate venation that result from indumentum (Fig. 1, B).

During the field work, it has been recorded that flower colour varies from yellow, white, pale green, pale yellow to cream in *P. vulgaris* subsp. *vulgaris* (Fig. 2, E) and purple, pink, lilac, white, pale yellow to cream in *P. vulgaris* subsp. *sibthorpii* (Fig. 3, E). Besides, some of the colour forms (white, pale yellow to cream) from both subspecies are present in the same habitat and elevations. However, the dominant colour is yellow for *P. vulgaris* subsp. *vulgaris* and purple for *P. vulgaris* subsp. *sibthorpii*.

As seen in dendrogram resulting from UPGMA (Fig. 4), all examined populations were grouped into two distinct groups corresponding to *P. vulgaris* subsp. *sibthorpii* and *P. vulgaris* subsp. *vulgaris* without depending on colour form and altitudinal gradient.

Key to the subspecies of *Primula vulgaris* in Turkey was prepared mainly following Lamond (1978).

1a. Scape not (or very seldom) developed, flowers arising singly from basal rosette ……..…….…….. ***P. vulgaris* (2)**
1b. Scape well developed, inflorescence a few to many-flowered umbel or rarely 2-whorle ……. ***Primula spp.* (*P. algida, P. auriculata, P. davisii, P. elatior, P. longipes, P. magaseifolia, P. veris*)**
2a. Basal leaves hairy on the veins beneath, reticulate venation distinct, flowers yellow, pale green, pale yellow to cream or white ……. …….. **subsp. *vulgaris***
2b. Basal leaves glabrescent beneath, reticulate venation indistinct, flowers pink, purple to pale lilac, pale yellow to cream or white ……. ……..…… **subsp. *sibthorpii***

The total lengths of the ITS (ITS1. 5.8S. and ITS2) regions range from 708 to 713 bp and GC % content varies between 52.8 and 53.2 in *P. vulgaris* subsp. *vulgaris*. On the other hand, the length of ITS region are between 711 and 716 bp, and GC % content varies between 53.1 and 53.3 in *P.* subsp. *sibthorpii*. The ratio of Purine/Pyrimidine varies from 1.002 to 1.022 in *P.* subsp. *vulgaris* and from 1.011 to 1.019 in *P. vulgaris* subsp. *sibthorpii*. As well, the ITS similarities among samples were determined through "Pairwise Distance" analysis. The pair-wise distances obtained by Kimura's 2-parameter model for the examined populations vary from 0.0 % to 1.7 %.

According to MP tree (Fig. 5), the investigated populations, excluding outgroup taxa, fall into two clusters with a bootstrap value of 96% corresponding to *P. vulgaris* subsp. *sibthorpii* and *P. vulgaris* subsp. *vulgaris*. Besides, populations belonging to *P. vulgaris* were distinctly separated with a high bootstrap value (96%) from the rest of the Turkish *Primula* taxa.

DISCUSSION

Flower colour is considered an important taxonomical character in *P. vulgaris* and some researches divided the *P. vulgaris* into several species at Caucasian regions (Shipunov *et al.* 2011; Volkova *et al.* 2013). In Flora of Turkey and the East Aegean Islands

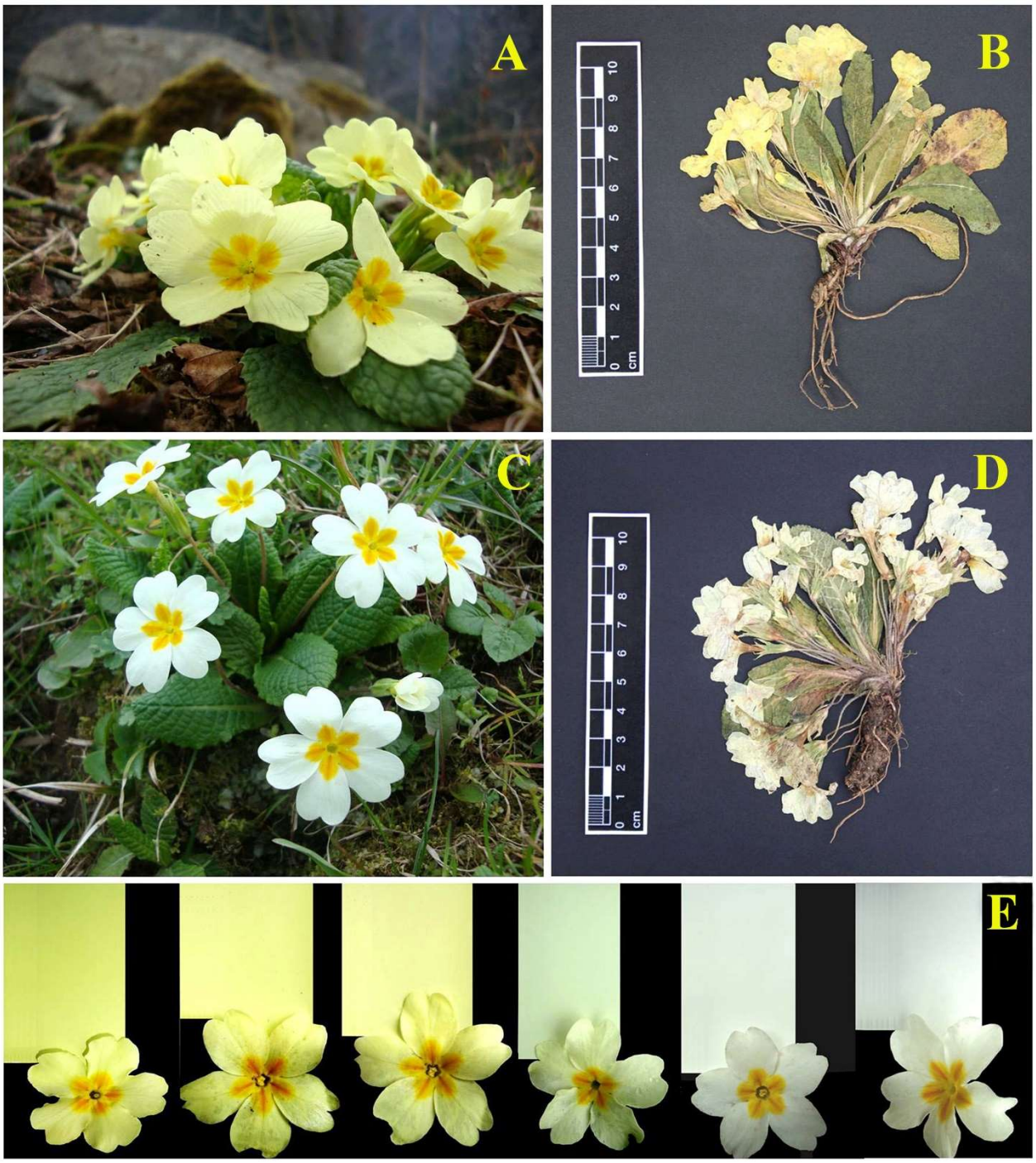

Fig 2. ***Primula vulgaris*** Huds. subsp. ***vulgaris***, **A**, **C.** Overview, **B**, **D.** Herbarium samples, **E.** Colour polymorphism

(Lamond, 1978), *P. vulgaris* subsp. *vulgaris* and *P. vulgaris* subsp. *sibthorpii* were distinguished from each other according to their flower colour and distribution ranges. However, two subspecies are mostly found in the same habitat and range with the same flower colour in North East of Anatolia. Thus, it is not always possible to separate these two subspecies based on solely flower colour and distribution ranges. It is well known that plants producing different pigments against the ultraviolet rays depend on altitudinal ranges (Mori *et al.* 2005). Shipunov *et al.* (2011) state that coloration from dark to light in flowers of *P. vulgaris* increase parallel to altitudinal ranges. Additionally, Shipunov *et al.* (2011) reported that flower colour is not a useful character to

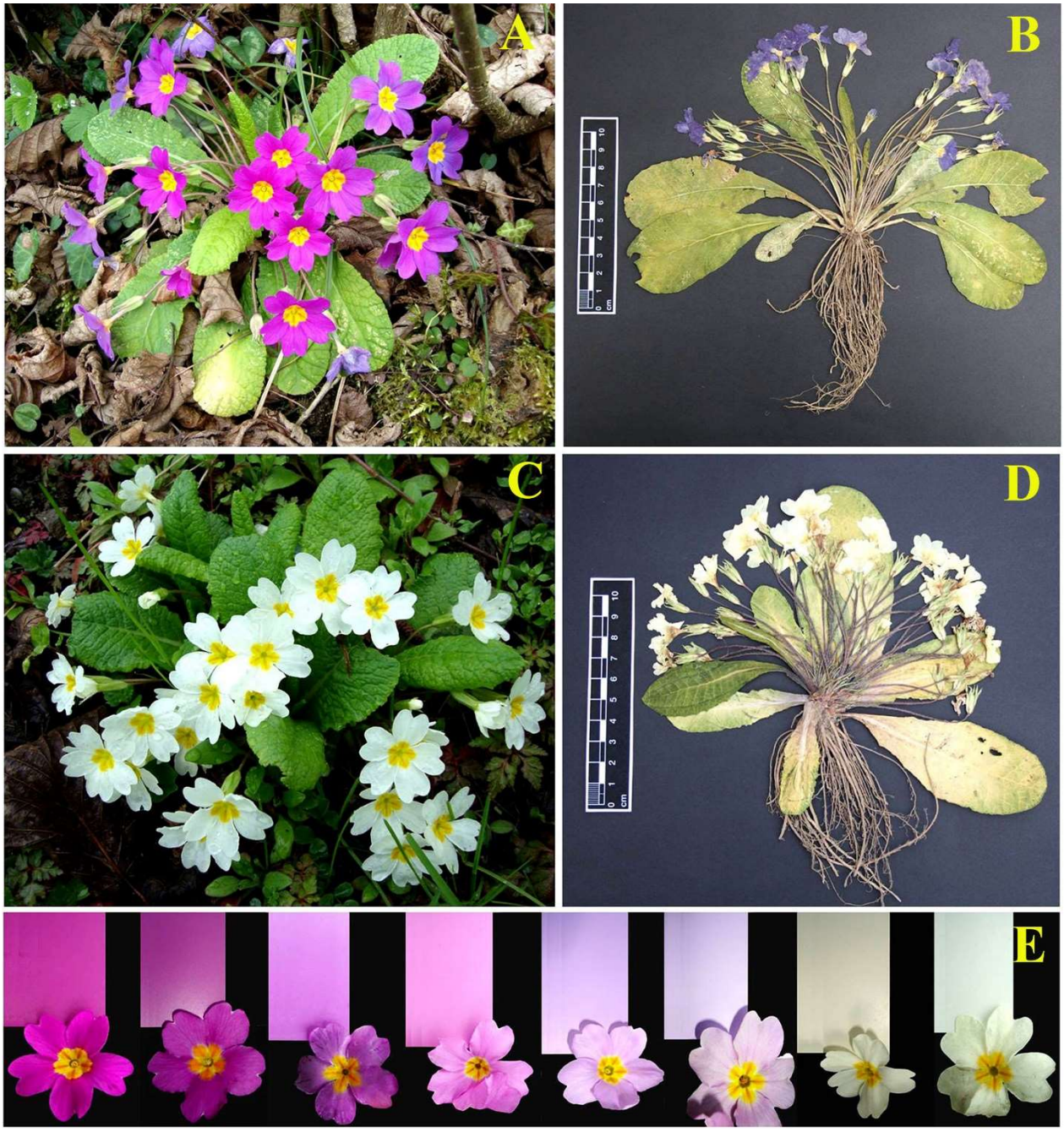

Fig 3. ***Primula vulgaris*** Huds. subsp. ***sibthorpii*** (Hoffmanns.) W.W.Sm. & Forrest. **A**, **C**: Overview, **B**, **D**: Herbarium samples, **E**: Colour polymorphism.

distinguish the subspecies of *P. vulgaris* in Northeastern Black Sea Coast. Present observations showed that light-coloured flowers are more abundant at higher altitudes in North East of Anatolia. Thus, flower colour is not also suitable trait for separating the examined subspecies of *P. vulgaris* in North East Turkey. Finally, additional morphological characters are necessary to separate each subspecies of *P. vulgaris.*

The shape of leaf basis towards the petiole was considered an important character to distinguish the subspecies of *P. vulgaris* by Valentine and Kress (1972) in Flora Europaea. Present findings related to leaf pubescence and leaf venation type contribute to and confirm the results reported by Valentine and Kress (1972). In addition, it has been observed that the states of venation and pubescence of leaves beneath do not vary in contrast to flower colour depending on altitudinal gradient within each subspecies. Lamond (1978) keyed

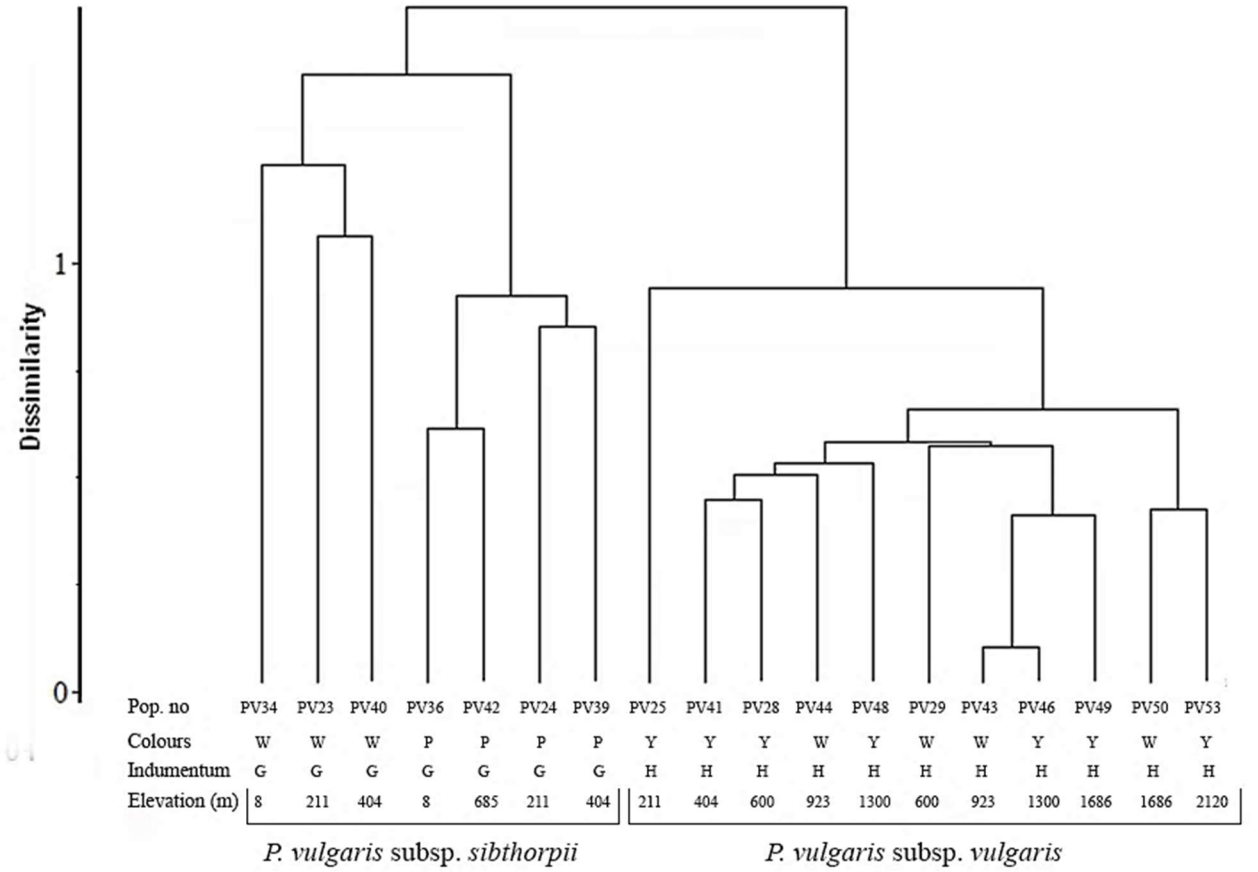

Fig. 4. Dendrogram resulting from UPGMA. (W: white flowered. P: purple flowered. Y: yellow flowered. G: Leaf beneath glabrous. H: Leaf beneath hairy). Explanation of the population numbers is in table 1.

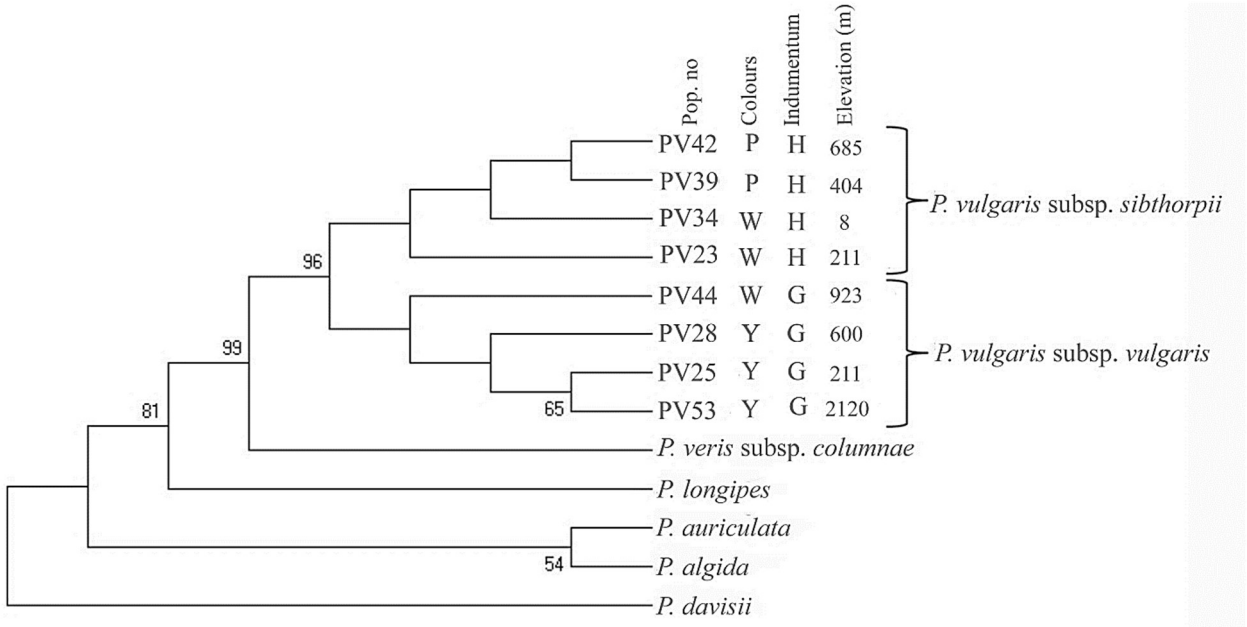

Fig. 5. Most parsimonious phylogenetic tree based on ITS sequences. Numbers above branches are bootstrap support in percent based on 1000 replicates (W: white flowered. P: purple flowered. Y: yellow flowered. G: Leaf beneath glabrous. H: Leaf beneath hairy).

out both subspecies according to flower colour and altitudinal gradient. But our results both from UPGMA (Fig.4) and MP tree (Fig. 5) are not congruent with Lamond's viewpoint related to flower colour and altitudinal gradient. White, pale yellow to cream flowers are shared with both subspecies. However, indumentum of leaf beneath and venation distinctly differ in two subspecies even in the same populations (Fig. 1).

Although the subspecies of *P. vulgaris* formed a clade with lower support in MP tree (Fig. 5), results are congruent with phenetic dendogram obtained from morphological analysis. However, flower colours did not resolve relationships between the subspecies. Similarly, Gültepe *et al.* (2010) and Schmidt-Lebuhn *et al*. (2012) reported that *P. vulgaris* formed a weakly supported clade based on the ITS and molecular studies revealed geographical proximity. Besides, flower colours show no correlation with the subspecific disjunction as indicated by Shipunov *et al.* (2011) and *Volkova et al.* (2013). Present results also revealed that flower colour is not adequate for the separation of both subspecies as suggested by Richards (2003). Marsden-Jones and Turrill (1944) reported that flower colour is heritable character and coded by several allele-genes in common primrose. Colour polymorphism in the subspecies of *P. vulgaris* can be caused by genetic drift, natural selection and pollinators as stated by Shipunov *et al.* (2011). Considering the field-work, flower colour polymorphism may be originated from the different ecological features explained by Shipunov *et al.* (2011). According to Çolak (2012), the colour polymorphism is not related with soil factors (physical and chemical), fatty acid methyl ester profiles and altitude. Furthermore, Yaylı *et al*. (2016) found that there are no meaningful changes in the content of essential oils, fatty acid methyl esters, and antimicrobial activities among both subspecies of *P. vulgaris*. Likewise, molecular results of the present study do not contribute to delimit the subspecies associated with colour dimorphism. Additionally, Shipunov *et al.* (2011) believed that there are not enough evidences to accept the colour morphs within the subspecies in *P. vulgaris.*

CONCLUSIONS

The leaf pubescence is one of the most important traits in distinguishing the subspecies of *P. vulgaris*. Furthermore, there is no significant difference in ITS profile within the populations of subspecies of *P. vulgaris* based on the flower colour and altitudes.

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Supplemental table: ITS sequences used in the phylogenetic analysis of figure 5.

Taxa	Code	Accession number	Source
Primula vulgaris subsp. *sibthorpii*	PV42	MT158770	This study
Primula vulgaris subsp. *sibthorpii*	PV39	MT158771	This study
Primula vulgaris subsp. *sibthorpii*	PV34	MT158772	This study
Primula vulgaris subsp. *sibthorpii*	PV23	MT158773	This study
Primula vulgaris subsp. *vulgaris*	PV44	MT158774	This study
Primula vulgaris subsp. *vulgaris*	PV28	MT158775	This study
Primula vulgaris subsp. *vulgaris*	PV25	MT158776	This study
Primula vulgaris subsp. *vulgaris*	PV53	MT158777	This study
Primula veris subsp. *columnae*		EU643649	GenBank
Primula longipes		EU643662	GenBank
Primula auriculata		EU643658	GenBank
Primula algida		EU643660	GenBank
Primula davisii		EU643664	GenBank