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Morphological, ecological and palynological studies on *Sempervivum sosnowskyi* Ter-Chatsch. (Crassulaceae) with a new distribution record from Turkey

Fergan Karaer[1], *Ferhat Celep[2] & H. Güray Kutbay[1]

[1]Ondokuz Mayıs University, Department of Biology, Kurupelit, Samsun-Turkey
[2] Middle East Technical University, Department of Biological Sciences, 06531, Ankara-Turkey

***Corresponding author: ferhat_celep@hotmail.com, fcelep@metu.edu.tr**

Abstract

This study presents investigations on the morphology, ecology and palynology of *Sempervivum sosnowskyi* Ter-Chatsch. For morphological studies, a detailed description of *S. sosnowskyi* is given. In addition, diagnostic morphological characters of similar species (*S. armenum* and *S. glabrifolium*) are discussed. The species, known as Georgian endemic, is reported for the first time from Turkey. It is restricted to Artvin region in Northeast Anatolia where it grows on metamorphic rocks and moist subalpine steppe. Notes are also presented on its ecology and conservation status. Pollen micromorphology of *S. sosnowskyi* and the other species is presented using light (LM) and Scanning Electron Microscopy (SEM). A diagnostic key and a new distribution map of the species are also given.

Keywords: Crassulaceae; New Record; *Sempervivum*; Turkey

Introduction

Although the family Crassulaceae is nearly cosmopolitan, it has radiated extensively in five regions of the world: Southern Africa, Mexico, Macaronesia, the Mediterranean region and the Himalayas (Sponberg, 1978). Within each of these centers of diversity endemic genera of Crassulaceae are found. Mort et al. (2001) suggested that the family spread through from southern Africa to the Mediterranean and into Eastern Europe and Asia, and species from northern Africa dispersed to Macaronesia, where they subsequently diversified. Sempervivums (houseleeks, stonecrop) have horticulture potential in landscape design and applications such as home gardens, rock and dry wall gardens as succulent ground cover plant (Mitchell 1979 a, b). As well as, some *Sempervivum* species are used as folk medicine. Fresh juice from squeezed leaves of *Sempervivum* is treating to skin complaints such as burns, wounds, sores and also on painful areas (Abram and Donko, 1999). Drinking tea prepared from leaves of *S. tectorum* is recommended for ulcer treatment (Bremness, 1996). Basal leaves of *Sempervivum sosnowskyi* are consumed as salad by local people in NE Anatolia. However, there is no direct information about *S. sosnowskyi* on its medicinal effect. *Sempervivum* is a taxonomically difficult and highly polymorphic genus (Muirhead, 1972). In general, identification of *Sempervivum* species is very difficult and the nomenclature of the species is complicated. Although over 200 species have been described, only ca. 40-50 species are generally recognized ('t Hart et al., 2003). The genus is mainly distributed in mountainous regions of the Mediterr- anean, Southwest Asia, Caucasia and Central and Southern Europe (Topalov et al., 2006). Its centre of diversity lies in the Euro-Siberian, Mediterranean and Irano-Turanian phytogeographic regions (Konop, 1987). The first revision of *Sempervivum* in Turkey was made by Muirhead (1969, 1972) who recognized 12 taxa. Since the publication of the flora, five new taxa and one hybrid have been described from Turkey (Davis et al., 1988; Neeff, 2005; Karaer and Celep, 2007). The total has now reached 19 with this record, 13 of which are endemic to Turkey. Since 1999, as a part of a revisional study on the genus *Sempervivum* in Turkey, a large number of specimens have been collected through the project funded by TÜBİTAK (project number: 1747) by the authors. In addition, population size, phenological and ecological properties were observed. During a field trip to Yanıklı village near Şavşat (Artvin, NE Turkey) in scheme of revisional work for *Sempervivum*, we encountered an unusual population of *Sempervivum*. At first glance, it looked very similar to Turkish endemics *S. armenum* Boiss. & A. Huet and *S. glabrifolium* Boriss. Further studies highlighted that it was quite different from these species. The specimens were cross-checked with the keys provided by Muirhead (1972) and the *Sempervivum* accounts given in the relevant literatures e.g., Borissova (1939), Grossheim (1950), Jansson and Rechinger (1970), Mitchell (1979a, b), Parnell and Favarger (1993) and Zonneveld (1979). As well as, the specimens have been cross-checked with material housed at various herbaria (ANK, GAZI, HUB, ISTE, E, K, LE, BM). Eventually, it was identified as *S. sosnowskyi*, which was only known from Southwest Georgia. This is a new record for the Flora of Turkey. According to literature, the new record is not surprising since this species also grows nearby Georgia-Turkish border. While some morphological and palynological studies have been carried out on *Sempervivum*

Table 1. A comparison of selected characters of *S. sosnowskyi* between *S. armenum* and *S. glabrifolium*

Characters	*S. sosnowskyi*	*S. armenum*	*S. glabrifolium*
Rosette diam. (cm)	(8)-10-15 (-20)	2-8	2-3.5
Number of offsets	2-3	3-4	many
Basal leaves length (cm)	6-8	1-4	1.5-2.5
Stem leaves	Widely ligulate	Non-ligulate	Non-ligulate
Flower diameter (cm)	3-3.5	1.5-2.5	1.5-2
Calyx lobes	Oblong-ovate	Lanceolate	Swollen and strongly incurved ovate
Filament colour	Lavender or violet	Pale lilac to violet	Yellow-white
Number of flowers	(40-) 60-210	15-50 (-60)	10-20
Scales	Horizontal	Erect	Slightly erect

and other genera by Afshari et al. (2008), Kaviani (2008), Muirhead (1969, 1972), Oybak et al. (1997) and Parnell (1991), ecological, anatomical and micromorphological studies of most *Sempervivum* species have not yet been investigated. Although the European taxa are relatively well-known, most Asian taxa are still insufficiently known (‘t Hart et al., 2003). Studies on morphology, ecology and pollen micromorphology of *S. sosnowskyi* are very limited. Thus, the present study aims to give a comprehensive description of the morphological, palynological and ecological properties of the species. The taxonomic significance of morphological and palynological properties is also discussed with its closely similar species. Besides, distribution area and conservation status of *S. sosnowskyi* are re-evaluated.

Fig 1. Habit of *Sempervivum sosnowskyi*

Materials and methods

The specimens collected from near Şavşat (Artvin, NE Turkey) were identified after critical examination and comparison with the original description and the type specimens photos available at the herbaria E. In the field, geographic coordinates, population size, habitat and ecological characteristics were recorded and the species vulnerability was estimated according to IUCN threat categories (2001). For palynological investigations, pollen material was obtained from herbarium samples. The pollen slides were prepared according to Wodehouse’s (1935) technique. For light microscopy (LM) studies, pollen grains were dissected from herbarium samples and placed on clean microscope slides. Glycerin-gelatin and basic fuchsin were added to the pollen and were then mixed with a clean pin to be scattered. The polar length (P), the equatorial length (EA), the colpus length (CLG), colpus width (CLT), the exine and the intine thickness for 30 pollen grains were measured under the light microscope (1000x) and P/E ratios were calculated. For scanning electron microscopy (SEM), unacetolyzed pollen grains were first mounted on doublesided carbon tape affixed to aluminum stubs, covered with gold with an Hummle VII sputter coater and photographed at a magnification of 2000x to 15000x with a JEOL-5600. SEM micrographs were used determine exine sculpturing of the pollen. Pollen terminology followed Punt et al. (2007). Soil analyses were carried out by using standard methods (Allen et al., 1986). During field studies, we aimed to visit as many as different habitats and populations to ensure representative geographical coverage of the species in Turkey. In the field, habitat and relevant field observations were also recorded. When the species was detected, we observed following criteria: the area of occupancy and distribution, populations and their size and the number of mature individuals. Threat categories are proposed according to IUCN Red List Categories Version 3.1 (2001) and the Application of IUCN Red List Criteria at Regional Levels (Gardenfors et al., 2001). According to the results, we assessed its both international and national red list category.

Results and Discussion

Sempervivum sosnowskyi Ter-Chatsch.--Zametki Sist. Geogr. Rast. [Not. Syst. (Tbilisi)] 13: 17. 1947; (Fig. 1). The following description of the species was based on the type specimens collected from Bakuriani region in Georgia and Turkish specimens collected from Şavşat (Artvin) in Turkey. Perennial, 30-45 (-60) cm. Rosettes (8-) 10-15 (-20) cm. diam., offsets few. Rosette leaves oblong-spatulate, 60-80 x 20-25 mm, glabrous, margins ciliate, 0.6-0.8 mm, with dark purple tips. Scape leaves oblong-oblanceolate, glabrous and widely ligulate with reddish tips, 60-90 (-100) x 25-35 mm, imbricate, scape c. 1.5-2 cm diam. Inflorescence dense, (40-) 60-210 flowered, pedicels 2-3 cm, flowers c. 30-35 mm diam.; calyx lobes oblong-ovate and glandular hairy, tips dark purple, (12-) 14-16 (-18)-merous; petals greenish yellow, linear-lanceolate and glandular hairy outside, purplish towards the base, filaments (24-) 28-32 (-36), pale lavender or violet, anthers yellow; carpels (12-) 14-18, glandular hairy, scales horizontal and sub-quadrate. *Type:* Georgia: Mount Gvirgin near Tsagvery in the Borzhomo-Bakuriani region, rocky outcrop A. Dzhavahishvily, 1938. holotype TBI (photo !) and isotype E (photo !). Turkish specimens: A9 Artvin (Turkey): Northeast Anatolia, Artvin

Table 2. Summary of pollen characters for species: x: minimum, y: mean, z: maximum; Ap.: type of apertures, P: polar axis (µm), EA: equatorial axis (µm), E: Exine (µm), I: Intine (µm), Cl: Colpus (µm), $P_{1:}$ Pore (µm), PS: Pollen shape

		S. armenum	*S. sosnowskyi*	*S. glabrifolium*
P	y	22.5	19.2	18.7
	x	19.8	14.5	16.6
	z	27.1	26	21.8
E	y	23.2	19.7	21.8
	x	18.7	16.6	18.7
	z	29.1	28.1	24.2
E	Meso	1.3	1.6	1
	Cuti	1.8	2.3	1.3
I		0.3	0.4	0.4
Cl	clg	18.7	16.6	17.1
	clt	7.3	7.8	7.2
PS		Suboblate to oblate-spheriodal	Suboblate to oblate-spheriodal	Oblate-spheroidal
Aperture		2% Syncolporate 98% Tricolporate	Tricolporate	Tricolporate

to Şavşat, Yanıklı Village, high plateau, alpine, 16.07.2000, 1700 m, *F. Karaer* 8681-8683! (OMUB); ibid., 11.08.2000, 2100 m, *F. Karaer* 8844-8857! (OMUB); ibid. 30.07.2002, 2200-2400 m, *F. Karaer* 9594-9603! (OMUB).

S. sosnowskyi, previously known as a species endemic to Georgia, is closely related to Turkish endemics *S. armenum* and *S. glabrifolium* (Mitchell, 1979a). These three species are all compared on the basis of their vegetative and reproductive organs in Table 1. *S. sosnowskyi* can be distinguished from them by using the following key, adapted from part of the keys found in Muirhead (1972) and Mitchell (1979a). Key for the species of *S. sosnowskyi*, *S. armenum* and *S. glabrifolium*:

1. Filaments white or pale yellow……..... ***S. glabrifolium***
1. Filaments pale lavender to violet.....**2**
2. Rosettes 2-8 cm diameter; scape leaves not ligulate; inflorescence 15-50 (-60) flowered; pedicel 1-1.5 cm; flowers 1.5-2.5 cm diameter..... ***S. armenum***
2. Rosettes (8-) 10-15 (-20) cm diameter; scape leaves widely ligulate; inflorescence many flowered (40-) 60-210; pedicel 2-3 cm; flowers 3-3.5 cm diameter....***S. sosnowskyi***

Distribution, Habitat and Ecology

Literature surveys indicated that *S. sosnowskyi* was previously known from Bakuriani (Mitchell, 1979a), Meskheti and Kartli region in Southwest Georgia (Akhalkatsi *et al.,* 2005). Recently, it has been found in Şavşat region (Artvin) in Northeast Turkey by us (Fig. 2).

S. sosnowskyi grows on limestone rocks in Georgia (Akhalkatsi et al., 2005) and on metamorphic rocks and moist sub-alpine steppe in Turkey. Its estimated altitudinal ranges between 1500 and 2400 m in Turkey and Georgia. Soil texture, taken from Şavşat region in Turkey, was sandy clay. Soil pH was 6.8. Soil N (%), P (%) and K (%) concentrations were found 0.47, 0.046 and 0.73, respectively. This species occurs on slightly acidic soils. The vegetation in these places is mainly herbaceous. It shares its habitat in Georgia with *Pinus kochiana* Klotzsch ex K.Koch, *Trisetum flavescens* (L.) P.Beauv., *Trisetum rigidum* Roem. & Schult., *Dianthus*

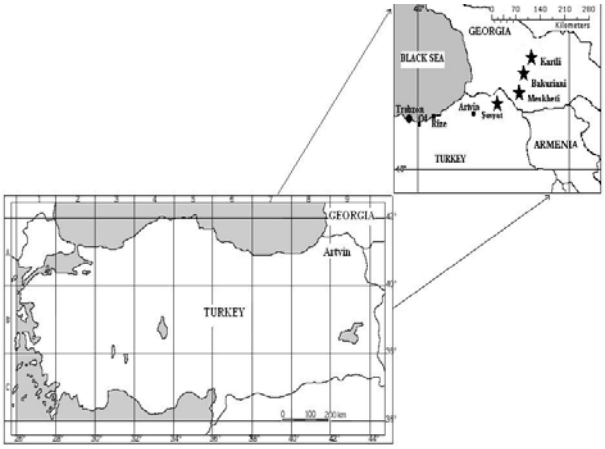

Fig 2. Distribution map of *S. sosnowskyi* in Turkey and Georgia. (★) indicates the distribution areas of the species

cretaceus Adam, *Asphodeline taurica* Kunth, *Daphne transcaucasica* Pobed., *Daphne glomerata* Lam., *Juniperus communis* L., *Poa pratensis* L., *Festuca ovina* L. and *F. woronowii* Hack. (Akhalkatsi et al., 2005), and in Turkey with *Festuca woronowii* Hack., *Sedum alpestre* Vill., *Hylotelephium telephium* (L.) H.Ohba subsp. *telephium*, *Anthemis marschalliana* Willd. subsp. *pectinata* (Boiss.) Grierson, *Centaurea appendicigera* K.Koch (from 2400 m), *Coronilla orientalis* Mill. subsp. *balansae* (Boiss.) Uhrova, *Potentilla oweriniana* Rupr. ex Boiss., *Valeriana saxicola* C.A.Mey. and *Valeriana alpestris* Steven . Both Georgian and Turkish sites belong to the Euro-Siberian phytogeographic region (Davis et al., 1965) and the locality has a relatively humid climate with cool winters and mild summers. Mean annual precipitation is approximately 654 mm at the Georgian-Turkish border in Meskheti (Georgia), where the majority of the precipitation falls between April and October, with May and June being considered the months with most rainfall (82 mm/month and 88 mm/month, accord-

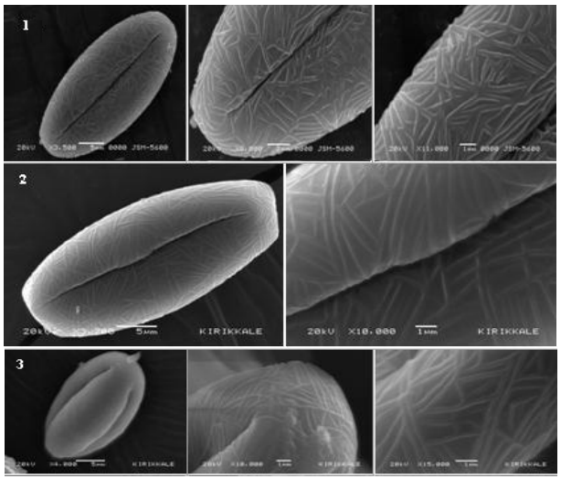

Fig 3. Pollen ornamentation (SEM).1- *S. armenum.* 2- *S. sosnowskyi.* and 3- *S. glabrifolium*

ingly). The driest months of the year in these parts are December (32mm/month) and January (30 mm/month) (Akhalkatsi et al., 2005). Climatological data were obtained from Şavşat Meteorological station (Turkey) between 1971 and 1996 (DMIGM, 2001). The mean annual temperature at 1100 m is 9.8 ^{0}C and precipitation is 737.9 mm. The values of the hottest and coldest months were 29.3 ^{0}C and -6.0 ^{0}C. The coldest month is February with a minimum temperature of -19.9 ^{0}C and the hottest month is August with a maximum temperature of 38.1 ^{0}C.

Conservation Status

S. sosnowskyi is known from only two close locations (Şavşat) in Northeast Turkey and known from several locations (Meskheti, Kartli and Bakuriani) in Southwest Georgia (Akhalkatsi et al., 2005). The distance between Şavşat (Turkey) and Kartli (Georgia), the furthest locations, is approximately 180 km. It is estimated area of occupancy is less than 2000 km^2 and extent of occurrence less than 5000 km^2. According to its distribution areas, it should be classified as Vulnarable (VU B2ab(i,ii,iv)) using IUCN criteria (IUCN, 2001) at international level. In Turkey, the two locations do not have any conservation priority although they contain the majority of the members of *S. sosnowskyi* and many isolated endemic taxa. According to our field works, it is estimated area of occupancy is less than 200 km^2, extent of occurrence less than 1000 km^2 and estimated the proportion of the global population occurring about 20% in Turkey. Moreover, the populations of *S. sosnowskyi* contain less than 500 individuals in Turkey. Therefore, it should be classified as Endangered (EN B2ab(i,ii,iv)) at national level.

Palynological properties

Comparing the pollen characteristics of these three species under SEM and light microscopy revealed general similarities among the species (Fig. 3). The details are provided in Table 2. The pollen of *S. sosnowskyi* is suboblate to oblate-spheriodal with *P* = 19.2 ± 8.5 µm and *E* = 19.7 ± 9 µm and P/E = 0.85-0.90. The aperture is tricolporate. The exine is 2 ± 0.4 µm thick and the intine is 0.4± 0.03 µm. The pollen of *S. armenum* is suboblate to oblate-spheriodal shape with *P* = 22.5 ± 4.8 µm and *E* = 23.2 ± 6 µm and P/E = 0.83-0.90. The aperture is 3.33% syncolporate and 96.67% tricolporate. The exine is 1.5 ± 0.3 µm thick and the intine is 0.3± 002 µm. The pollen of *S. glabrifolium* is oblate-spheroidal with *P* = 18.7 ± 3 µm and *E* = 21.8 ± 3 µm and P/E = 0.92. The aperture is tricolpate. The exine is 1.2 ± 0.1 µm thick and the intine is 0.4 µm. The striate-rugulate ektexine, as seen under the SEM, is characteristic of the genus, as previously stated by Parnell (1991). However, it is difficult to discern clearly the sculpturing on the surfaces of the pollen grains using LM (Oybak et al., 1997). According to average length of polar axis, the largest pollen grains are found in *S. armenum*, the smallest in *S. glabrifolium* although the size range overlaps. While the pollen shape in *S. glabrifolium* is oblate-spheroidal, pollen shape in *S. sosnowskyi* and *S. armenum* are suboblate to oblate-spheroidal. The shape of the pollen grains may be significant in distinguishing the species.

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