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Rediscovery and Neotypification of *Festuca decolorata* (Poaceae), an Endemic Species from Mount Uludağ (Turkiye) with Amended Species Description and Notes on Ecology, Distribution and Conservation

Uludağ'dan (Türkiye) Endemik Bir Tür Olan Festuca decolorata'nın (Poaceae) Yeniden Keşfi ve Neotipifikasyonu, Güncellenmiş Tür Tanımı ve Ekolojisi, Dağılımı ve Korunması Üzerine Notlar

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ABSTRACT

Festuca decolorata Markgr.-Dann. is a rare, endemic species from Mount Uludağ in Bursa province, Turkiye. It was described in 1981 based on a single scant herbarium sheet. No other specimen has been recorded ever since, and the species remained known only from the holotype. While conducting the revision of the genus *Festuca* in Turkiye, we learned that the type specimen itself was no longer present in the collection where it was deposited. During our field surveys in Mount Uludağ, we discovered a small population of *F. decolorata* for the first time after the type gathering in 1968. Considering that there is no other existing original material on which the name was based, we assigned a neotype for the species. In addition, we performed a detailed examination of the new material and provided an amended taxonomic description and additional data about anatomy, micromorphology, phenology and ecology. We also estimated the species' potential distribution area using soil wetness, soil type, and forest cover as environmental predictors, and we used the resulting distributional data to assess the Red List status of the species. We found that the species is in grave danger of extinction with a maximum distribution area of only 6.15 km^2 and urgent conservation measures are needed to ensure its existence.

Keywords: Neotype, Poaceae, IUCN Red List assessment, Critically Endangered

ÖZ

Festuca decolorata Markgr.-Dann. Türkiye'nin Bursa ilinde bulunan Uludağ'a özgü nadir, endemik bir türdür. 1981'de tek bir yetersiz herbarium örneğine göre tanımlanmış olup ve günümüze kadar holotip kaydı dışında kayıt alınmamıştır. *Festuca* cinsinin Türkiye revizyonu yapılırken tip örneğin artık depolandığı koleksiyonda bulunmadığı öğrenilmiştir. Uludağ'da gerçekleştirilen arazi çalışmaları sırasında, 1968'deki kayıttan sonra ilk kez küçük bir *F. decolorata* popülasyonu tespit edilmiştir. Ayrıca, yeni materyallerin ayrıntılı incelemesi yapılmış ve düzeltilmiş bir taksonomik tanım hazırlanarak türün anatomisi, mikromorfolojisi, fenolojisi ve ekolojisi hakkında ek veriler sunulmuştur. Çevresel belirleyiciler olan toprak nemi, toprak türü ve orman örtüsü kullanılarak türün potansiyel dağılım alanı tahmin edilip ve elde edilen dağılım verileri, türün Kırmızı Liste durumunu değerlendirmek için kullanılmıştır. Maksimum dağılım alanı sadece 6.15 km^2 olan türün ciddi bir yok olma tehlikesiyle karşı karşıya olduğu ve korunması için acil önlemlere ihtiyaç duyulduğu tespit edilmiştir.

Anahtar Kelimeler: Neotip, Poaceae, IUCN Kırmızı Liste değerlendirmesi, Kritik tehlikede

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INTRODUCTION

The species *Festuca decolorata* Markgr.-Dannenb. is an endemic Euxine element from Mount Uludağ, in Bursa province, Turkiye. It was described by Markgraf-Dannenberg (1981) from a single herbarium specimen, collected by P. A. Quézel in 1968 and deposited in the MARS herbarium collection in Marseille, France. In the additional notes on the species description, Markgraf-Dannenberg (1981) stated that even though the only available specimen was very scanty, it differed from all other Turkish taxa of the *F. violacea* group. Thus, Markgraf-Dannenberg described it as a new species, hoping that better material for a detailed study would be provided in the future. *F. decolorata* was included in the Flora of Turkey and the East Aegean Islands, vol. 9., within *F.* sect. *Festuca*, *F*. subsect. *Rubrae* Weihe and *F. violacea* Ser. ex Gaudin group (Markgraf-Dannenberg, 1985). In addition, the author pointed out once again that the species is known only from the type specimen, which is in poor condition (not a single whole spikelet was seen), and an effort should be made to collect proper material. However, no other specimen was recorded afterwards. In the recent literature, fescue species characterized with extravaginal shoots, often flat leaves, specific anatomy of vegetative leaves (multifaceted leaf cross-sections, more than three sclerenchyma strands and deep grooves between ribs on adaxial surface), and, in certain species, hairy ovary at apex and sheaths closed to the mouth, are placed within the *F.* sect. *Aulaxyper* Dumort. (Angelov & Bednarska, 2018; Tzvelev, 2006, 2010; Devesa et al., 2013; Ortúñez, 2020). Thus, *F. decolorata* belongs to *F.* subgen. *Festuca*, *F.* sect. *Aulaxyper* and *F. violacea* group.

While trying to obtain the type specimen's digital image, we sent a query to the MARS herbarium. They directed us to the Museum of Aix-en-Provence (AIX herbarium), where Quézel's collection from Greece and Turkiye is currently stored. After AIX herbarium staff examined the collection on our behalf, we learned that the type specimen of *F. decolorata* is missing.

During our field studies for the contemporary revision of the *Festuca* L. genus in Turkiye, we visited Mount Uludağ a few times and discovered a small population of *F. decolorata*. Fresh material allowed us to examine the species' taxonomical features more closely and provide an amended description, as the previous description was based only on one poorly conditioned specimen and was partly incomplete. Moreover, considering that the holotype's fate is unknown, and there are no other specimens documented from the same collection nor collected for more than 50 years, we have decided to assign a neotype for the *F. decolorata* using the recently collected material.

Here, we present the neotype and an amended species description, and provide information about the species' anatomy, micromorphology, phenology and ecology. In addition, we have estimated the species' potential distribution area using soil type, soil wetness and forest cover as environmental predictors known to affect the distribution of the species and plants in general. Finally, we used the estimated distribution to calculate the area of occupancy and extent of occurrence according to the International Union for Conservation of Nature's (IUCN) Red List status assessment guidelines (IUCN, 2022). Based on our findings and following the IUCN criteria, we suggest the Critically Endangered (CR) category for the *F. decolorata*.

MATERIAL AND METHODS

Plant material

Fresh material of *F. decolorata* was collected as a part of the countrywide sampling for the ongoing systematic revision of the genus *Festuca*. We visited the type location of the species, Mount Uludağ, several times and eventually detected a small population of *F. decolorata*. A few samples were collected, dried and pressed using standard herbarium techniques (Simpson, 2010; RBG Edinburgh, 2017). Afterward, we stored the specimens in the Plant Systematics Laboratory at the Department of Biological Sciences, Middle East Technical University (herb. J. Erdal). We identified the specimens using Flora of Turkey and the East Aegean Islands (Markgraf-Dannenberg, 1985).

To examine the type specimen and compare it with our material, we contacted AIX herbarium, where Quézel's collection is currently stored. However, the specimen of our interest was not found, and we were informed that the holotype of *F. decolorata* ("Turkey, A2 (A) Bursa, Ulu Dağ, nardaies, 1968, *Quézel UD 2 (blue stripe label nr. 49.)*") was not present in the collection. We also surveyed MARS, BM, K, MPU and P herbaria online catalogues, since those herbaria host samples from P. A. Quézel's collection. But we did not encounter *F. decolorata* specimens. Therefore, it seems that there are no other *F. decolorata* samples known from the same gathering. Photographs and illustrations of the original material do not exist, either. Under these circumstances, we decided to assign one of the samples we recently collected as a neotype for *F. decolorata*. Thus, we prepared an herbarium sheet following the standard procedures (Simpson, 2010; RBG Edinburgh, 2017), and it will be deposited in the Ankara University Herbarium (ANK). In addition, we provided a digital image of the neotype (Figure 1).

We also surveyed collections or contacted Turkish herbaria ANK, GAZI, ISTE, ISTF, NGBB and BULU in order to detect any other specimen of *F. decolorata*, but there was none.

Localities of the samples were cited according to Davis's grid square system and phytogeographic regions, established in the Flora of Turkey and the East Aegean Islands, vol.1 (Davis, 1965).

Morphological, anatomical and micromorphological examination

Examinations and measurements of the vegetative and floral characters were made using an OPTIKA SLX-3 stereo microscope. We used assessment and terminology in

Figure 1. Neotype of *Festuca decolorata* (voucher specimen J. Erdal, G. Yaprak & M. Doğan 16064)

accordance with Markgraf-Dannenberg (1981, 1985). We performed the examinations on five different specimens, which were: Turkiye, A2 (A) Bursa: Osmangazi, Mount Uludağ, tourism development zone 2, wet *Nardus stricta* meadow, 1795 m, 19. 07. 2021, *J. Erdal*, *G. Yaprak & M. Doğan 16064* (ANK!), J. Erdal *16065*!, J. Erdal *16066*!, J. Erdal *16067*!, J. Erdal *16070*!. For each quantitative feature, we took five measurements on each specimen whenever possible. We applied "Haeckel's convention", a method traditionally used for measuring *Festuca*'s spikelets. According to this method, the spikelet is reduced to 4 florets and measured from the glume base to the 4th lemma apex, excluding the awn (Connor, 1998). We used the measurements obtained to provide an updated description of the species, and we presented all quantitative features as ranges. The images of floral and leaf features were taken using an OPTIKA C-B5 microscope camera (Figure 2, Figure 3).

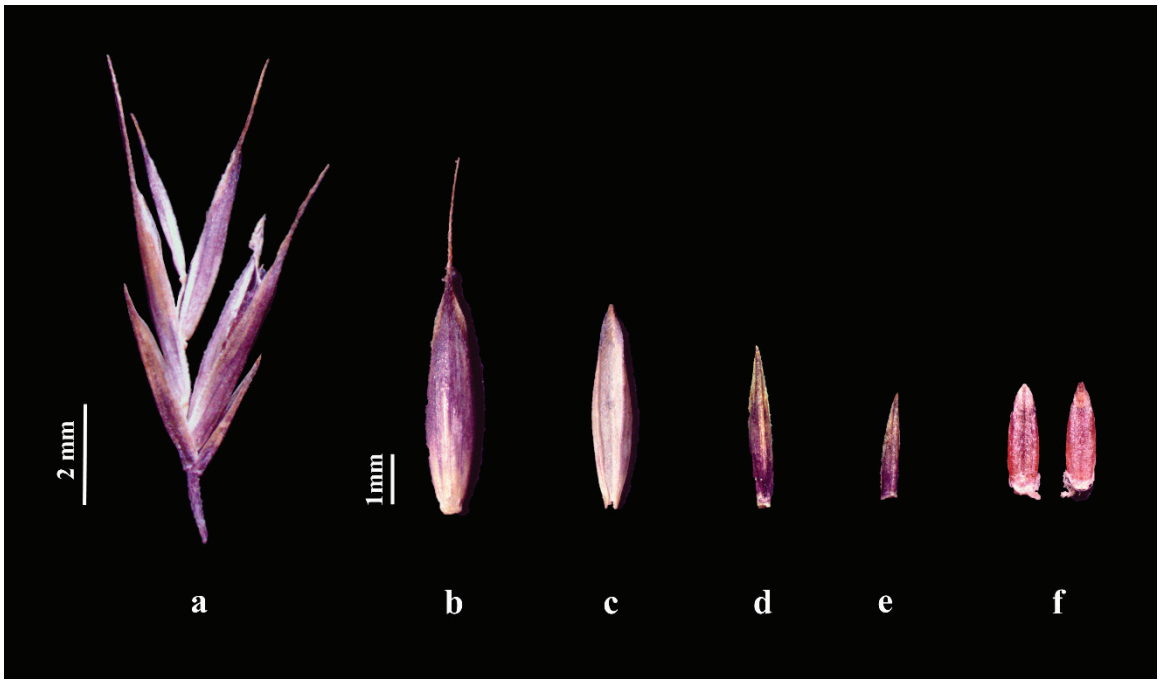

Figure 2. *F. decolorata*: spikelet (a), lemma (b), palea (c), upper glume (d), lower glume (e), caryopsis (f). Voucher specimen J. Erdal, G. Yaprak & M. Doğan 16065

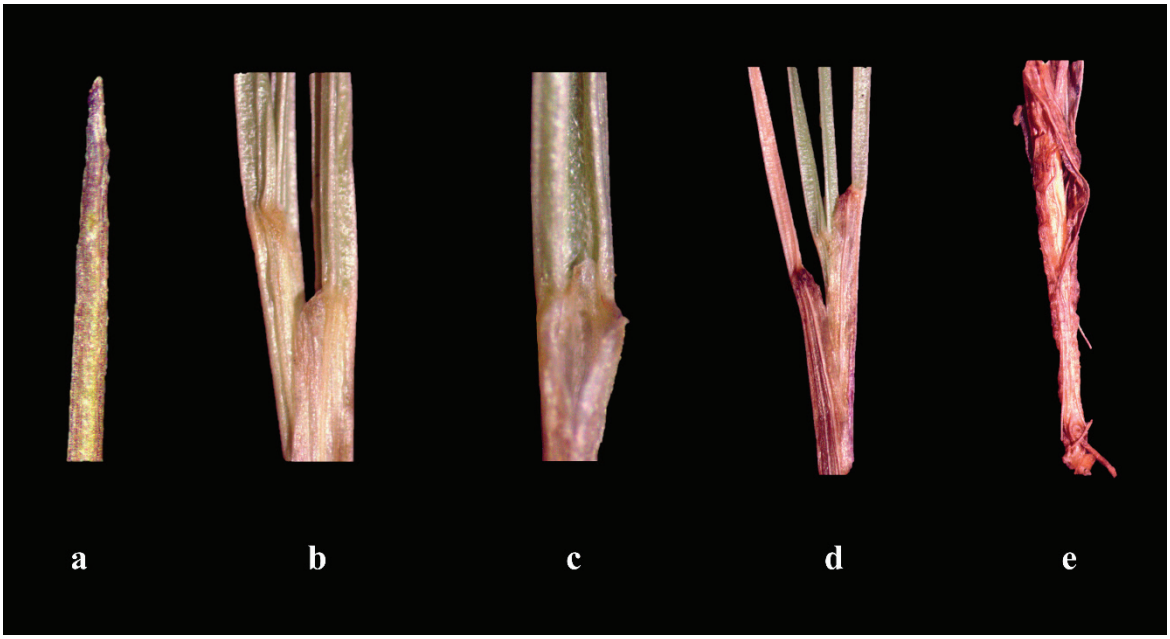

Figure 3. *F. decolorata*: leaf blade (a), auricles (b), ligule (c), leaf sheaths (d), leaf sheaths decayed into fibers (e). Voucher specimen J. Erdal, G. Yaprak & M. Doğan 16065

For leaf anatomy examination, we used herbarium material. Dry leaves were hydrated in hot water and free-hand cross-sections were made at the middle part of the leaf blades (Martínez-Sagarra et al., 2017). We observed the anatomical features under the OPTIKA SLX-3 stereo microscope. The images of leaf cross-sections were taken using an OPTIKA C-B5 microscope camera (Figure 4).

For the scanning electron microscopy (SEM) analyses, we chose fully developed spikelets and green leaf blades. The second lemma in the spikelet was always used for analyses. Leaf blade tissue samples were taken from the middle part of the leaf. All samples were cleaned with trichloromethane for 24 h (Ortúñez & Fuente, 2010). In the following steps, samples were fixed on aluminum stubs with double sided carbon tapes and coated with gold particles, and surfaces were scanned with a QUANTA 400F Field Emission Scanning Electron Microscope (SEM) using 500X and 1000X and 1500X magnifications (Figure 5). The terminology regarding

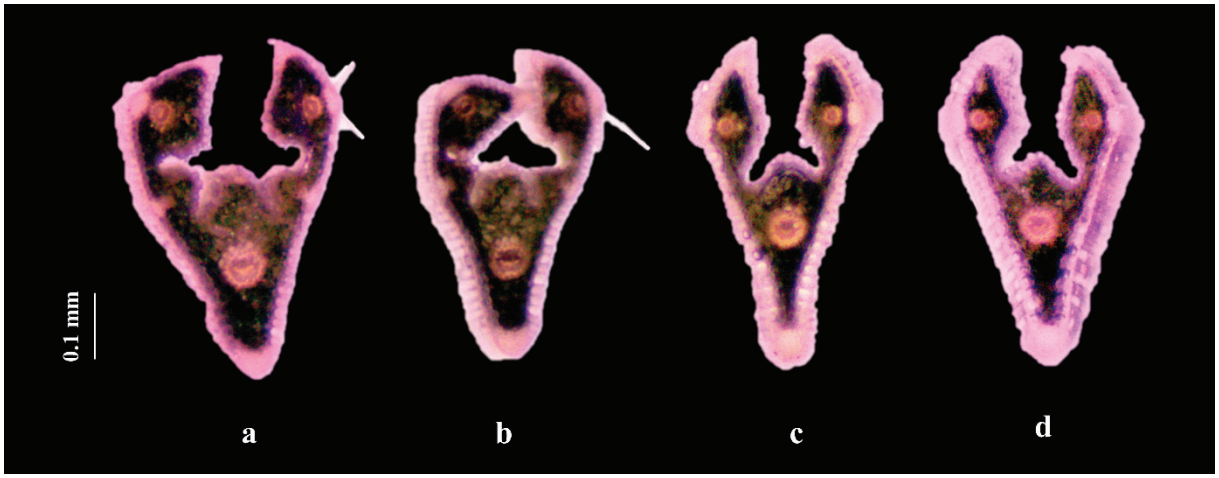

Figure 4. *F. decolorata*: leaf blade cross-section (a-d). Voucher specimens: J. Erdal, G. Yaprak & M. Doğan 16065, J. Erdal 16070

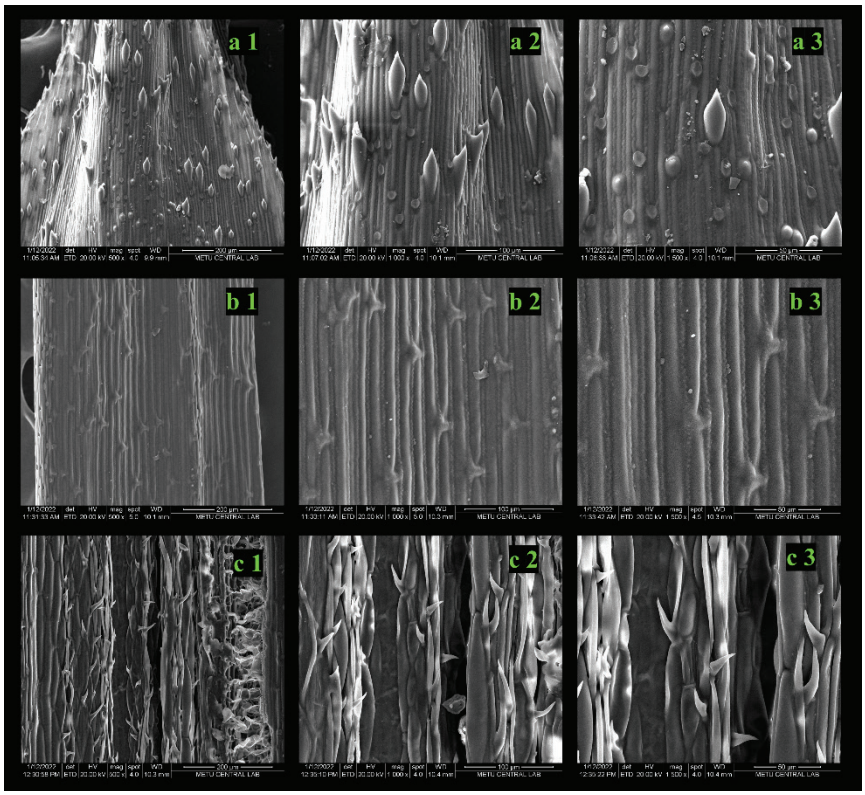

Figure 5. *F. decolorata:* micromorphology of abaxial lemma epidermis (a1, a2, a3); micromorphology of abaxial leaf blade epidermis (b1, b2, b3); micromorphology of adaxial leaf blade epidermis (c1, c2, c3). Magnifications: 500X (1), 1000X (2), 1500X (3). Voucher specimen J. Erdal, G. Yaprak & M. Doğan 16065

micromorphology was adopted from Stančík & Peterson (2007) and Ortúñez & Fuente (2010).

Habitat examination

We examined the species' habitat and identified other species that grow together with *F. decolorata*, using Flora of Turkey and the East Aegean Islands (Davis, 1965-1985).

Delimitation of the potential distribution area

Our field observations and records of the previous sample suggest that *F. decolorata* exists in close association with *Nardus stricta* L. (Poaceae) meadows (Markgraf-Dannenberg, 1985), which are mainly found above the tree line and in damp soil (Chadwick, 1960; Güleryüz et al., 1998; Kissling et al., 2004). It is also known that, in general, topographically, and biologically controlled soil moisture is an important driver of plant distributions (Dwire et al., 2006; Moeslund et al., 2013). Therefore, to find the localities that are suitable for *F. decolorata* at Mount Uludağ and its surroundings, we calculated the Topographic Wetness Index (TWI; Sørensen et al., 2006) by using a digital elevation model with 30 m spatial resolution (Shuttle Radar Topography Mission (SRTM) 1 Arc-Second Global dataset - Data available from U.S. Geological Survey's Earth Resources Observation and Science Center) in R 4.0.2 software (R Core Team, 2020). We used topoWetnessIndex function from the Envirem package (Title & Bemmels, 2018) to calculate the index. After obtaining TWI values for the area above the tree line, we checked our sampling location and its immediate surrounding's TWI values and TWI values for our other sampling stations where the species was absent. Then, we set a minimum soil wetness threshold above which the land was considered "suitable" for the species. The sampling locations of *F. decolorata* and other sampling stations from Mount Uludağ, where *F. decolorata* was not present, are given in Table 1. We delimited the area above the tree line using satellite imagery and ArcGIS Pro 2.3.0 software (Esri, 2018). We also used soil type as a predictor to detect suitable areas for the species because soil type is another factor that is known to be an important driver of plant distributions (Williams et al., 2009). For this purpose, we used the Food and Agriculture Organization of the United States' (FAO) World Reference Base for Soil Resources 2006 classification (FAO, 2006), which has a spatial resolution of 250 m, to detect the type of soil *F. decolorata* lives in, and to exclude uninhabitable areas from our final distribution area estimate. In the end, we picked suitable sites from the Mount Uludağ area alone, as the Mount Uludağ range is the only mountain habitat island in the region. We designated the resulting area as the potential distribution area of the species.

IUCN red list status assessment

We suggested the IUCN category for the species according to the guidelines listed in the IUCN Red List Categories and Criteria, Version 15, (IUCN, 2022).

The potential distribution area we produced was the approximation of the maximum suitable area of the species; consequently, the realized distribution area or the area of occupancy (IUCN, 2022) is probably smaller than the potential area. Nevertheless, we decided to treat the potential distribution area as the area of occupancy because the species is wind-pollinated and dispersed and the distribution area is already very small and made up of patches that are spatially very close to each other. Therefore, we believe that it is not farfetched to assume that *F. decolorata* is found in most of the potential distribution area. The extent of occurrence was defined as the smallest convex polygon enveloping all of the area of occupancy (IUCN, 2022). We calculated the area of occupancy and extent of occurrence in ArcGIS 2.3.0. (Esri, 2018).

Table 1. List of the sampling locations from Mount Uludağ

Location	*F. decolorata*
A2 (A) Bursa, Osmangazi, Mount Uludağ, tourism development zone 2, wet *Nardus stricta* meadow, 40°6' 27.09"N, 29°9'0.94"E, 1795 m	present
A2 (A) Bursa, Osmangazi, Mount Uludağ, tourism development zone 1, meadow, 40°6'11.97"N, 29°7'46.10"E, 1910 m	absent
A2 (A) Bursa, Osmangazi, Bursa-Uludağ road, *Quercus* forest, 40°8'5.00"N, 29°1'33.00"E, 937 m	absent
A2 (A) Bursa, Osmangazi, Bursa-Uludağ road, open rocky area within *Abies* forest, 40°6'47.51"N, 29°4'8.35"E, 1351 m	absent
A2 (A) Bursa, Osmangazi, Bursa-Uludağ road, open rocky area with *Juniperus*, within *Abies* forest, 40°6'30.37"N, 29°6'15.37"E, 1727 m	absent
A2 (A) Bursa, Osmangazi, Mount Uludağ, tourism development zone 1, mountain meadow, 40°6'12.02"N; 29°7'50.12"E, 1893 m	absent
A2 (A) Bursa, Osmangazi, Mount Uludağ, tourism development zone 2, sandy, rocky slope, 40°86'32.36"N, 29°9'3.25"E, 1796 m	absent
A2 (A) Bursa, Osmangazi, Mount Uludağ, road to observation platform, open areas within *Abies* forest, with *Juniperus* and *Vaccinium*, 40°7'2.00"N, 29°8'35.00"E, 1765 m	absent
A2 (A) Bursa, Osmangazi, Mount Uludağ, close to the observation platform, open rocky area within mixed forest, 40°8'17.79"N, 29°8'12.45"E, 1716 m	absent

RESULTS AND DISCUSSION

Neotypification

We found out that no original material of *F. decolorata* remains. The only known specimen of *F. decolorata*, the holotype "Turkey, A2 (A) Bursa, Ulu Dağ, nardaies, 1968, *Quézel UD 2 (blue stripe label nr. 49.)* (MARS)", is lost. There is no other material from the same gathering, nor are there any original material photos or illustrations. Therefore, we designated a neotype in accordance with the International Code of Nomenclature for algae, fungi and plants, Article 9.8, 9.13, 9.16 and 9.19 (Turland et al., 2018).

***Festuca decolorata* Markgr.-Dann.,** Willdenowia 11(2): 203 (1981). - **Neotype (designated here):** Turkiye, A2 (A) Bursa, Osmangazi, Mount Uludağ, tourism development zone 2, wet *Nardus stricta* meadows, 1795 m, 19 July 2021, *J. Erdal, G. Yaprak & M. Doğan 16064* (ANK!), Figure 1.

Updated description of *F. decolorata*

As previously mentioned, the species description was made on a single scanty specimen without a single preserved whole spikelet. Observations of the species in the field, sampling of the fresh material, and the higher number of specimens gave us a chance to examine the species in detail and to provide an updated complete description as follows:

Caespitose perennial. Stems few, 27-40 cm long, occasionally geniculate at the base, erect, thin, scabrid only below the panicle, violet-colored in the upper part. Basal innovation shoots mainly extravaginal. Young leaf sheaths light green; old sheaths brownish, decaying into fibers. Leaf sheaths closed for most of their length, with flat pubescent margins. Ligule very short. Leaf-blades filiform, green; with sparse long hairs; slightly scabrid at the tip, diameter 0.2-0.5 mm; angular in cross-section, contain 3-5 veins (vascular bundles), 3-5 ribs (inner ridges) with long hairs above, and 5-7 subepidermal, subequal, sclerenchyma strands. Panicle 3.5-7 cm long, lax, interrupted, lanceolate, branches pilose. Spikelets lanceolate-elliptic, violet, with 3-5 florets, 5-7 mm long, slightly pruinose. Glumes unequal, oblong-lanceolate, with broad scarious margins, shorty acuminate and slightly scabrid at the tips. Lower glume, 1.5-2.5 mm. Upper glume 2.5-3.5 mm long. Lemma broadly lanceolate, 4-4.5 mm long, violet, 5-veined, with broad scarious margin, shortly hairy towards the tip, acuminate; awned. Awn 1-2.5 mm long. Palea equal in length with lemma; 2-veined; densely pilose in the upper half. The ovary is pubescent on the apex. Caryopsis with adherent pericarp, hairy at apex, hilum linear.

Phenology

Markgraf-Dannenberg (1981, 1985) indicated July as a flowering time of *F. decolorata*. However, when we collected plants in the third week of July they were already in the late reproductive stages and some florets had even formed seeds. There are two possible explanations for this shift in flowering time. First, Markgraf-Dannenberg (1981, 1985) very often directly referred to the month of the voucher specimens' collection date as a flowering time, without using estimations based on the developmental stage of the specimen. This approach seems to be problematic, particularly when flowering time is determined on a single or a few samples. Second, even if we assume that the previous flowering time estimation was accurate in the past, it may be inapplicable to the current conditions. Since it has been shown that springs are becoming warmer and arriving earlier in Turkiye due to climate change (Tayanç et al., 2009), the flowering time of the study species, like many other alpine/subalpine plants, might have been advancing (Gordo & Sanz, 2010; Dorjia et al., 2020). With the projected changes in the climate, it will probably keep advancing. Eventually, we suggest that flowering time for *F. decolorata* will be during June rather than July.

Distribution and habitat

The location of the only known *F. decolorata* population is given in Figure 6, according to Davis' grid square system (Davis, 1965).

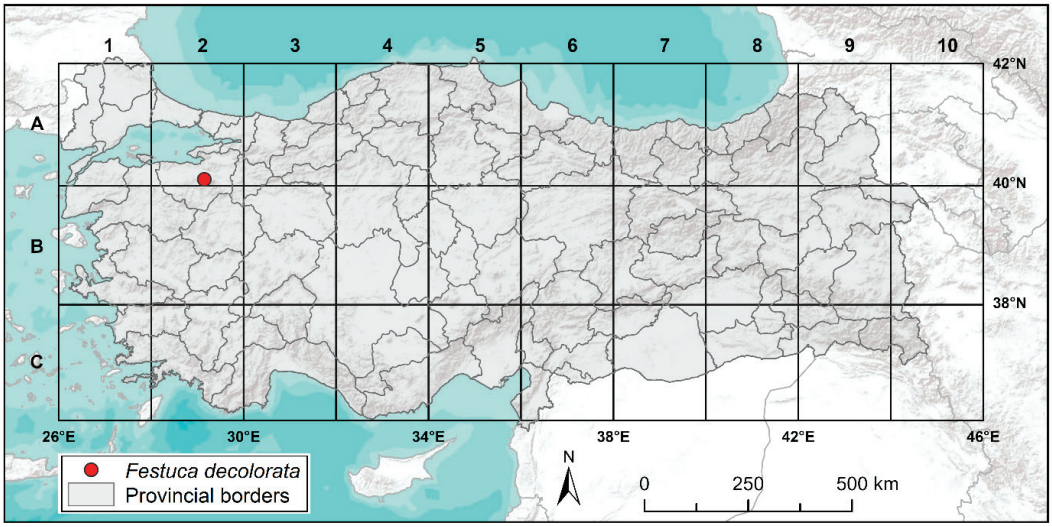

Figure 6. Distribution of the *F. decolorata* according to Davis' grid square system

There are three main vegetation types and plant communities above the tree line at Mount Uludağ: *Nardus stricta* meadows, *Juniperus communis* dwarf shrub, and hard *Festuca* communities (Güleryüz et al., 1998). *F. decolorata* was found within the *Nardus stricta* meadows. It prefers very wet, bog ground around the stream beds in the alpine zone, right above the forest level. The species grows together with *Nardus stricta* L., *Polytrichum commune* Hedw. *var. commune*, *Carex echinata* Murray, *Pinguicula balcanica* Casper, *Vaccinium myrtillus* L., *Calluna vulgaris* (L.) Hull, *Campanula olympica* Boiss, etc.

Our TWI calculations confirmed our observation that *F. decolorata* exists in very damp soil. The sample collection location and its immediate surrounding (closer than 60 m) had very high TWI values (average TWI = 7.58). Conversely, other sampling locations where the species was absent consistently had lower TWI values (maximum TWI = 6.85). We set the TWI threshold, over which the land was considered damp enough for the species to exist, as TWI = 7.00. This threshold is a little bit low in order to be more inclusive and flexible in our final distribution estimates.

Regarding the soil types present in the mountain, we found that the peak area is made up of two major soil types Cambisols, which have incipient soil formation with originating horizon formation and brownish color, and Podzols, which have light grey coloring as a result of bleaching by loss of organic matter and iron oxides and very low levels of available moisture, making it unsuitable for plants growing in damp soil (FAO, 2006). We saw that the place from which that the sample was collected has Cambisol type of soil, and considering this and the unsuitable nature of Podzols, we excluded Podzol areas from our final distribution area estimate. Our final potential distribution area estimate, which included areas above the tree line with high enough TWI values and Cambisol soil type, was only 6.15 km^2, and the extent of occurrence was only 178.54 km^2. The potential distribution map is given in Figure 7.

Conservation recommendations

F. decolorata is an endemic species known from a single location, Mount Uludağ, and the mountain is very close to the city of Bursa (around 20 km) and easily accessible by car

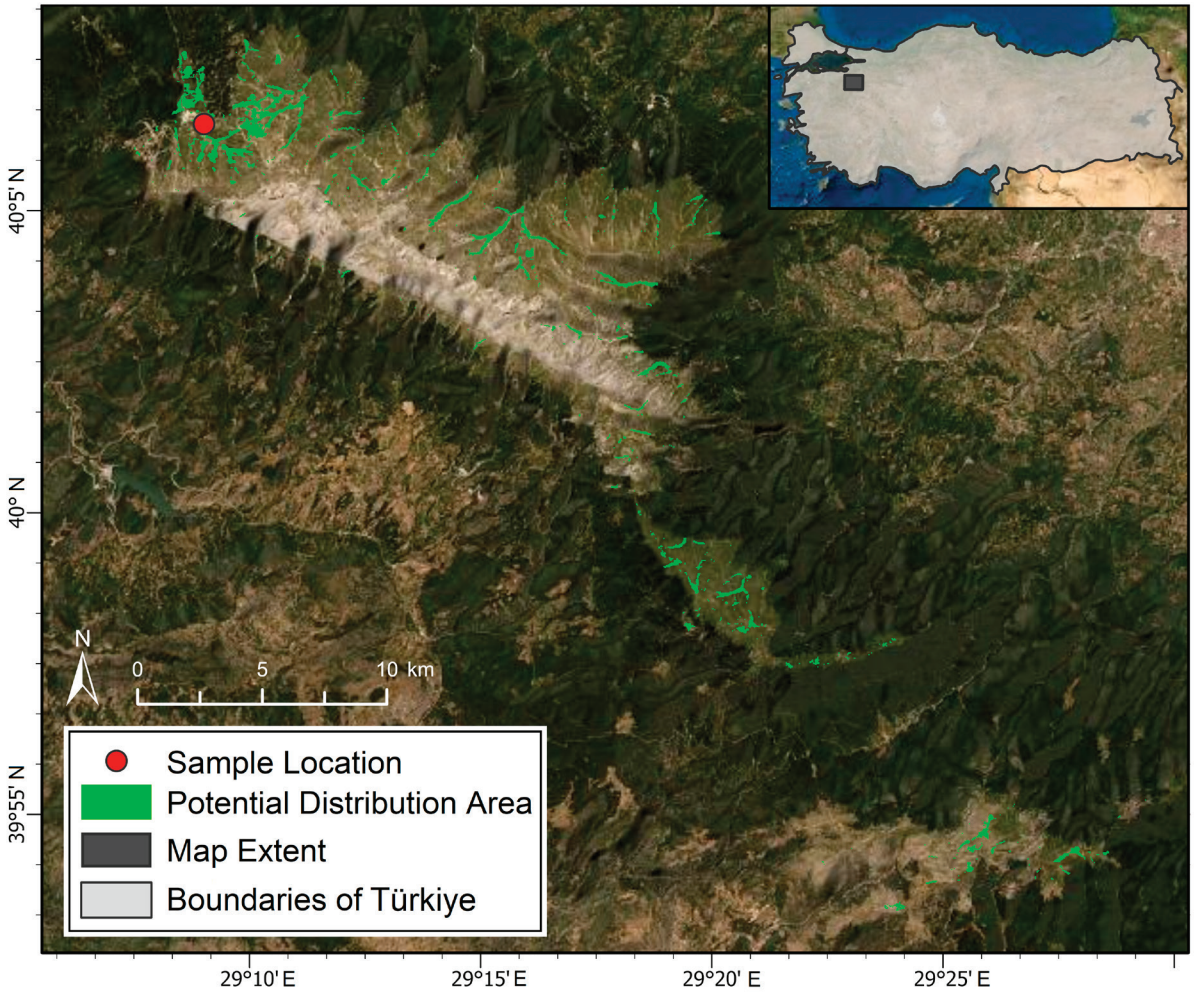

Figure 7. The potential distribution area of the *F. decolorata* (green polygons) and the location where the samples were found (red dot). Basemap sources: Esri, DigitalGlobe, GeoEye, Earthstar, Geographics, USDA, USGS, AeroGRID, IGN, and the GIS user community

or cable-car. Due to its position, it is a very popular skiing center, picnic and camping area, with numerous hotels, restaurants and summer houses. Tourism activities are very intense throughout the year. The population of *F. decolorata* appears to be very small, and it is located within zone two of the tourism development area; it will be seriously threatened by its expansion in the future. Also, during our field trips, we observed that the population is under severe grazing pressure, which is a common problem for many of the plants in Turkiye (Şekercioğlu et al., 2011). What is even worse, the species' range, like the range of many other high-elevation plants, is expected to shift upwards and contract due to global climate change (Cotto et al., 2017). Considering its low population size, very limited distribution, and pressure from tourism, grazing, construction and climate change, a declining population trend is certainly expected in the future. In light of all of our findings, and according to the IUCN guidelines, we suggest CR status for the species at the global and national scale based on the following criteria: [B2 ab (i, ii, iii, iv)]; area of occupancy less than 10 km^2; known to exist at only one location; continuing decline observed, estimated, inferred or projected in (i) extent of occurrence, (ii) area of occupancy, (iii) area, extent and/or quality of habitat and (iv) number of locations or subpopulations (IUCN, 2022). The CR status at the national level was suggested in the past as well (Ekim et al., 2000).

Comments on the methods

The method we used to delimit the potential distribution area was a simple and efficient way to define the potential area. A species distribution model (Elith & Leathwick, 2009) or an occupancy estimation model (MacKenzie et al., 2017) would probably be more reliable methods to define the species' potential occupancy. However, since our target species is known only from a single location (i.e., we have a single "present" data), and since both of these methods require a lot of data as input to operate, we decided to use ecological features of the species and other species which occur in sympatry with it to come up with a potential distribution area. We believe that we made the most of the available data and have used relevant predictors of plant distributions to make our final distribution map. Considering that thousands of species are going extinct every year without even going through any Red List assessments (Barnosky et al., 2011), our method, which does have some scientific ground to it, can serve very well until enough data is collected in further studies to build occupancy estimation or species distribution models, or to map the distribution of the species. Such studies should be supported urgently, especially considering the range contraction high-elevation plants are expected to experience due to climate change, which may happen faster than the plants can cope with (Cotto et al., 2017).

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