A new Scorzonera (Asteraceae) species from South Anatolia, Turkey, and its taxonomic position based on molecular data

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Abstract: Scorzonera zorkunensis Coskuncelebi & S.Makbul (Asteraceae) is described and illustrated from Turkey. It grows on serpentine in alpine steppe vegetation together with several endemic Scorzonera L. species in southern Anatolia at altitudes of 2075-2100 m. It is morphologically similar to S. pisidica Hub.-Mor., but it differs in its habit and stem and leaf pubescence. Additionally, the size and micromorphological characters of the achenes and some anatomical traits of the leaves, stems, and roots are helpful in distinguishing these closely related taxa. The phylogenetic analyses based on nrDNA ITS sequence data indicate that S. pisidica is a sister species of the new taxon.

Key words: Compositae, Scorzonera, systematics, taxonomy, Turkey

Güney Anadolu, Türkiye'den yeni bir Scorzonera (Asteraceae) türü ve moleküler verilere dayalı taksonomik durumu


Anahtar sözcükler: Compositae, Scorzonera, sistematik, taksonomi, Türkiye

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Introduction

*Scorzonera* L., a member of the Asteraceae, is known as a taxonomically difficult genus. It grows mainly in dry areas throughout the Mediterranean region and central Asia and includes about 175 species (Nazarova, 1997; Bremer, 1994; Lack, 2007). Since the genus *Scorzonera* s.str. was revised by Chamberlain (1975) for the *Flora of Turkey*, many new species have been recorded from Turkey. While this genus is represented by more than 50 species in Turkey (Parolly & Kilian, 2003; Doğan & Duran, 2010; Doğan et al., 2011), there are only 28 in *Flora Europaea* (Chater, 1976). This means that Turkey is an important centre of diversity of the genus. Some species of *Scorzonera* have been used as vegetables and in traditional medicine in Europe, Asia, and Anatolia (Ertuğ, 2000; Zidorn et al., 2003).

During field studies on Amanos Mountain, Osmaniye (south Anatolia), the authors collected an unusual specimen growing in alpine meadows in 2010. These specimens have been compared with many specimens in the herbaria of GAZI, HUB, KTUB, KATO, and RUB and with the main literature by Lipschitz (1964), Chamberlain (1975), Chater (1976), Rechinger (1977), Davis et al. (1988), Güner (2000), and Parolly and Kilian (2003), but the material did not key out. Observations and studies showed that our specimens apparently belong to a new species. In the present study, the new taxon is described and illustrated and compared to the related species. Additionally, the new and related species were compared using micromorphological characters of pollen, achenes, and leaf surfaces using scanning electron microscopy (SEM) while the anatomy of the leaf, stem, and root was subject to light microscopy (LM). The nrDNA ITS region was also sequenced in order to verify the phylogenetic status of the new species.

Material and methods

Plant material

Specimens of *Scorzonera zorkunensis* were collected on Amanos Mountain, south Anatolia, Turkey, in 2010 (Figure 1). Voucher specimens are stored in the herbaria of Karadeniz Technical University and Rize University. Morphological features were determined from the herbarium specimens and field observations. The sources of *S. pisidica* Hub.-Mor. (Figure 2) used for morphological comparison are given in Appendix 1.

![Figure 1. *Scorzonera zorkunensis*. a - habitat (Photo: S. Makbul), b - holotype (Makbul 242).](image-url)
SEM studies

SEM examination was carried out on the achenes, leaf surface, and pollen obtained from the herbarium specimens of *Scorzonera zorkunensis* (Makbul 242) from C6 Osmaniye and *S. pisidica* (Makbul 229) from C2 Muğla. Acetolysed pollen (Erdtman, 1952) and a minimum of 4 achenes and leaves from each species were coated with 12.5-15 nm of gold before observation with a Zeiss EVO LS10 scanning electron microscope (SEM). SEM micrographs were taken using an acceleration voltage of 10 kV. Seed surface terminology followed Barthlott (1981) and pollen terminology mainly followed that of Punt et al. (2007) and Türkmen et al. (2010).

Anatomical studies

The material for anatomical study (Makbul 229, Makbul 242) was fixed in FAA (formaldehyde:acetic acid:alcohol) for 24 h and then preserved in 70% alcohol in the field. Transverse sections of roots, stems, and leaves were taken using a freezing microtome. All sections were stained with haematoxylin for 30 min and mounted in glycerine-gelatine in order to obtain permanent slides (Vardar, 1987). Well-stained sections were photographed using an Olympus BX-51 microscope including BAB Bs200Pro analysis software.

Molecular studies

Total genomic DNA was extracted from silica-gel dried leaves following a modified CTAB extraction procedure of Doyle and Doyle (1987) according to Gültepe et al. (2010). PCR amplifications of the nrDNA ITS regions were performed using universal ITS4 and ITS5 primers designed by White et al. (1990). PCR product purification and nrDNA ITS were sequenced by Macrogen Inc. (Seoul, Korea). The sequencing process was carried out with BigDye terminator cycling protocols (Applied Biosystems Inc., Foster City, CA, USA). Sequencing of 5’ end of ITS region was carried out using the primer ITS4. Sequences with ambiguous sites were re-sequenced from the 3’ end with the primer of ITS5. For the phylogenetic reconstruction, 13 accessions belonging to *Scorzonera* were used and 3 additional accessions, *Tolpis proustii* Pit. (AJ633439), *Hieracium microtum* Boiss. (FJ613414), and *Lactuca canadensis* L. (GU818575), were obtained from GenBank to be used as outgroups in the present study. The locality information of the Turkish *Scorzonera* taxa used in phylogenetic analysis and GenBank numbers are given in Appendix 2.

The nucleotide sequences were automatically aligned using BioEdit v. 7.0 software (Hall, 1999).

Figure 2. *Scorzonera pisidica*. a - habitat (Photo: K. Coşkunçelebi), b - herbarium specimen (Makbul 229).
Phylogenetic analyses were conducted using the PAUP* 4.0 b10 (Swofford, 2002) with gaps treated as missing data. Maximum parsimony (MP) analyses were conducted using heuristic searches with 100 random additions replicates with no more than 100 trees saved per replicate. Support for clades was estimated using 1000 bootstrap replicates each with 100 random addition replicates, saving no more than 1500 trees per bootstrap replicate, TBR branch swapping, and Multrees option in effect.

**Taxonomic treatment**

*Scorzonera zorkunensis* Coskuncelebi & S.Makbul sp. nov. (Figure 1).

**Type:** Turkey. C6 Osmaniye: Amanos Dağı, Zorkun Yayasından 5 km sonra, Keldaz tepesi, Halep gören mevkii, 2075 m, 36°58’388’’N, 36°24’583’’E, 05.07.2010, S.Makbul 242 & K.Coskuncelebi (holotype:Karadeniz Technical Univ. Herb., isotypes: Rize Univ. Herb., ANK).

Diagnosis: *Scorzonera zorkunensis* is closely related to *Scorzonera pisidica*, which is restricted to south-west Anatolia. It mainly differs from *Scorzonera pisidica* because it has erect stem (not suberect or procumbens), leaves greyish, tomentose-lanate (not sericeous-lanate), indistinct main vein (not 3-5 distinct main vein), capitulum 1 per flowering stem (not 1-2 per flowering stem), pappus violet-brownish (not cream-yellowish), and achenes c. 7 mm (not 9-10 mm).

Description: Perennial herbs, subscapigerous to short caulescent, sometimes sub-caespitose, 9-16 cm tall. Rootstock thick, cylindrical (c. 6 mm diam.), remains of old leaves scarcely persistent. Flowering stem weakly decumbent to erect, solid, densely greyish, adpressed or subadpressed, tomentose-lanate throughout, c. 2 mm diameter below, bearing a single capitulum. Basal leaves entire, mostly lanceolate, rarely linear-lanceolate, at least some leaves slightly recurved, 5-10 × (-0.3)0.5-0.8(-1.3) cm, greyish, adpressed or subadpressed, tomentose-lanate, apex acute to acuminate, margins plane, gradually narrowed towards base, main vain indistinct; cauline leaves very similar to basal leaves, but smaller, decreasing towards capitula, upper bract-like. Capitula homogamous, ligulate, 15-20 × 8-10 mm at flowering; outer phyllaries 6-10 (-12) × 1-2 mm below, straight, lanceolate with a long (c. 4 mm) narrowly acuminate apex, outer surface crisped-pubescent, margins slightly violet, inner surface glabrous; inner phyllaries 15-19 × 3-4 mm, very similar to outer phyllaries both in shape and pubescence but with wider scarious margins. Ligues yellow and slightly longer than inner phyllaries, c. 12 mm long, 3 mm wide, tube 2 mm, 5-toothed, teeth c. 0.2 mm long; style branches filiform, c. 3 mm long, obtuse, shorter than ligule. Achenes c. 7 mm long, cylindrical, yellowish to greenish, typically ridged, and smooth, glabrous; pappus violet at least at apex (immature achenes), light brownish when mature, c. 10 mm long, setae plumose below and barbellate above. Flowering in July, fruiting in July-August.

In the key of the entire-leaved subscapeigerous *Scorzonera* species of the Turkish flora (Parolly & Kilian, 2003), *S. zorkunensis* may be inserted as follows, with some modification:

1. Leaves glabrous; plants of base-rich or sub-saline marshes.…………………………S. parviflora

Leaves lanate, sericeous or tomentose; plants of dry habitats.……………………………………2

2. Leaves broadly ovate to ovate-lanceolate, <2.5 times as long as wide.…………………S. boissieri

Leaves linear to narrowly lanceolate >3-4 times as long as wide.…………………………..3

3. Hairs on leaves and flowering shoots up to 1 mm long.………………………………………S. cinerea

Hairs on leaves and flowering shoots 3-5 mm long.………………………………………………4

4. Leaves 3-5 distinctly veined, sericeous-lanate; achenes 9-10 mm long…………………..S. pisidica

Leaves not distinctly veined, tomentose-lanate; achenes 6-7 mm long………………….. S. zorkunensis

**Habitat and ecology:** *Scorzonera zorkunensis* grows in alpine serpentine steppe and shares its habitat with several endemic species such as *Scorzonera yildirimlii* A.Duran et Hamzaoğlu, *Scorzonera tomentosa* L., *Alyssum oxycaarpum* Boiss. et Bal., and *Thuuya capitata* Boiss. et Bal. as well as non-endemic plants including *Silene spergulifolia* (Desf.) M.Bieb., *Genista albida* Wild., *Hypericum sp.*, *Salvia tomentosa* Mill., *Anthemis sp.*, *Centaurea sp.*, *Onosma sp.*, and *Allium sp.*
**Distribution and conservation status:** *Scorzonera zorkunensis* is endemic to Turkey. It is only known from type locality in south Anatolia (Figure 3) and should be regarded as Endangered (EN: B2a iii, iv) according to the IUCN guidelines (2001) based on the number of populations, habitat quality, and overgrazing by goats.

**Eponymy:** The names refer to the type and only known locality of the new species.

**Results and discussion**

*Scorzonera zorkunensis* belongs to a natural group of subscapigerous and subcaulescent perennial herbs. This group consists of more than 18 species in Turkey, including *S. pisidica* Hub-Mor. (Chamberlain, 1975; Parolly & Kilian, 2003). *Scorzonera zorkunensis* is morphologically similar to *S. pisidica*, treated under the Sect. *Nervosae* Lipsch. together with *S. karabelensis* Parolly & N.Kilian, a recently described record from Turkey (Parolly & Kilian, 2003). *Scorzonera zorkunensis* has the following characters in common with other members of Sect. *Nervosae* (Lipschitz, 1964): entire leaves, vertical cylindrical rootstock, and a more or less dense indumentum. However, it differs from other species in the section by its subcaulescent habit. According to Lipschitz (1964), the section consists of large perennial herbs with densely leafy stems although several subcaulescent taxa from Anatolia, such as *S. ulrichii* Parolly & N.Kilian, and *S. cinerea* Boiss., were treated under this section by Parolly and Killian (2003). Plant habit, rootstock type, and indumentum are critically important in separating the sections and their taxa in *Scorzonera*. In addition, achene indumentum is also very important in delimiting and determining the taxa belonging to *Scorzonera* (Chamberlain, 1975). Therefore, there is a need to modify the description of sect. *Nervosae* by adding characters related to the state of achenes (glabrous or pubescent) and stem type (subcaulescent to caulescent perennials).

*Scorzonera zorkunensis* prefers stony habitats in alpine steppe vegetation above 2000 m in southern Anatolia, but *S. pisidica* mainly grows in pine forests in south-western Anatolia, at altitudes between 1100 and 1300 m. *Scorzonera zorkunensis* flowers in July and fruits in July-August while *S. pisidica* flowers in June-July and fruits in July.

The leaves are dense pubescent, concealing the surface in *Scorzonera zorkunensis* but they are slightly pubescent and do not conceal the surface in *S. pisidica* (Figure 4).

Both species have yellow ligules and glabrous-smooth achenes (Figure 5). Both outer and inner phyllaries are nearly glabrous in *Scorzonera zorkunensis* while they are typically covered with long spreading sericeo-tomentose hairs in *S. pisidica* (Figure 6). Additional combinations of morphological traits that can be used to delimit these 2 taxa are given in Table 1.

We also recorded the micromorphological characters of the achene of the new and the relative species (Figure 7) by SEM. It seems that the general

![Figure 3. Distribution map of Scorzonera zorkunensis (□) and Scorzonera pisidica (●) in Turkey.](image-url)
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Structure of the achenes looks very similar, but the general appearance of the achene epidermal cells further distinguishes these 2 investigated taxa. The anticlinal cell walls are distinct and straight in *S. zorkunensis* but they are not obvious in *S. pisidica* (Figure 7). Additionally, the periclinal cell walls are densely striate in *S. pisidica* although there is no striation in those of *S. zorkunensis*.

Anatomical characters have a systematic value in many plants (Lersten & Curtis, 2001) and may also supply sufficient useful information in *Scorzonera* (Makbul et al., 2011). At first glance, *S. zorkunensis* and *S. pisidica* are anatomically quite similar (Figure 8). However, *S. zorkunensis* differs from *S. pisidica* in terms of cork size, the presence of secretory cells in the stem phloem, the number
of rows of palisade parenchyma, and the density of sclerenchymatic tissue in the leaf midrib (Table 2). The cork is also extremely tough and persistent in the root of *S. zorkunensis* while it is much thinner and less persistent in *S. pisidica* (Figure 8). Schulte and Nobel (1989) determined that the periderm is an important barrier for plants growing in dry habitats. *Scorzonera zorkunensis* grows in steppe vegetation of high altitude whereas *S. pisidica* grows in open forest area in the lower altitude.

Makbul et al. (2011) stressed that the distribution of the fibres in Turkish *Scorzonera* is taxonomically important, but there are no differences between *S. zorkunensis* and *S. pisidica* in terms of fibre distribution. There are, however, some valuable anatomical differences between *S. zorkunensis* and *S. pisidica*, as

![Figure 6. LM micrographs of phyllaries. *Scorzonera zorkunensis*: a - outer phyllaries, b - inner phyllaries; *Scorzonera pisidica*: c - outer phyllaries, d - inner phyllaries.](image)

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>S. zorkunensis</em></th>
<th><em>S. pisidica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem</td>
<td>rarely caespitose</td>
<td>never caespitose</td>
</tr>
<tr>
<td>Leaf</td>
<td>greyish tomentose-lanate</td>
<td>densely sericeous-lanate</td>
</tr>
<tr>
<td>Leaf surface</td>
<td>not distinctly veined</td>
<td>distinctly 3-5-veined</td>
</tr>
<tr>
<td>Capitula</td>
<td>1 per stem</td>
<td>1-2 per stem</td>
</tr>
<tr>
<td>Pappus</td>
<td>violet (immature) and brownish (mature)</td>
<td>cream to yellowish</td>
</tr>
<tr>
<td>Achene length</td>
<td>6-7 mm</td>
<td>9-10 mm</td>
</tr>
<tr>
<td>Flowering</td>
<td>July</td>
<td>June-July</td>
</tr>
<tr>
<td>Fruiting</td>
<td>July-August</td>
<td>July</td>
</tr>
</tbody>
</table>
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Figure 7. SEM micrographs of achenes. *Scorzonera zorkunensis*: a - overview, b - surface detail; *Scorzonera pisidica*: c - overview, d - surface detail.

Table 2. Palynological and anatomical comparison of *Scorzonera zorkunensis* and *Scorzonera pisidica*.

<table>
<thead>
<tr>
<th>Characters</th>
<th>S. zorkunensis</th>
<th>S. pisidica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polar axis (μm)</td>
<td>24.25 ± 1.42</td>
<td>24.12 ± 1.36</td>
</tr>
<tr>
<td>Equatorial axis (μm)</td>
<td>25.40 ± 1.63</td>
<td>26.54 ± 1.39</td>
</tr>
<tr>
<td>Polar axis (P)/ Equatorial axis (E)</td>
<td>0.95 ± 0.04</td>
<td>0.92 ± 0.04</td>
</tr>
<tr>
<td>Pollen grain</td>
<td>echinolophate</td>
<td>echinolophate</td>
</tr>
<tr>
<td>Pollen shape</td>
<td>oblate-spheroidal</td>
<td>oblate-spheroidal</td>
</tr>
<tr>
<td>Aperture type</td>
<td>tricolporate</td>
<td>tricolporate</td>
</tr>
<tr>
<td>Lacunae ornamentation</td>
<td>perforate-microreticulate</td>
<td>perforate-microreticulate</td>
</tr>
<tr>
<td>Lophae ornamentation</td>
<td>echinate-perforate</td>
<td>echinate-perforate</td>
</tr>
<tr>
<td>Root pith</td>
<td>absent</td>
<td>Present</td>
</tr>
<tr>
<td>Root cortex thickness: Min.-Max. (M ± S) (μm)</td>
<td>90-105 (102 ± 7)</td>
<td>410-440 (417 ± 7.8)</td>
</tr>
<tr>
<td>Old periderma remains</td>
<td>non-persistent</td>
<td>persistent</td>
</tr>
<tr>
<td>Cortex bundle in the stem</td>
<td>present</td>
<td>present</td>
</tr>
<tr>
<td>Phloem secretory cells</td>
<td>present</td>
<td>absent</td>
</tr>
<tr>
<td>Xylem sclerenchyma in stem</td>
<td>present</td>
<td>absent</td>
</tr>
<tr>
<td>Collenchyma in stem</td>
<td>dense</td>
<td>sparse</td>
</tr>
<tr>
<td>Sclerenchyma in leaf bundles</td>
<td>sparse</td>
<td>dense</td>
</tr>
<tr>
<td>Mesophyll</td>
<td>heterogeneous symmetrical</td>
<td>heterogeneous symmetrical</td>
</tr>
<tr>
<td>Width of mesophyll: Min.-Max. (M ± S) (μm)</td>
<td>425-440 (432 ± 9.6)</td>
<td>145-155 (146 ± 5.8)</td>
</tr>
<tr>
<td>Row number of upper/lower palisade</td>
<td>3/3</td>
<td>2/2</td>
</tr>
</tbody>
</table>
seen in Table 2. These results are in accordance with the previous findings of Makbul et al. (2011).

The leaves of both of the investigated taxa have heterogeneous symmetrical mesophyll with anomocytic stomata, but there are distinct differences in their stomata indices. The stomata index is 10.7 ± 0.74 and 9.7 ± 0.82 for the upper and lower surface in *S. zorkunensis* and 9.1 ± 0.31 and 11.4 ± 1.3 in *S. pisidica*. The present results are similar to the findings of Makbul et al. (2011), but it is important to note that stomata index is easily influenced by environmental factors, as indicated by Özörgücü et al. (1991).
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Figure 9. SEM micrographs of pollens. Scorzonera zorkunensis: a - overview, b - surface detail; Scorzonera pisidica: c - overview, d - surface detail.

Figure 10. Phylogenetic tree based on the internal transcribed spacer. Numbers above the branch are bootstrap values, in percentages, based on 1000 replicates.
Pollen grains of $S.$ zorkunensis and $S.$ pisidica are echinolophate, isopolar, radially symmetric, tricolporate, oblate-sphericaloid (Figure 9). These types of grain are generally observed in Turkish Scorzonera, as noted by Türkmen et al. (2010). Blackmore (1982) reported that the number and arrangement of lacunae in the Scorzonerae is very diverse and provides a number of useful taxonomic characters. According to Blackmore (1982), whose groups were based on lacunae properties, both of the examined taxa fall into the $S.$ laciniata-type, characterised by 6 abporal lacunae, 6 equatorial lacunae, and 6 interapertural lacunae. All of the palynological observations (seen in Table 2) showed that there are no distinct differences between $S.$ zorkunensis and the morphologically related taxa.

In the phylogenetic analysis, 13 subscapigerous taxa were evaluated along with the new species. The length of the nrDNA ITS sequences varied from 598 to 642 bp in all of the examined taxa. In total, the aligned sequences with outgroups provided 391 (58%) parsimoniously informative and 511 (76%) variable characters. The MP analysis resulted in 6 most parsimonious trees of 804 lengths with a consistency index (CI) of 0.751 and a retention index (RI) of 0.766. In the MP tree (Figure 10), Scorzonera zorkunensis occupies a position with moderate supported clade (with 54% BP) that is composed of $S.$ semicana, $S.$ boissieri, $S.$ sublanata, and $S.$ pisidica. Although $S.$ zorkunensis is linked to $S.$ pisidica with a 100% bootstrap value, several base substitutions and indels occurred between $S.$ zorkunensis and $S.$ pisidica. The position of $S.$ zorkunensis in the reconstructed molecular phylogeny is congruent with our interpretation of its morphological characters and confirms its close affinity to $S.$ pisidica.

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Appendix 1.

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