

Molecular systematic characteristics of *Rhododendron* L. (Ericaceae) taxa in Kaçkar Mountains National Park (Rize/Turkiye)

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Abstract: In this study, the molecular systematic features of *Rhododendron* L. (Ericaceae) taxa distributed in the Kaçkar Mountains National Park area were investigated. The examined plant materials were collected during field studies between 2013 and 2014. The collected louse samples were transformed into herbarium samples. Then, in order to determine the ITS profiles, DNA isolation was made from the leaf samples taken from each sample by appropriate methods. As a result of the study, morphological structure characterization results of *Rhododendron* specimens and ITS DNA analysis data confirm that specimens belonging to *R. caucasicum*, *R. ponticum*, and *R. luteum* species are found in Kaçkar Mountains National Park. In addition, ITS DNA analyzes clearly distinguishes the *R. luteum* species in the selected samples.

Keywords: ITS, leaf samples, morphology, PCR, species, taxa

INTRODUCTION

Taxa belonging to the genus *Rhododendron* are found in many parts of the world [Curtis, 2001; Browicz, 1983; Gelderen, Smith, 1992; Swisher, 1979; Zohary, 1973; Withers, 1992]. In terms of plant geography, Turkiye is divided into 3 different floristic sub-regions, namely Irano-Turanian, Mediterranean and Euro-Siberian, located in the Holarctic region. The phytogeographical region of Europe-Siberia is divided into 2 sub-regions as Euxsin and Colchik provinces. The research area is within the borders of the Kaçkar Mountains National Park, which is located within the provincial borders of Rize, and is located in the A8 square according to Davis's grid system [Davis, 1972].

Rize is a province located in the Eastern Black Sea Region of the Black Sea Region, surrounded by Trabzon in the west, Erzurum in the south, Artvin in the east, the Black Sea in the north, between 40°-20° east and 41°-

20° north parallels. The total area of Rize, excluding lakes, is 3920 km (Fig. 1).

Rize consists of wavy, hilly rough lands due to its land structure. The undulating and slightly hilly terrain is dominant up to 200 m above sea level.

From the coast, the altitude suddenly reaches 150-200 meters. In the following parts, the land is deeply cut by the gradually narrowing river valleys. Although sharp, ridges close to each other and "V" profile valleys with steep slopes come. It creates the characteristic topographic appearance of this area, up to an altitude of approximately 2000 m. After an altitude of 2000 m, in the region where high mountainous and glacial topography is dominant, steep and very steep sloping land structure dominated by 'U' type valleys is dominant [High, 2011].

The Kaçkar Mountains, where the study was carried out, were declared a National Park in 1994 and cover an area of 51.500 hectares. A large part of the National Park is located within the borders of Çamlıhemşin district in Rize province, a small part is located within the borders of Yusufeli district of Artvin province (Fig. 1). Storm Creek is located in the west of Kaçkar Mountains, Hemşin Creek in the east is covered with a rich flora (Fig. 3). The vegetation in the Kaçkar Mountains is characterized by karst flora and contains both lower and upper flora endemic species.

Rhododendron caucasicum found in Turkiye only reach 3000 m a.s.l.

The climate of Rize is mild in winters, cool in summers and has a climate type that receives abundant precipitation in all seasons. According to the meteorological studies carried out in observations for fifty years, the average temperature is around 14°C. The lowest temperature recorded for fifty years is -7°C and the highest is 38.2°C. January has a temperature of about 6.7°C and is the coldest month. July has a temperature of about 22.2°C and is known as the hottest month.

Average monthly temperature curve graph in Rize is above 5°C throughout the year and the average temperature of 4 months is below 10°C. In just two months, the average temperature exceeds 20°C. From all these results, it is concluded that the temperature

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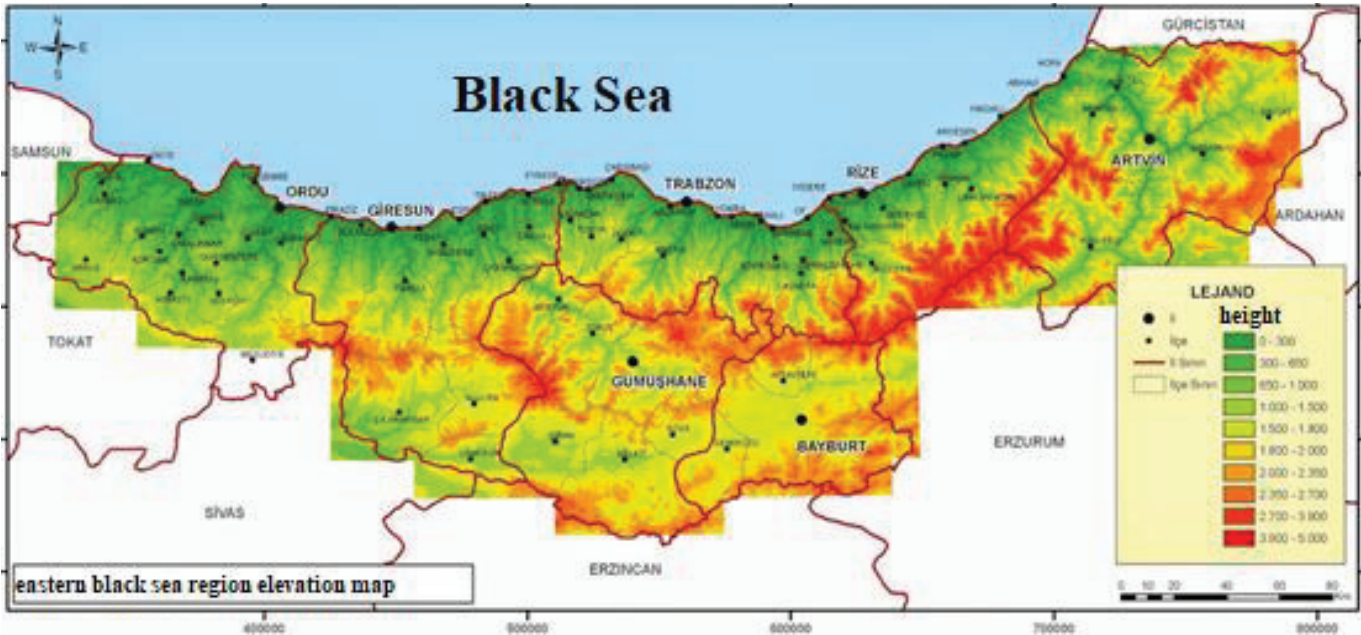


Figure 1. Physical map of Rize province.

regime of Rize has a very regular structure.

Rize is the wettest province of Turkiye with an annual precipitation of over 2300 mm. Balanced precipitation occurs throughout the seasons. Therefore, there is no dry season in Rize. Spring is the least rainy season with a total precipitation of 367.9 mm, which is above the drought value.

For this reason, the dry month is not seen in any season in Rize. When the curves in the temperature and precipitation graphs are followed, the precipitation curve graph does not fall below the temperature curve graph in any month (Fig. 2).

Since there is no meteorology station in the Kaçkar National Park region, the temperature and precipitation graphs were prepared using the data of the Sunday station, which is the closest to the region.

The vegetation type common in the Black Sea region is forest vegetation. Forest vegetation starts from the sea coast and spreads to subalpine regions. It is possible to encounter forest units up to 2000-2200 m, and at later altitudes mixed subalpine and alpine meadows are encountered. Grassland associations are also common in high mountain areas. In particular, shrubs such as *Rhododendron caucasicum*, *Vaccinium myrtillus* form

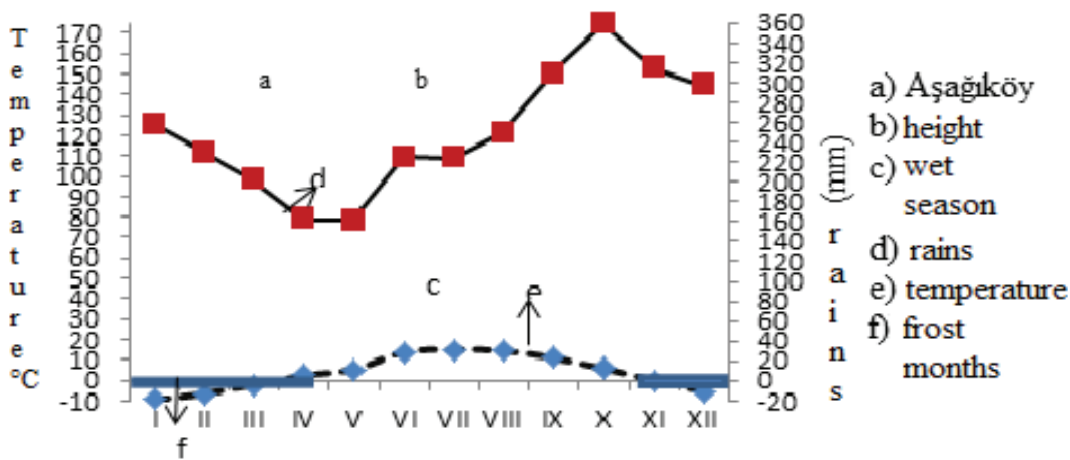


Figure 2. Climate diagram of Kaçkar Mountain National Park (Aşağıköy location).

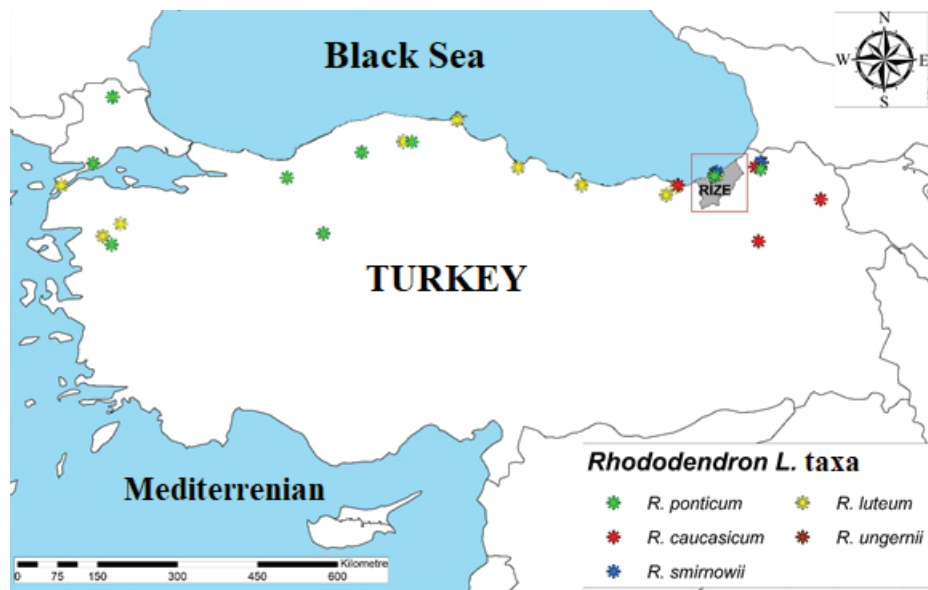


Figure 3. Distribution map of *Rhododendron* taxa in Turkey.

pure and sometimes mixed associations.

According to the 6th volume of Flora of Türkiye [Davis, 1972] within the borders of Türkiye, the genus *Rhododendron* L. with the following 9 taxa: 1. *R. luteum* Sweet, 2. *R. ungeronii* Trautv., 3. *R. x rosifaciensis* R. Milne, 4. *R. smirnovii* Trautv., 5. *R. x davisianum* R. Milne, 6. *R. caucasicum* Pallas, 7. *R. ponticum* L., 8. *R. x filidactylis* R. Milne, 9. *R. x sochadzeae* Charadze et Davlianidze is represented by 6 taxa within the research area: *R. ponticum*, *R. luteum*, *R. caucasicum*, *R. ungeronii*, *R. smirnowii* and *R. x sochadzeae* (Fig. 3, 6).

As can be seen from this map, these species spread in all districts of Rize, starting from the sea coast, in all parts of Kaçkar Mountain, and especially in mountainous and high mountain areas.

A. Guner et al. [1987] and M. Vural [1996] classified the vegetation of the Rize region as syntaxonomic and noted that the taxa belonging to the genus of *Rhododendron* have formation-forming properties.

M. Vural [1996] examined and classified the vegetation of the high mountain sections of Rize. In this classification, he stated that the taxa belonging to the *Rhododendron* genus are an important coenoses-forming taxon in the vegetation.

Important researches on plant diversity, flora and vegetation in the areas within the borders of Kaçkar Mountains National Park have been carried out recently [Baykal, 2015; Baykal, Atamov, 2017; Çobanoğlu, 2012; Çobanoğlu, Atamov, 2019; Demir, Atamov, 2020; Sarıgül, 2017; Süzen, 2018; Süzen, Atamov, 2022]. In these studies, plant associations and biodiversity of taxa

belonging to the *Rhododendron* genus were included.

Rhododendron ponticum L.

R. ponticum blooms in spring and maintains its inflorescence for a long time. The local name in the Black Sea region is “kara kumar/komar”, “black ag” or “gambling”. It can grow up to 8-10 m in its habitat and is known as a shrub or small tree species. *R. ponticum* is the best known of the *Rhododendron*. *R. ponticum*, which starts from the Istranca mountains and continues to the eastern Black Sea coastal mountains, is common in the Kaçkar mountains. It is used as firewood in a large part of the Black Sea region, especially by people living within the boundaries of the Kaçkar Mountains land, and in some areas it is used in the production of charcoal [Taşkın, 1987].

R. ponticum takes its place among the six *Rhododendron* defined by Linnaeus. Its leaves are bright green and have a length of 12-15 cm. It has purplish-pink flowers with 5-20 coexisting. These flowers can also be found in white form in some individuals of *R. ponticum*. It is common in beech forests and widespread from sea level up to 1800 m. The species occurs as an euxine element starting from the Caucasus Mountains along the Black Sea coast in Turkey to the southwest of Bulgaria (Fig. 4.1). *R. ponticum* subsp. *ponticum* is accompanied by *Pinus pine* (pistachio pine) assemblages [Avcı, 2004].

The differences in leaf sizes and flower stalks of two subspecies of *R. ponticum* facilitated the discovery of fossils of these species. These two subspecies are in a

relationship with each other [Browicz, 1983].

Although *R. ponticum* in Turkiye is a shrub species, it prefers to live in areas where soil conditions are suitable (Fig. 3, 4.1).

R. ponticum forms the lower layer of the vast majority of pure beech forests and continues to spread with tree species belonging to the evergreen mixed forest vegetation in winter. *R. ponticum* forms dense communities with different fern species that cannot be entered [Sarigül, 2017]. In some regions, it also interacts with hornbeam (*Carpinus betulus* L.), chestnut (*Castanea sativa* Mill.) and alder (*Alnus glutinosa* L.) communities. It forms mixed associations with other *Rhododendron* species in coniferous forests such as Black Sea fir (*Abies nordmanniana*) or spruce (*Picea orientalis*) on the upper parts of broad-leaved trees within the boundaries of the Kaçkar Mountains National Park land borders.

Rhododendron luteum Sweet

R. luteum is popularly known as “curve flower”, “couple” [Güner, Duman, 1998] or “yellow net”. *R. luteum* is also known as a deciduous shrub species, and it differs from other species of *Rhododendron* in this respect. *R. luteum* is the only *Rhododendron* species that sheds its leaves in winter in Southwest Asia and Europe. It can be grown to a height of almost 4 meters, and at the same time, 5-15 of its yellow flowers are found together in groups (Fig. 3, 4, 2a, b). The distribution area of *R. luteum* varies between 400-2000 m. It is also a rhododendron that grows very rarely in places with trees. *R. luteum* is the species collected and identified by Tournefort around Trabzon in the 1700 s.

Although the yellow-flowered rhododendron spreads within the borders of Kaçkar Mountain National Park, it also has a wide distribution area as far as Balıkesir and Çanakkale (Fig. 4, 2a, b). *R. luteum* Öksin, which was collected from Çanakkale vicinity (Karacalar) by Tchihatchef in 1849, forms the flora element of the region [Avcı, 2004].

Rhododendron caucasicum Pallas

Mountain gambling is another name for Caucasian rhododendron in the Eastern Black Sea Region [Güner, Duman, 1998]. This species has white or cream colored flowers and is eaten by local people. This *Rhododendron* which can grow up to 1-1.5 m, is found on acidic soils with a pH of 3-4 within the borders of Kaçkar Mountain National Park and generally on the northern slopes of the mountains. Beech-fir, beech, or beech-spruce form rather dispersed communities in the lower part of

forest communities, growing conditions best known as subalpine belts and alpine belts [Browicz, 1983; Stevens, 1978].

Therefore, *R. caucasicum* is determined as the specific species of high mountain areas. It has a wide distribution area starting from the Caucasus Mountains to the west of Azerbaijan and up to the upper mountain border of the Samur River in Dagestan. Ferik Mountain, Kordevan Mountain, Tiryal Mountain, and Yalnızçam Mountains, where Caucasian rhododendron spreads in North East Anatolia, are among the rugged areas (Fig. 4, 3). In the vicinity of Gül Mountain, southwest of the Caucasian *Rhododendron* Murgul, Şavval Tepe forms communities in very high areas together with *Daphne glomerata* [Abay, 2000].

The flowers of the plant, which belongs to the *R. caucasicum* species, have a dull cream color and 5-8 flower bunches are found together, there are light hairs on the shoot tips (Fig. 4, 3). The distribution area of *R. caucasicum* is between 1800-3000 meters altitude (Browicz, 1983). The altitude range where the most favorable growth is seen is above 2000 m. Caucasian rhododendron can reach up to 3250 m in Kaçkar Mountains.

The Caucasian rhododendron was described by Pallas in 1784. It has increased its importance as an ornamental plant because it blooms 3 to 4 weeks earlier than other species [Gelderen, Smith, 1992].

Rhododendron smirnowii Trautv.

R. smirnowii, which was discovered by Baron Ungern Sternberg in 1885 around Artvin, was named as Red Kumar on the Eastern Black Sea coast [Gelderen, Smith, 1992]. It took its place in the scientific world with the name of Sternberg's friend, M. Smirnov. The distribution area of *R. smirnowii* is quite limited [Fig. 4.4]. It has been known as an endemic plant in Anatolia for a very long time. When it was identified in the Southwest of the Caucasus Mountains in 1962, its distribution area expanded and its information was updated and changed [Avcı, 2004].

R. smirnowii is an evergreen plant and can grow up to 4 meters, shoots, leaves are dark green and bear flowers, are densely covered with gray-white hairs. The flowers, with 7 to 15 of them together, are bright pink [Fig. 4. 4.]. This species, distributed at altitudes between 1600-2200 m, is found between 850-2300 m in Anatolia. *R. smirnowii* coexists with *R. ponticum* and *R. ungeronii*, forming mixed or solitary communities in beech forests and sustains its habitat at 1000-1600 m altitude. The cultivation of *R.*

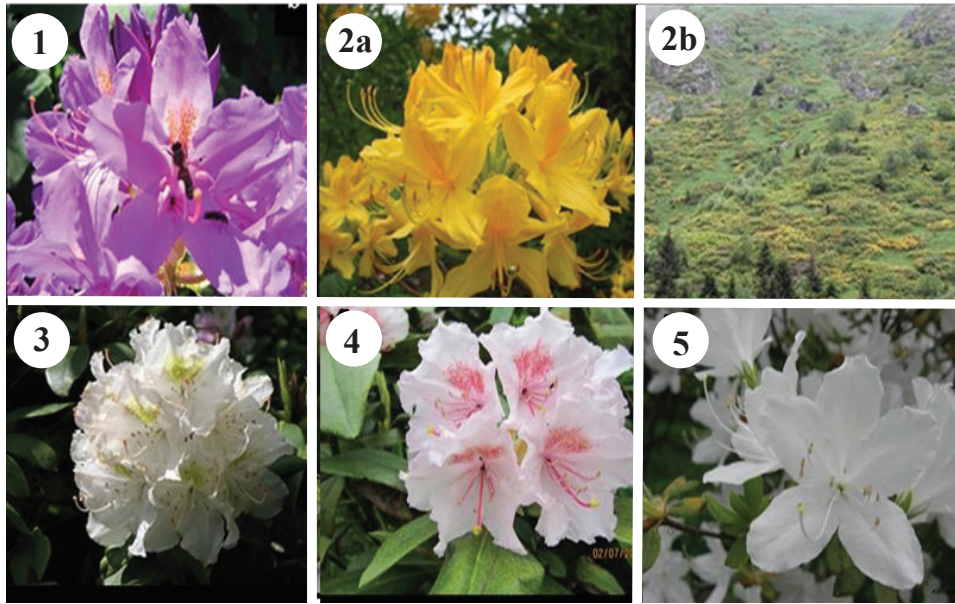


Figure 4. *Rhododendron* species: 1. *R. ponticum* in flowering phenophase, 2a. *R. luteum* in flowering phenophase, 2b. Natural distribution of *R. luteum* around Yayla village (Kaçkar Mountains National Park area), 3. *R. caucasicum* distributed in the Kaçkar Mountains, 4. *R. smirnowii* in the Kaçkar Mountains, 5. *R. ungeronii* distributed in the Kaçkar Mountains.

smirnowii took place one year after its discovery [Avcı, 2004].

R. ungeronii is also called white komar, which can grow up to 6 to 7 m in Turkey. The flower bunch, consisting of 12 to 24 flowers, is located on the flower stalk (Fig. 4, 5). Although the flowers of *R. ungeronii* are matte pink and white, this plant is a member of the Euxin flora region. The distribution area of *R. ungeronii* is similar to *R. smirnowii* [Avcı, 2004].

This mesophilic species usually occurs on north and

east facing slopes in mountainous areas. This species continues its distribution in spruce, beech or mixed forests. It forms mixed communities with *R. smirnowii*, usually in the belt between *R. ponticum* and *R. caucasicum*. *R. ungeronii* continues to spread between 800-2000 m, in some areas this height reaches up to 2200 m. In Türkiye, it has spread within the borders of Artvin in the Eastern Black Sea Region and along the Kaçkar Mountains (Fig. 5).

It has an area of distribution on Şavval hill in Murgul,

Table 1. Morphological features of *Rhododendron* taxa.

Species	Lifetime	Life Form	Blooming	Habitat	Altitude (m)	Endemic status
<i>R. luteum</i>	Perennial	Bush	4-9	Under the forest	0-2000	NE
<i>R. ungeronii</i>	Perennial	Bush or small tree	6-8	<i>Picea</i> forest	1000-2000	NE
<i>R. smirnovii</i>	Perennial	Bush	6-7	<i>Rhododendron</i> thicket	850-2000	E
<i>R. caucasicum</i>	Perennial	Bush	6-7	Upper limit of the forest	1830-3000	NE
<i>R. ponticum</i>	Perennial	Bush	3-8	in the forest	0-2000	NE
<i>R. sochadzeae</i>	Perennial	Bush	6-7	<i>Rhododendron</i> thicket	1700-2400	NE

Note: E: Endemic; NE: Not endemic

Tiryal mountain and Kaçkar mountains [Stevens, 1978; Browicz, 1983].

The data on life form, life span, flowering period, habitat, altitude and endemism of *Rhododendron* species distributed within the borders of Rize are summarized in table 1.

MATERIAL AND METHODS

Sampling. All *Rhododendron* samples were collected within the borders of Kaçkar Mountains National Park, in June-July between 2013 and 2014. These specimens are divided into two groups. The first group of the samples were used for herbarium material. They were firstly identified according to traditional methods and each species was stored in herbarium as taxa. The second group was used for gDNA extraction material. The samples used in the study were shown in table 2.

Molecular Studies. DNA Extraction and ITS-PCR Studies. While some of the plant samples were dried and turned into a herbarium sample, enough leaves were first subjected to surface sterilization against the risk of contamination for molecular studies. For this according to F.S. Beris et al. [2005], the leaves were first kept in 10%

sodium hypochlorite for 5 minutes. Then, it was passed through 70% ethanol series in 3 separate containers. After washing with ethanol, it was passed through distilled water to remove the alcohol on the leaves and kept at room temperature to remove water. Surface sterilization completed leaves were numbered and stored at -20°C until used in DNA isolation. Identification of the collected specimens was carried out by examining the herbarium materials. Diagnoses were made from flora of Turkey and other related flora. Species identified by morphological examinations and their collection information are given in table 2.

Genomic DNA's then were isolated from healthy leaves by using MN Plant NucleoSpin® Plant II Midi / Maxi (Macherey Nagel, Germany) according to manufacturer's protocol. The DNA samples were checked on 0.7% agarose gels and the concentrations of gDNA were determined by using a NanoDrop® ND-1000 Spectrophotometer (Thermo Fisher Scientific Inc. USA). Then, gDNA samples were stored at -20°C for further studies in polymerase chain reaction (PCR). PCR was used to amplify the nrITS DNA regions. The PCR mixture (50 µL) contained 10 ng (8 µL) of

Table 2. Collection information of the samples in the study.

Code	Collection area and sampling location
E1	A8 Rize: Zil Kale on Elevit Road, x: 665663 y:4534920
E2	A8 Rize: Gito Plateau, x:665896 y:4528270
E3	A8 Rize: Gito Plateau,x: 660829 y: 4530098
E4	A8 Rize: Gito Plateau, x:660155 y:4530069
E5	A8 Rize: Gito Plateau, x: 658493 y: 4527551
E7	A8 Rize: Zilkale, x:667719 y:4542231
E8	A8 Rize: Zilkale, x:665230 y:4536990
E9	A8 Rize: Zilkale, x:665544 y:4534408
E10	A8 Rize: Orta Yayla, x:660806 y:4518141
E12	A8 Rize: Zilkale, x:665663 y:4534920
E14	A8 Rize: Zilkale, x:665230 y:4536990
E16	A8 Rize: Zilkale, x:662695 y:4528451
E17	A8 Rize: Yukarı Kavron Plateau , x:679606 y:4528816
E18	A8 Rize: Gito Yaylası road, x: 660295 y:4528960
E19	A8 Rize: Yukarı Kavron, Plateau, x:679914 y:5530223
E20	A8 Rize: Gito Plateau road x:660295 y:4528960
E22	A8 Rize: Gito Plateau, x: 684184 y:4554074
E23	A8 Rize: Siraköy, x:660806 y:4518141
E24	A8 Rize: Gito Plateau, x:664016 y:5529999
E25	A8 Rize: Gito Plateau, x:660829 y:4530098
E27	A8 Rize: Çat Köyü x: 664194 y:4525573
E28	A8 Rize: Gito Plateau Yolu, x:660712 y:4531129
E29	A8 Rize: Gito Plateau, x:660155 y:4530069
E30	A8 Rize: Dereköy, x:645030 y: 4511562
E31	A8 Rize: Yukarı kavron Plateau, x:679585 y:4528165
E32	A8 Rize: Dereköy, x: 649375 y: 4511131

each template DNA, 5 µL of 5X PCR Buffer, 2.5 mM MgCl₂, 0.5 mM dNTPs, 1 µL of each primers (ITS4; 5'-CCTTATCATTAGAGGAAGGAG-3' and ITS5; 5'-GGAAGTAAAAGTCGTAACAAGG-3'), and 1 unit/µL Taq DNA polymerase. The following cycling program was used for ITS region, 3 min denaturation at 95°C followed by 36 cycles of denaturation of 95°C for 1 min, 30 s annealing at 52°C and extension at 72°C for 1 min 30 s, followed by elongation for 5 min at 72°C. The PCR was performed on a Bio-Rad T100™ gradient thermal cycler system (Bio-Rad Laboratories, Inc., CA, USA). The amplicons with a 100 bp DNA ladder (New England Biolabs, France) were size fractionated in a 1.4% agarose gel at a constant 120 V for 20 min and stained with EtBr. After visualizing the DNA bands on a UV-transilluminator, the agarose patterns were captured using UVP Bioimaging Systems (UVP, CA, USA). ITS regions of the examined samples were amplified by using universal primers and then sequenced by Macrogen Inc. (Amsterdam, Netherlands). Then, the DNA sequences were aligned and analyzed by using MEGA 6.05 software. Phylogenetic tree was formed in order to explain genetic relationship among the taxa.

Evaluation of Data of ITS Regions. PCR samples were sent to Macrogen Inc (Netherlands) and subjected to DNA sequence analysis with the same primers. Using the

NCBI (National Center for Biotechnology Information) BLAST (The Basic Local Alignment Search Tool) internet interface, the similarities with the data in GenBank and the CLUSTAL-W program were compared according to the sequence data obtained, and the results were evaluated (URL-4). Mega 7 program was used in the analysis of trees.

RESULTS AND DISCUSSION

Identification Key for Rhododendron Taxa. A map of the Rhododendron taxa showing the distribution localities based on the distribution coordinates taken during the field studies was presented (Fig. 5).

They are deciduous or evergreen shrubs or small trees. The leaves are revolute in the bud (in the species in Turkiye). The inflorescence is usually terminal, surrounded by more or less deciduous bud scales. Bracts and bracteoles are exfoliative. Flowers are 5-part, more or less zygomorphic. Sepals small, free or adjacent at the base. Corolla is more or less infundibular (Tubular or funnel-shaped), shed. The middle lobe is large. Anthers do not have extensions, they open with terminal pores; The pollens are in sticky rows called viscin. In the species in Turkey, the ovary has 5 eyes, the nectar is striking, the stigma is capitate (inflated in the shape of a head). The fruit is a septicidal capsule (opens from its walls called

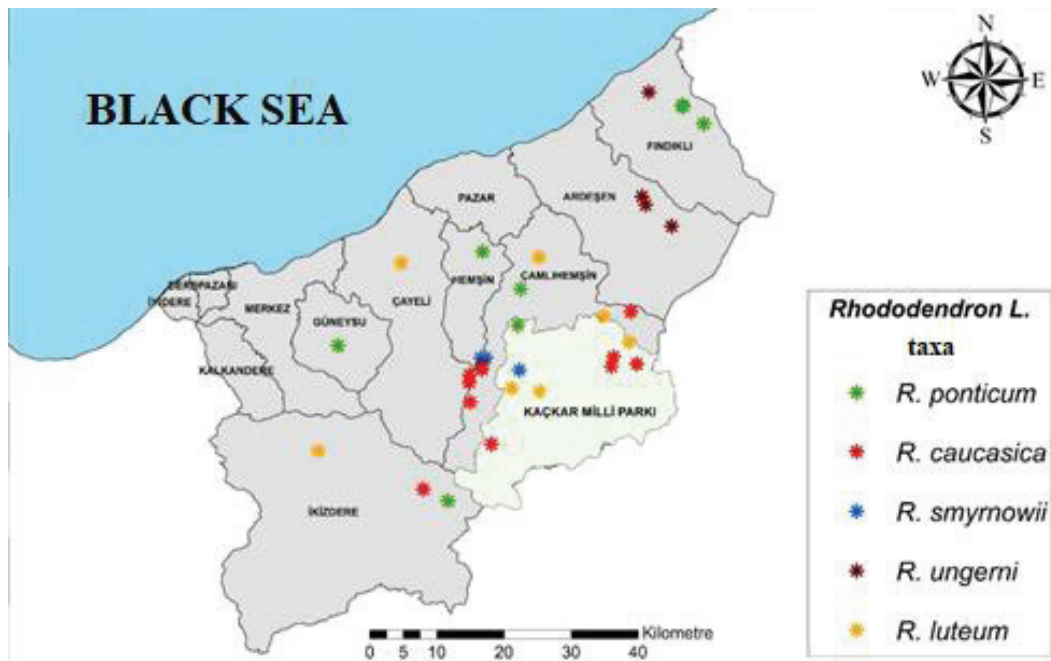


Figure 5. Locality map of *Rhododendron* taxa within the borders of Rize and Kaçkar National Parks.

Table 3. Morphological identification of *Rhododendron* samples with the amplicon sizes, their GC%, and their nBLAST results of nr-ITS PCR.

<i>Rhododendron</i> Samples	Morphological Identification Results	ITS Region (bp)	G+C%	nBLAST Similarity Results	nBLAST Similarity Index (%)
E1	<i>R. ponticum</i>	746	53.80	<i>R. ponticum</i>	100.00
E2	<i>R. caucasicum</i>	751	52.40	<i>R. caucasicum</i>	99.85
E3	<i>R. ponticum</i>	803	50.06	<i>R. ponticum</i>	99.85
E4	<i>R. caucasicum</i>	748	52.40	<i>R. caucasicum</i>	100.00
E5	<i>R. caucasicum</i>	762	53.20	<i>R. caucasicum</i>	99.85
E7	<i>R. ponticum</i>	738	51.30	<i>R. ponticum</i>	99.85
E8	<i>R. ponticum</i>	820	49.20	<i>R. ponticum</i>	99.08
E9	<i>R. ponticum</i>	753	53.60	<i>R. ponticum</i>	99.08
E10	<i>R. luteum</i>	792	54.50	<i>R. luteum</i>	99.83
E12	<i>R. ponticum</i>	740	51.70	<i>R. ponticum</i>	100.00
E14	<i>R. ponticum</i>	839	52.80	<i>R. ponticum</i>	98.92
E16	<i>R. ponticum</i>	818	53.30	<i>R. ponticum</i>	100.00
E17	<i>R. caucasicum</i>	749	52.20	<i>R. caucasicum</i>	100.00
E18	<i>R. ponticum</i>	749	51.50	<i>R. ponticum</i>	99.69
E19	<i>R. ponticum</i>	736	52.10	<i>R. ponticum</i>	100.00
E20	<i>R. ponticum</i>	799	51.80	<i>R. ponticum</i>	99.69
E22	<i>R. ponticum</i>	793	48.80	<i>R. ponticum</i>	99.85
E23	<i>R. luteum</i>	844	53.10	<i>R. luteum</i>	99.83
E24	<i>R. ponticum</i>	799	56.07	<i>R. ponticum</i>	99.69
E25	<i>R. ponticum</i>	703	53.40	<i>R. ponticum</i>	99.08
E27	<i>R. ponticum</i>	791	51.30	<i>R. ponticum</i>	99.69
E28	<i>R. ponticum</i>	742	54.04	<i>R. ponticum</i>	99.08
E29	<i>R. caucasicum</i>	745	52.60	<i>R. caucasicum</i>	98.98
E30	<i>R. luteum</i>	754	52.30	<i>R. luteum</i>	99.69
E31	<i>R. ponticum</i>	739	52.09	<i>R. ponticum</i>	90.05
Outgroup	<i>Vaccinium myrtillus</i>	750	56.20	<i>V. myrtillus</i>	100.00

the fruit septum); In the species in Turkey, the seeds are winged, the seed coat cells are thin-walled, and they are generally longer than their width.

The zygomorphism of the flower is not very pronounced, associated with the upper sepals and lower stamens being longer than the others. The honey bed is located in the upper corolla lobe (sometimes also in the adjacent lobes), and the pollinator bees take pollen from the lower part, from the bent lower stamens, as soon as they land in the lower lobes.

1. Plant defoliant; leaves are strigose (hairy pubescent); stamen 5 1. luteum

1. Plant evergreen; leaves (short, dense, felt-shaped hairs) tomentose, glandular hairy or glabros-like (glabrous, smooth); stamen 10

2. Calix lobes 4-8 m, ; ovary densely glandular hairy. 2. ungeronii

2. Calix lobes 0.6-1 (-2.5) mm; ovary glabros or reddish-whitish tomentose

3. When the lamina is fully expanded, the underside is tomentose hairy.

4. Corolla red-pink; ovary white tomentose 3. simirnovii

4. Corolla light cream; ovary reddish hairy 4. caucasicum
3. The lamina is glabrous on the underside when fully expanded.

5. Usually the lamina lateral veins are straight in the upper part; usually corolla pinkish-purple; ovarian glabrosis.

5. ponticum 5. In the upper part, lamina lateral veins are flattened; corolla usually light pink; ovary usually reddish hairy 6. X sochadzeae

As a result of the field studies carried out between 2013 and 2014, the genomic DNAs of *Rhododendron* taxa were amplified according to the procedure mentioned in the previous section. As a result of the morphological characterization of the *Rhododendron* specimens, 3 different species were observed in the Kaçkar National Mountains as caucasicum, ponticum, and luteum. In the molecular analysis, as a result of the rDNA-ITS sequencing of the species, the lengths of the sequences varied between 703-844 bps; G+C% content in the whole ITS nucleotide length was found to vary between 48.80-53.80 (Tab. 3). The similarity relationships between the ITS regions of all studied samples and nBLAST analysis results were revealed

using the Maximum Likelihood model analysis by MEGA 7 [Tamura et al., 2004; Kumar et al., 2016, respectively].

According to dendrogram by generated MEGA 7, although all luteum specimens cluster together, caucasicum and ponticum species have a separate distribution in the branches formed (Fig. 6). Only the samples determined to belong to the ponticum species by the morphological characterization of E8, E9, E25, and E28 were determined to be more similar by being separated from other samples. According to the dendrogram analysis, it was observed that the E31 sample was in a separate branch from the caucasicum group and other groups, and according to the difference table (Tab. 4), it was separated with an average value of 0.225.

CONCLUSIONS

The Kaçkar Mountains National Park within the borders of Rize province, which was chosen as the research area, is located in the Eastern Black Sea Region. According to Davis' grid system, it is in the A8 square.

With this research, the localities of *Rhododendron* L. taxa that grow naturally in Kaçkar Mountains National Park were determined and shown on the map. In the flora of Rize, 9 taxa belonging to the genus *Rhododendron* L. were determined.

Related to taxa of Rhododendron genus. S. Terzioğlu [1998] emphasized in his study on the flora and vegetation of Uzungöl (Trabzon-Çaykara) and its surroundings that the region is an area where *Rhododendron* species naturally spread.

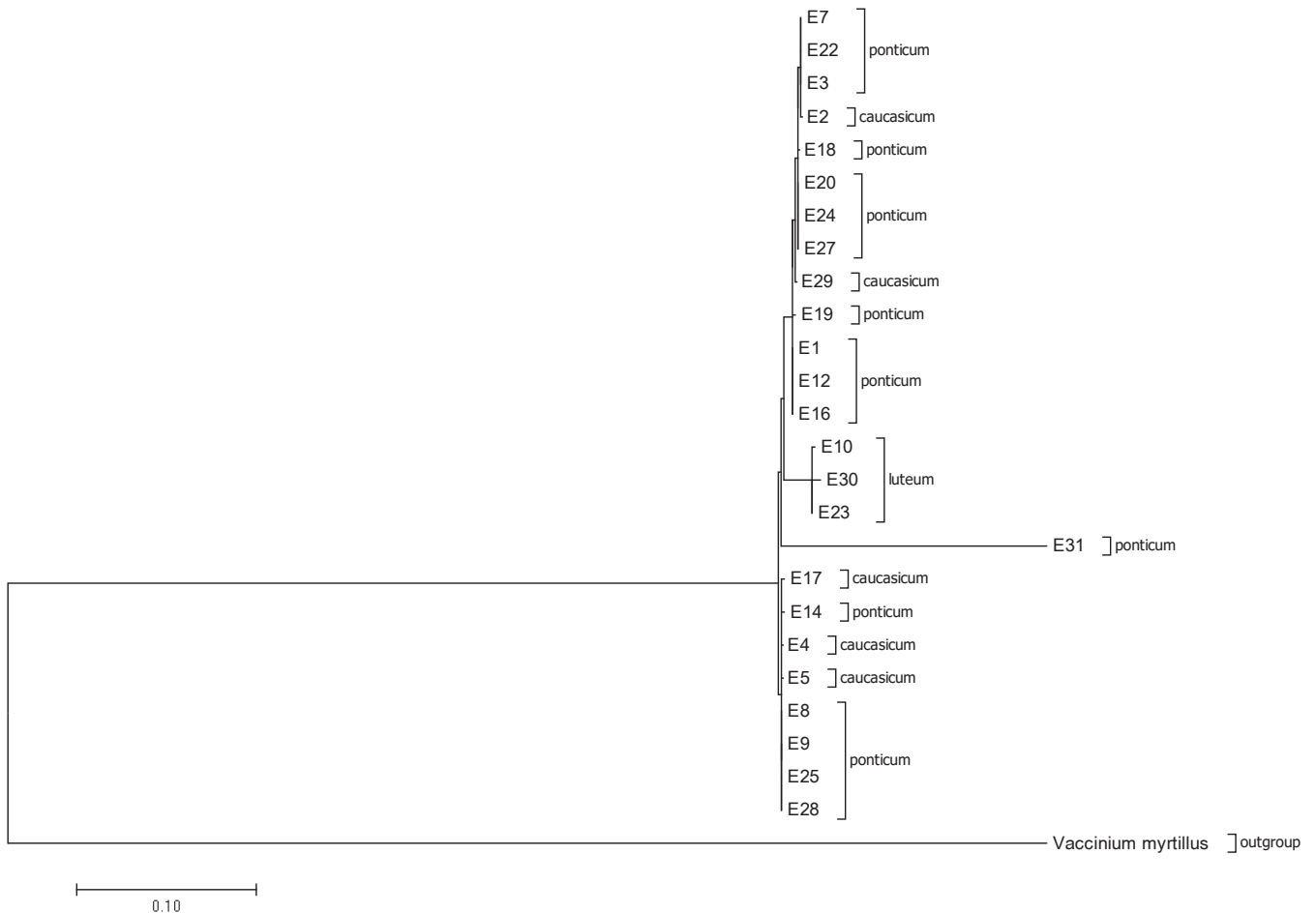


Figure 6. Proximal analysis of ITS regions with Mega 6.05 program by using the Maximum Likelihood method based on the Tamura-Nei model [1].

Table 4. Difference estimates between sequences were made in MEGA7 [Kumar et al., 2016].

Samples	E1	E3	E7	E8	E9	E12	E14	E16	E18	E19	E20	E22	E24	E25	E27	E28	E31	E2	E4	E5	E17	E29	E30	E23	E10	
E1	0,002																									
E3	0,002	0,000																								
E7	0,009	0,011	0,011																							
E8	0,009	0,011	0,011	0,000																						
E9	0,009	0,011	0,011	0,000	0,009																					
E12	0,000	0,002	0,002	0,009	0,009	0,011																				
E14	0,011	0,013	0,013	0,002	0,002	0,011																				
E16	0,000	0,002	0,002	0,009	0,009	0,000	0,011																			
E18	0,003	0,002	0,002	0,013	0,013	0,003	0,014	0,003																		
E19	0,002	0,003	0,003	0,011	0,011	0,002	0,013	0,002	0,005																	
E20	0,003	0,002	0,002	0,013	0,013	0,003	0,014	0,003	0,000	0,005																
E22	0,002	0,000	0,000	0,011	0,011	0,002	0,013	0,002	0,002	0,003	0,002															
E24	0,003	0,002	0,002	0,013	0,013	0,003	0,014	0,003	0,000	0,005	0,000	0,002														
E25	0,009	0,011	0,011	0,000	0,000	0,009	0,002	0,009	0,013	0,011	0,013	0,011	0,013													
E27	0,003	0,002	0,002	0,013	0,013	0,003	0,014	0,003	0,000	0,005	0,000	0,002	0,000	0,013												
E28	0,009	0,011	0,011	0,000	0,000	0,009	0,002	0,009	0,013	0,011	0,013	0,011	0,013	0,000	0,013											
E31	0,226	0,226	0,226	0,218	0,218	0,226	0,221	0,226	0,229	0,229	0,229	0,226	0,229	0,218	0,229	0,218										
E2	0,002	0,000	0,000	0,011	0,011	0,002	0,013	0,002	0,002	0,003	0,002	0,000	0,002	0,011	0,002	0,011	0,226									
E4	0,009	0,011	0,011	0,000	0,000	0,009	0,002	0,009	0,013	0,011	0,013	0,011	0,013	0,000	0,013	0,000	0,218	0,011								
E5	0,009	0,011	0,011	0,000	0,000	0,009	0,002	0,009	0,013	0,011	0,013	0,011	0,013	0,000	0,013	0,000	0,218	0,011	0,000							
E17	0,011	0,013	0,013	0,002	0,002	0,011	0,003	0,011	0,014	0,013	0,014	0,013	0,014	0,002	0,014	0,002	0,219	0,013	0,002	0,002						
E29	0,002	0,003	0,003	0,011	0,011	0,002	0,013	0,002	0,002	0,003	0,002	0,003	0,002	0,011	0,002	0,011	0,229	0,003	0,011	0,011	0,013					
E30	0,023	0,021	0,021	0,026	0,026	0,023	0,024	0,023	0,019	0,024	0,019	0,021	0,019	0,026	0,019	0,026	0,263	0,021	0,026	0,026	0,028	0,021				
E23	0,021	0,023	0,023	0,021	0,021	0,021	0,019	0,021	0,024	0,023	0,024	0,023	0,024	0,021	0,024	0,021	0,256	0,023	0,021	0,021	0,023	0,023	0,005			
E10	0,023	0,024	0,024	0,023	0,023	0,023	0,021	0,023	0,026	0,023	0,026	0,024	0,026	0,023	0,026	0,023	0,261	0,024	0,023	0,023	0,025	0,024	0,006	0,002		
Vaccinium myrtillus	3,995	3,995	3,995	4,002	4,002	3,995	4,002	3,995	3,990	4,000	3,990	3,995	3,990	4,002	3,990	4,002	5,206	3,995	4,002	4,002	4,032	3,990	3,895	3,905	3,934	

M. Avcı [2004] conducted a study on the natural distribution of Rhododendrons (*Rhododendron* L.) in Turkey. According to this study; It is represented by 9 different taxa in areas under the influence of the Black Sea climate type. However, it was stated that the concentration of rhododendrons in the Eastern Black Sea region, where precipitation and temperature values differ in terms of climatic conditions, is not accidental. Morphological and molecular studies reveal that ecological characteristics are important in natural hybridization among *Rhododendron* species [Milne et al., 1999]. Three hybrid rhododendrons, which were not known until recent years, were also found in the same distribution area. This situation also reveals that rhododendrons prefer areas with high rainfall but also high summer rainfall as their habitat.

R. ponticum, recognized by foresters as a "tenacious living cover", is an important trench plant. For this reason, it both prevents the rejuvenation of other plants in its distribution area and leads to the impoverishment of the soil fauna [Şahin, Cevahir, 1991].

A study was conducted by S. Sönmez [2000] on the determination of a New Distribution Area of the Yellow Flowering *Rhododendron* (*R. luteum*) in Western Anatolia (Balıkesir-Burhaniye), and in this study, it was not related to the climatic conditions that the yellow-flowered rhododendron was grown in Western Anatolia, has been placed on the grass. Because it has been concluded that this species is outside the limits of distribution and climate.

Also, T.B. Ozbucak et al. [2009] conducted an ecological study on some *Rhododendron* species distributed around Ordu.

N. Tezgul Cakir et al. [2005] conducted studies on the anatomical, phytochemical and antimicrobial properties of the endemic *R. smirnovii* taxon.

A. Tutar et al. [2012] conducted a study on the characterization of humic acid collected from *Rhododendron* humus in the Western Black Sea region. M. Zohary [1973], P. Quezel et al. [1980] and R. Anşın [1980] classified the forest vegetation of the Eastern Black Sea region and stated that the species belonging to the *Rhododendron* genus are common in the information part about the flora contents of the vegetation types in this classification.

Considering the studies on phylogenetic analyzes of *Rhododendron* species, Y. Liu et al. [2012] DNA barcoding technique in Rhododendrons grown in China, Y. Liu et al. [2011] Microsatellite DNA markers in *R. aureum*, *R. dauricum* and *R. brachycarpum* species. Delmas et al. [2011] Investigation of genetic structure

of *Rhododendron ferrugineum* population, again microsatellite DNA markers, C. Huang et al. [2011] ITS DNA markers to determine the genetic structure of *R. pseudochrysanthum* population, R.I. Milne [2004] Mitochondrial and chloroplast DNA markers and P.G. Wolf et al. [2004] investigated the genetic structure of *R. ferrugineum* species with the AFLP technique and revealed their genetic structures.

The flowers of some species of the *Rhododendron* L. genus are very similar, making it difficult to diagnose between species, and species are systematically mixed. In recent years, phylogenetic analyzes based on ITS regions of rDNA have been used in taxonomic position studies. These analyzes are also used in the reconstruction of the phylogenetic of many taxonomically complex genera, species or groups containing a large number of taxa [A. Özad, 2010].

Morphological characters, especially in the diagnosis of *Rhododendron* L. taxa; stem form, leaf characteristics, pubescence and flower characteristics are of great importance. It is possible to collect the *Rhododendron* L. specimens examined in three groups as shrub, small tree and tree. *R. simirnovii*, *R. caucasicum*, *R. ponticum*, *R. sochadzeae* are shrub-shaped and perennial, *R. ungeronii* is shrub-shaped or small-tree-formed perennial, and *R. luteum* is shrub-shaped and perennial. Among these species, only *R. simirnovii* is an endemic species. Species of *Rhododendron* genus are distributed in forest bottoms, spruce forests, *Rhododendron* thicket, areas higher than the tree line, in forests and degraded *R.* thickets. Flowering times are seen between 4-8 months. Their altitude distribution is 0-3000 m. spread among.

In our study, *Rhododendron* samples collected from Kaçkar Mountains National Park were found to belong to 3 *Rhododendron* species: *R. caucasicum*, *R. ponticum* and *R. luteum*. The nBLAST analysis of the DNA base sequences obtained by the ITS-PCR method applied to the samples also confirms the data obtained by morphological characterization. In addition, as a result of morphological and ITS DNA analyzes, the results of *R. caucasicum* and *R. ponticum* samples were found to be compatible with the results of the study by R.I. Milne et al. [1999]. Although the ITS DNA analysis of the *R. luteum* samples in our study allows us to see this group together, different molecular markers must be used in order to clearly distinguish the other species, *R. caucasicum* and *R. ponticum*.

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Kaçkar Dağları Milli Parkında (Rize/Türkiye) *Rhododendron* L. (Ericaceae) taksonlarının moleküler sistematik xüsusiyyətləri

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Kaçkar Dağları Milli Parkı ərazisində yayılmış *Rhododendron* L. (Ericaceae) taksonlarının moleküler sistematik xüsusiyyətləri tədqiq edilmişdir. Tədqiq olunan bitki nümunələri 2013-2014-cü illər arasında çöl tədqiqatları zamanı toplanmışdır. Toplanmış bitki nümunələri Universitetin Herbariumuna depozit edilib. Daha sonra ITS profillərini müəyyən etmək üçün müvafiq üsullarla hər bir herbaridən götürülmüş yarpaq nümunəsindən DNT ekstraksiya edilmişdir. Tədqiqat nəticəsində Kaçkar Dağları Milli Parkında tapılan növlər morfoloji xarakteristika və ITS DNT analizi məlumatlarına əsasən *R. caucasicum*, *R. ponticum* və *R. luteum* kimi təyin edilmişdir. Qeyd edək ki, ITS DNT analizləri seçilmiş nümunələrdə *R. luteum* növünü aydın şəkildə fərqləndirməyə imkan verir.

Açar sözlər: *ITS, yarpaq nümunələri, morfolojiya, PSR, növ, taksa*

Молекулярно-систематическая характеристика таксонов *Rhododendron* L. (Ericaceae), распространенных в национальном парке Качкарские горы (Ризе/Турция)

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В статье приводятся молекулярно-систематические особенности таксонов *Rhododendron* L. (Ericaceae), распространенных в Национальном Парке Качкарских гор. Исследуемый растительный материал был

собран в течение полевых исследований в период с 2013 по 2014 год. Собранный растительный материал депонирован в Гербарий университета. Для определения профилей ITS, соответствующими методами производили выделение ДНК из листьев каждого гербарного образца. В результате исследования видов, обнаруженных в национальном парке Качкарских гор, были идентифицированы как *R. caucasicum*, *R. ponticum* и *R. luteum* на основании морфологической характеристики и данных анализа ДНК ITS. Кроме того, анализ ДНК ITS четко различает *R. luteum* в отобранных образцах.

Ключевые слова: ITS, образцы листьев, морфология, ПЦР, вид, такса