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Contributions to the description and molecular properties of *Erodium hendrikii* Alpınar (Geraniaceae), endemic to Turkey

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Abstract: A Turkish endemic, *Erodium hendrikii* Alpınar, was firstly described from NE Anatolia in 1994 based on insufficient specimens. In the present study, the description of this poorly known taxon was emended by adding several macro- and micromorphological traits of its leaf and fruit observed under LM and SEM. Furthermore, it was compared with the closely related species *E. malacoides* (L.) L'Hér. based on both morphological and molecular data and a modified identification key for the *Flora of Turkey* was prepared. The conservation status was also re-assessed according to the IUCN threat criteria.

Key words: cpDNA, *Erodium*, Geraniaceae, ITS, IUCN, Turkey

Introduction

The genus *Erodium* L'Hér. (Geraniaceae) includes more than 60 species worldwide (Alarcon et al., 2003). Approximately 40 of them are widespread throughout the Mediterranean region (Raduloviç, 2009), defined as the centre of biodiversity of the genus (Sharawy & Badr, 2008). According to Davis (1967), 31 taxa naturally grow in Anatolia and 16 of them are endemic to Turkey. *E. hendrikii* Alpınar, an endemic species, was firstly recorded from NE Anatolia and described based on very limited traits of insufficient materials (Alpınar, 1994). It is well known that the identification process needs adequate specimens and also the specimens should be collected in a different vegetation period for precise identification. Therefore, the description of the species needed additional traits. In the present study, *E. hendrikii* was re-collected from a second locality in 2009 and 2010. Phenetic properties were obtained from both recently collected specimens and the isotype material stored in Edinburgh Herbarium (E!). The emended description and images provided here are based upon extensive observations in the field. The conservation status was determined based on size of the habitats, population dynamics, and field observations based on the IUCN (2001) criteria. We also investigated the micromorphological properties of the leaf and fruit of this species by SEM together with those of a closely related species, *E. malacoides* (L.) L'Hér. Additionally, nrDNA ITS and the cpDNA *trnL-F* intergenic spacer were sequenced in order to verify the relationship of *E. hendrikii* with *E. malacoides*.

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Materials and methods

Specimens

Specimens of *E. hendrikii* were collected from NE Anatolia (A8 Trabzon: Uzungöl) in 2009 and 2010. They were dried according to standard herbarium techniques and deposited in the herbaria of both the Faculty of Forestry (KATO) and Biology Department (KTUB) at Karadeniz Technical University. Morphological features were noted from herbarium specimens of *E. hendrikii* (KATO; 18790, 18791, KTUB; Coskuncelebi 759) and isotype specimens stored in E.

Micromorphological studies

Mature fruits (mericarps) and healthy leaves were selected from herbarium specimens of *E. hendrikii* distributed in NE Anatolia (A8 Trabzon) and *E. malacoides* distributed in W Anatolia (C2 Muğla). Fruits and leaves from each taxon were mounted on metal stubs, sputtered with gold (Pelco S.C. 6 coating system), examined and photographed by a Zeiss EVO LS10 scanning electron microscope (SEM). Shape, and surface patterns of each mericarp were analysed on 5 micrographs for each taxon. Surface terminology of the mericarps followed Barthlott (1981). A minimum of 4 mericarps and leaves from each species were coated with 125-150 Å of gold before observations with SEM. Photographs were taken using an acceleration voltage of 10 kV. The terminology for indumentums followed Lawrence (1958).

Molecular studies

Total genomic DNAs were extracted from herbarium specimens following the modified CTAB extraction procedure of Doyle and Doyle (1987) according to Terzioğlu et al. (2012). The nrDNA ITS and the cpDNA *trnL-F* intergenic spacer were amplified by the polymerase chain reaction (PCR) using a Biometra personal thermal cycler. The amplification reactions for ITS were performed using universal ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) primers, developed by White et al. (1990). The universal primers trnTe (5’ -GGTTCAAGTCCCTCTATCCC-3′) and trnTf (5’ -ATTTGAACTGGTGACACGAG-3′) designed by Taberlet et al. (1991) were used to amplify the *trnL-F* intergenic spacer.

Sequencing of ITS regions and the *trnL-F* intergenic spacer was carried out by Macrogen Inc. using universal forward and reverse primers (ITS5, ITS4, trnTe, and trnTf), respectively. Sequence data were submitted to GenBank, accession no. trnL JN711470 (*E. hendrikii*) and JN711471 (*E. malacoides*), accession no. for ITS JN711469 (*E. hendrikii*). ITS sequence of *E. malacoides* (accession no.: EF185383) was obtained from GenBank. The nucleotide sequences were automatically aligned by using BioEdit v.7.0 software (Hall, 1999). G+C% content was calculated by FastPCR (Kalender, 2009) and indels were determined by Molecular Evolutionary Genetics Analysis (MEGA v.5.0) software (Tamura et al., 2011).

Taxonomic treatment

Erodium hendrkii Alpınar in Edinburgh J. Bot. 51: 68 (1994) (Figures 1-2)

Type: [Turkey] A7 Gümüşhane: Yağmurdere, 1800 m, 10.8. 1989. *K.Alpınar & H.’t Hart* (ISTE 61049), (İso. E!).

Annual plant, caulescent, with short root. Stem up to (-5) 15-27 (-35) cm, ascending, with short retrorse hairs. Basal leaves ovate to deltoid, nearly cordate at base, coarsely lobed, crenate, pubescence at both sides, 2.5-3.5 × 1.8-2.5 cm, long petioled, cauline leaves ovate to deltoid ovate, ±5-7 lobed, irregularly crenate-dentate, 1-2 × 0.7 cm, densely short glandular-pubescent, stipule triangular, both sides eglandular hairs, 4-5.5 cm, inflorescences axillary 5-7 (-9) flowered, pedicels 11-21 mm with glandular hairs, peduncles 40-70 mm with short arachnoid hairs, bracts 0.1-0.2 mm wide and 0.3-0.5 mm long, triangular with hairs at the margins. Sepals ovate to oblong, 4-5.5 mm long and 1.1-1.7 mm wide at anthesis, 6-7.5 mm long and 2-3 mm wide at the fruiting time, glandular pubescent, shortly ciliate along margins, awn c. 1 mm, long hairs absent at the tips. Petals whitish, 6-8 (-10) mm, claw ciliate. Beak of fruit 22-32 mm long, beak hairy, beak bursting from the top, mericarp 6-7.5 mm, mericarp with one apical pit, mericarp surface plumose, with glandular trichome, mericarp stalk long-haired, seed 2-2.2 mm long and 0.5-0.7 mm wide, oblong.

In the key of the *Flora of Turkey* (Davis, 1967), *Erodium hendrikii* may be inserted as follows:

Figure 1. *Erodium hendrikii.* a- herbarium specimen, b- in nature.

1. All leaves ovate, mericarp with a furrow

2. Petal white, sepals ciliate, mericarp 6-7.5 mm with glandular surface, stalk long haired. ***E. hendrikii***

2. Petal mauve-pink, sepals non-ciliate (except at the tip), mericarp 4-5 mm with papillate surface, stalk glabrous,..... ***E. malacoides***

1. All leaves suborbicular, mericarp without furrow..... ***E. chium***

Habitat and ecology

Type locality of *Erodium hendrikii* is stony slopes at about 1800 m a.s.l. (Alpınar, 1994). Furthermore, the studied materials were obtained from the population

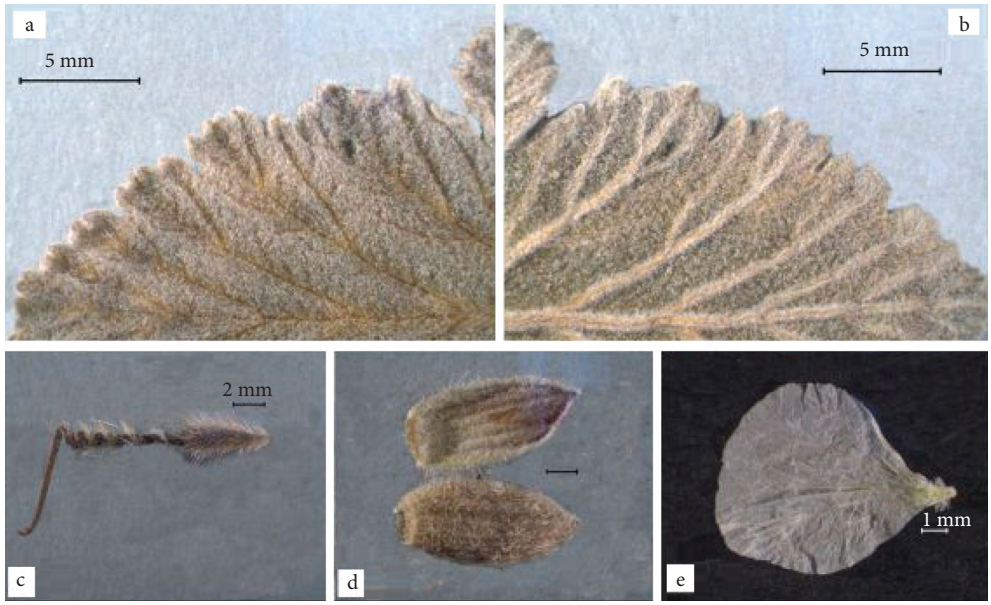

Figure 2. *Erodium hendrikii.* a- upper surface of basal leaf, b- lower surface of basal leaf, c- stalked mericarp, d- inside and outside of sepal, e- petal.

growing in subalpine meadows on stony slopes in Uzungöl Special Environmental Protection Area (SEPA) (Figure 3). This habitat is classified as "**H2.33**-*Southeast European Mountain Siliceous Screes*" according to the European Nature Information System (Davies et al., 2004). *E. hendrikii* shares its habitat in this biotope with several endemic (E), rare (R), and non-endemic plant species such as *Euphrasia minima* (E), *Geranium ibericum* subsp. *jubatum* (E), *Trifolium polyphyllum* (R), *Achillea millefolium*, *Digitalis ferruginea*, *Lapsana communis*, *Rhamnus microcarpus*, *Saxifraga paniculata*, *Orobanche nana*, *Pedicularis nordmanniana*, *Thymus praecox* subsp. *grossheimii*, *Sedum album*, *Sedum spurium*, *Thalictrum minus*, *Viburnum lantana*, *Teucrium chamaedrys*, *Silene odontopetala*, *Asperula pontica*, and *Clinopodium vulgare*.

Distribution and conservation status

Many rare and endemic species are at risk of extinction because of various human activities (Işık, 2012) and a precise assessment of the conservation status of endemic plant species is necessary in order to prevent their extinction. *E. hendrikii* is endemic to Turkey and it is a Euro-Siberian element. It is known from 2 localities (1800 and 2345 m) together with the type locality in NE Anatolia (Figure 3) and should be regarded as Critically Endangered (**CR**: B2b (ii, iii, iv)) according to IUCN threat criteria (2001) based on size of the habitats, the number of sub-populations, and habitat quality. Its habitat has been negatively affected by over-grazing.

Results and discussion

Erodium hendrikii was first collected by Alpınar (Alpınar, 1994) from A7 Gümüşhane: Yağmurdere, NE Turkey. Due to the lack of some necessary parts (e.g., basal leaves and mericarp) the complete description of the species was not carried out (Güner, 2000). The importance of leaves' and mericarps' properties was highlighted for the genus *Erodium* by Davis (1967). In 2009, we observed different plant specimens that at first glance resembled the genus *Geranium* due to fruit beaks, but they differed from *Geranium* based on leaf properties. After close observation, they keyed out as a species of *Erodium*. The genus *Erodium* is represented by many endemic species in Turkey and its taxonomic treatment was prepared by Davis (1967). After that many new taxa including *E. hendrikii* were recorded from Turkey (Güner, 2000). *E. hendrikii* was reported as a

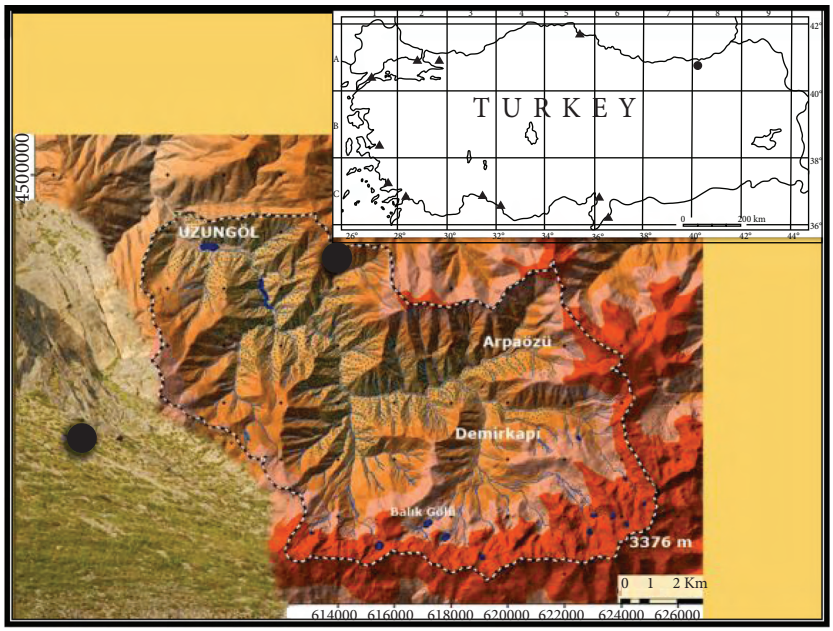

Figure 3. Habitat of *Erodium hendrikii* in Uzungöl SEPA (●) and the distribution of *Erodium malacoides* (▲) in Turkey.

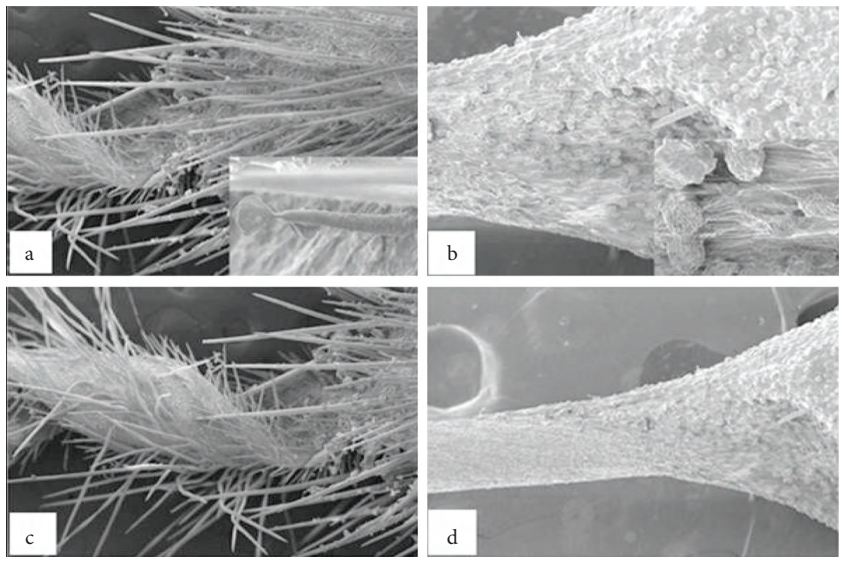

Figure 4. SEM micrographs of mericarp. Pit and glandular trichome of *Erodium hendrikii* (a), pit and papillate trichome of *Erodium malacoides* (b), hairs on the mericarp stalk of *E. hendrikii* (c), glabrous stalk of *Erodium malacoides* (d).

closely related taxon to *E. malacoides* with respect to characters of life cycle, stem, petiole of basal leaves, and mericarp (Alpınar, 1994). The most important differences are distribution of glandular trichomes and long plumose hairs on the mericarp surface (Figure 4) and petal colour (Table 1). Furthermore, hair type differs between the basal leaf and stem (Figure 5). Other important comparisons between *E. hendrikii* and *E. malacoides* are given in Table 1.

The length of the nrDNA ITS region was found to be 628 bp for *E. hendrikii* and *E. malacoides*. There are 4 base deletions in the ITS sequences for the investigated specimens, but number of deletions and base variations differ from region to region and taxon to taxon as seen in Table 2.

The length of the cpDNA *trnL-F* intergenic spacer was found to be 426 bp for *E. hendrikii* and 412 bp for *E. malacoides*. There are 21 base deletions in *E. malacoides* and 7 base deletions in *E. hendrikii*. These results reveal that there is a distinct discrimination between these 2 taxa.

GC% content of ITS was evaluated as a stable character in plants (Baldwin et al., 1995). Molecular data indicated that GC% contents of taxa in terms

Table 1. Comparison of *Erodium hendrikii* and *E. malacoides*.

	Erodium hendrikii	***Erodium malacoides***
Basal leaves	pubescence (velvety hairy)	sparsely hairy
Stem indumentum	short retrorse hairs	long hairs
Mericarp	6-7.5 mm long, with long glandular hairy	4-5 mm long, with short eglandular hairs
Trichome type on mericarp surface	glandular	papillate
Beak of fruit (mm)	22-32	22-25
Petal	white	mauve-pink
Sepal length (mm)	4-5.5 at anthesis, 6-7.5 at the fruiting time, with ciliate	2.5-3.5 at anthesis, 4.5-5 at the fruiting time, without ciliate except tip
Altitude (m)	1800-2345	0-300
Phytogeography	Euro-Siberian	Mediterranean

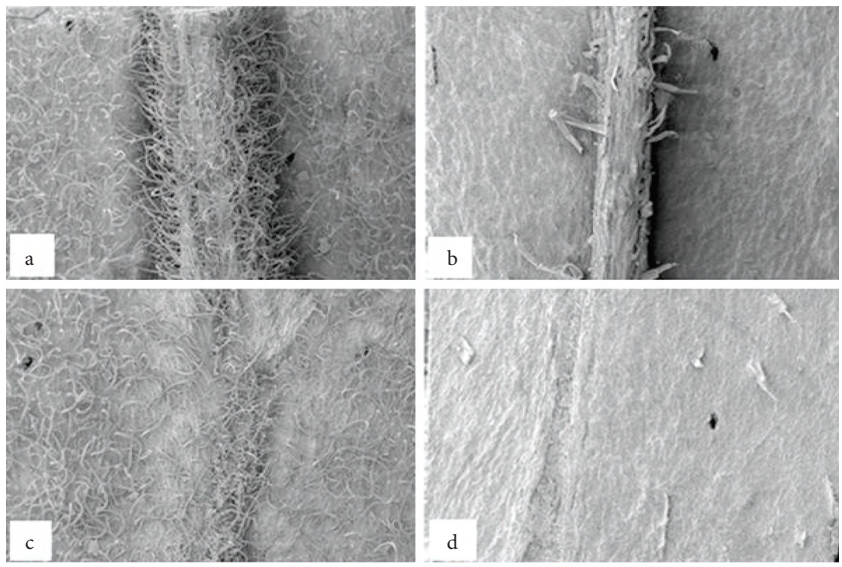

Figure 5. SEM micrographs of leaf indumentums. Pubescence hairs on the lower (a) and upper (c) leaf surface of *Erodium hendrikii*, sparse hairs on the lower (b) and upper (d) leaf surface of *Erodium malacoides.*

of the observed regions were different, but it was coherent within the same region. GC% content of ITS is 61.8 in *E. hendrikii* and 64.2 in *E. malacoides.*

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Table 2. Comparison of *Erodium hendrikii* and *Erodium malacoides* in terms of molecular data.

	E. hendrikii	***E. malacoides***
ITS 1 length (bp)	230	231
Number of deletions	3	2
Base variation (for ITS 1)	≈ **19.7**% **(46/233)**	
5.8 S length (bp)	163	164
Number of deletions	1	0
Base variation (for ITS 5.8 S)	≈ 1.8% **(3/164)**	
ITS 2 length (bp)	235	233
Number of deletions	0	2
Base variation (for ITS 2)	≈ **11.4**% **(27/235)**	
trnL-F length (bp)	426	412
Number of deletions	7	21
Base variation (for *trnL-F*)	≈ 16.8% **(73/433)**	
GC% (for ITS)	61.8	64.2
GC% (for *trnL-F*)	46.6	38.8

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