

TAXONOMICAL STUDIES ON ENDEMIC *SCORZONERA PYGMAEA* VAR. *PYGMAEA* AND VAR. *NUTANS* STAT. NOV. (ASTERACEAE) FROM TURKEY

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Abstract

The taxonomic status of *Scorzonera pygmaea* var. *pygmaea* and var. *nutans* belonging to the tribe. cichoreae (Asteraceae). *S. pygmaea* samples were collected from Arayit mountain. We suggest that these two subspecies should be classified as varieties because of their morphological and anatomical characteristics, ecological and geographical similarities. Moreover being together in the same localities of these under species taxa supports our opinion, i.e. *S. pygmaea* Sibth. & Sm. var. *pygmaea* stat. nov. and *S. pygmaea* Sibth. & Sm. var. *nutans* (Czeczott) O. Koyuncu & Yaylaci, stat. nov.

Introduction

Genus *Scorzonera* L. (Tribe cichoreae -Asteraceae) has approximately 180 species in the world (Bremer, 1994; Makbul *et al.*, 2011a). Although it originates from ancient Mediterranean, it distributes from arid regions of Eurasia to Northern Africa. This genus is fairly in intricate (Bremer, 1994; Nazarova, 1997; Lack, 2007; Makbul *et al.*, 2011a,b). The most significant taxonomical problem of this genus is having too much morphological variation (Bremer, 1994; Makbul *et al.*, 2011b). Taxonomic problems of this genus are not investigated sufficiently and there are still many controversial issues (Nazarova, 1997; Türkmen *et al.*, 2010).

A complete classification of genus *Scorzonera* was made by De Candolle (1805). Then, the genus was revised again by Boissier (1875) and Lipschitz (1935, 1939). Among these revisions, the work of Lipschitz (1939) was the most comprehensive. *Scorzonera* is represented by 28 species in Europe (Chater, 1976). Chamberlain (1975) reported 39 taxa from Turkey. After Chamberlain a few new taxa were added (Davis *et al.*, 1988; Güner *et al.*, 2000; Duran, 2002a,b; Kilian & Parolly, 2002; Parolly & Kilian, 2003; Duran & Hamzaoglu, 2004; Duran & Hamzaoglu, 2004; Dinç & Bağcı, 2009; Özhatay *et al.*, 2009; Doğan & Duran, 2010; Hamzaoglu *et al.*, 2010; Doğan *et al.*, 2011; Özhatay *et al.*, 2011; Coşkunçelebi *et al.*, 2012; Makbul *et al.*, 2012). Currently, *Scorzonera* is represented by 47 species, 7 subspecies and 4 varieties in Turkey, 28 of these taxa are endemic. Anatolia is a diversity centre for the genus *Scorzonera* with more than 50 taxa.

Some studies have been performed on anatomical, morphological, palynological, genetical, pharmacological, ethnobotanical, distribution and conservational status in genus *Scorzonera* (Metcalf & Chalk, 1950; D'amato, 2000; Meo & Khan, 2004; Makbul, 2006; Karaer & Celep, 2007; Qureshi *et al.*, 2008a,b; Dinç *et al.*, 2008; Dinç & Bağcı, 2009; Makbul *et al.*, 2010, 2011a,b, 2012; Türkmen *et al.*, 2010; Martin *et al.*, 2012; Coşkunçelebi *et al.*, 2012; Yildirim & Şenol, 2012).

Anatomical characters are quite important in systematics of *Scorzonera*. Although classification of *Scorzonera* seems easy, showing too much variation of the genus makes its identification harder (Makbul *et al.*, 2011a, b).

In addition, Chamberlain (1975) while revising the genus *Scorzonera* for Flora of Turkey observed some taxonomic problems in *S. pygmaea*. "The Kastamonu and Zonguldak plants of *S. pygmaea* consistently differ in being generally larger than those from Bursa and Isparta but may only represent peripheral variant populations. Czeczott described the capitula of *S. nutans* as nodding. This is surely an artifact; the specimens from Ilgaz Dağı do not show this feature" (Chamberlain, 1975).

The first specimens belonging to *S. pygmaea* were collected by Sithorbium from A2(A) Bursa, Turkey in the Olympus (Uludağ) and described as a new species by Sithorbium and Sm. in 1806 (Chamberlain, 1975). The first specimens belonging to *S. nutans* were collected by Czeczott from A4 Çankiri, Turkey in the Ilgaz Mountain in 1925 and was described as a new species in 1939. Later this species was reduced as *S. pygmaea* subsp. *nutans* by Chamberlain (1975).

In order to ascertain the taxonomic status of both the endemic taxa anatomic, morphologic, palynologic, distribution and conservation status was investigated.

Material and Methods

Specimen samples: We collected some *Scorzonera pygmaea* specimens in the Arayit Mountain (Eskişehir), Uludağ Mountain (Bursa) and Ilgaz Mountain (Çankiri) in 2009-2011.

Morphological studies: Fresh plant samples were identified with the help of relevant literature on Lipschitz (1935, 1939), Czeczott (1939), Chamberlain (1975) and Chater (1976). Morphological studies were conducted with stereo microscope Olympus CH 50. In addition, photos of various parts were taken by Nikon SZ120. The morphological characters were compared with that of Chamberlain (1975).

Anatomical studies: The samples were kept in 70% alcohol until the sections were prepared. We obtained some cross-sections of roots, stems and leaves manually. We also obtained lower and upper cross-sections from the fresh leaves. The sections were photographed by the Kameram™ digital camera and an Nikon 80i type microscope in our Department.

Palynological studies: For palynological studies pollen material was obtained from fresh flower specimens. The pollen morphology was investigated through Light Microscope (LM) and Scanning Electron Microscope (SEM). Faegri and Iversen's terminology were used for the naming of the exine layers (Faegri & Iversen 1975). Under light microscope the pollen acquired from the samples was investigated by Wodehouse (1935) and Erdtman (1952) methods. Pollen identifications were obtained by Prior binocular microscope. The materials were prepared according to Wodehouse's and Erdtman's methods; the exine and intine thickness pertaining to taxa are measured a minimum 20 and a maximum 50 times. From these obtained measurements, a natural mathematical mean is calculated. Microphotographs were taken at the Eskişehir Osmangazi University Science and Art Faculty, Department of Biology by Kameram™ digital camera and a Nikon 80i microscope.

For scanning electron microscopy (SEM) investigations, unacetolyzed pollen grains were directly placed onto stubs, sputter-coated with gold, and examined

with a Jeol 5600 LV scanning electron microscope (Walker, 1974a,b). Terminologies for pollen morphology proposed by Wodehouse (1935), Erdtman (1952, 1954, 1966, 1969), Pokrovskaja (1958), Kuprianova (1965), Skvarla (1966), Kapp (1968), Aytuğ *et al.*, (1971), Charpin *et al.*, (1974), Walker (1974a,b) and Faegri and Iversen (1975), Moore *et al.*, (1991) and Pehlivan (1995) were employed.

Result and Discussion

Morphological characteristics: Some morphological characters of *S. pygmaea* were examined and compared with the description of Chamberlain, (1975). Some specific characters in the type description were corrected (flowering stem 1,5-11 cm, leaves 1,2-7 x 0,2-03 cm, capitula straight or nodding and pappus lower part sparsely plumose) and some characters which were not mentioned in type description were mentioned for the first time with this study (achene length, fruiting) (Figs. 1-3, Table 1).

Table 1. Comparative morphology of *Scorzonera pygmaea* Sibth. & Sm. var. *pygmaea* and var. *nutans* (Czeczott) O. Koyuncu & Yaylaci

Characters	<i>S. pygmaea</i> var. <i>pygmaea</i>	<i>S. pygmaea</i> var. <i>nutans</i>
Habitus	cushion-forming, with numerous scapiform stems	cushion-forming, with numerous scapiform stems
Stem	all leaves basal, 1,5-5 (7)	all leaves basal, (3-) 5-11
Leaf	Leaves entire, 1,2-1,9 x 0, 2-0,3 cm Linear to linear lanceolat, sparsely lanat, ± amplexicaule	Leaves entire, 1,8-7 x 0, 2-0,3 cm Linear to linear lanceolat, sparsely lanat, ± amplexicaule
Capitula	1 per stem, straight	1 per stem, Nodding
Outer phyllary	pubescent, 3-5 mm long, ovat to lanceolat	pubescent, 3-5 mm long, ovat to lanceolat
Inner phyllary	pubescent, acute	pubescent, acute
Lobes of ligule	0,5-1,5 long	0,5-1,5 long
Branches of style	c. 5 mm long	c. 5 mm long
Pappus	pink tinged	pink tinged
Hairs of pappus	plumose below, barbellate above	sparsely plumose below, barbellate above
Achene length	6-7mm	7-8mm
Flowering	6-7(-8)	6-7(-8)
Fruiting	7-8(-9)	7-8(-9)



Fig. 1. General appearance. (a) *Scorzonera pygmaea* Sibth. & Sm. var. *pygmaea* (habit, in Uludağ-Bursa), (b) *Scorzonera pygmaea* Sibth. & Sm. var. *nutans* (Czeczott) O. Koyuncu & Yaylaci (habit, in Ilgaz Mountain-Çankiri).



Fig. 2. LM micrographs. *Scorzonera pygmaea* Sibth. & Sm. var. *pygmaea*: a- capitulum, b- achene; *Scorzonera pygmaea* Sibth. & Sm. var. *nutans* (Czeczott) O. Koyuncu & Yaylaci c- capitulum, d- achene.

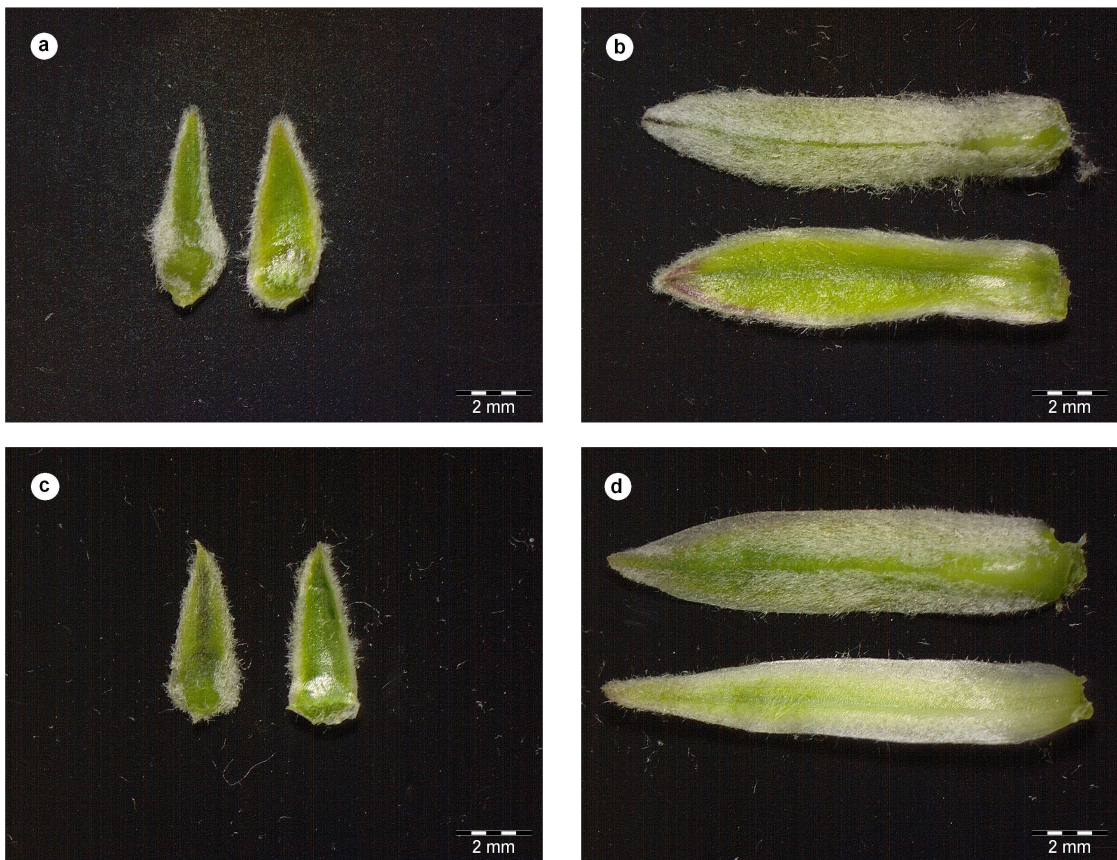


Fig. 3. LM micrographs of phyllaries. *Scorzonera pygmaea* Sibth. & Sm. var. *pygmaea*: a- outer phyllaries, b- inner phyllaries; *Scorzonera pygmaea* Sibth. & Sm. var. *nutans* (Czeczott) O. Koyuncu & Yaylaci: c- outer phyllaries, d- inner phyllaries.

Taxonomic treatment

A detailed morphological examinations of two taxa, revealed that both were similar in terms of many characters (habit, outer phyllary, inner phyllary, lobes of ligule, branches of style, flowering and fruiting). On the other hand, some differences were found in terms of some characters: subsp. *pygmaea*; stem; 1,5-5 (7) cm, leaf; 1,2-1,9 x 0,2-0,3 cm, capitula; straight, hairs of pappus; plumose below, barbellate above, achene length; 6-7mm, subsp. *nutans*; stem; (3-) 5-11 cm, Leaf; 1,8-7 x 0,2-0,3 cm, capitula; nodding, hairs of pappus; sparsely plumose below, barbellate above, achene length; 7-8mm (Figs. 1-3; Table 1).

In view of morphological similarity of both the taxa and sympatric distribution (Fig. 7) *Scorzonera pygmaea* Sibth. & Sm. subsp. *nutans*.

Scorzonera pygmaea Sibth.&Sm. var. *pygmaea*

Synonyms: *Scorzonera pygmaea* Sibth. & Sm. Prodr., Fl. Graec. 2:122 (1806)
= *Scorzonera pygmaea* Sibth. & Sm. subsp. *pygmaea*, Ic: Sibth. & Sm., Fl. Graeca 8: t. 783 (1833).

Type: Turkey A2(A) Bursa: Olympos Bithynio (Ulu Da.), cacumine, Sibthop.

Flowering stems 1,5-5 (7) cm, straight; leaves 12-19 mm; pappus lower part plumose var. *pygmaea*
Flowering stems (3-) 5-11 cm, nodding; leaves (18-) 30-70 mm; pappus lower part sparsely plumose var. *nutans*

Anatomical characteristics

***Scorzonera pygmaea* var. *Pygmaea*: leaf:** It is ecvifacial type and mesophyll consists of 2-3 layers of palisade inside of upper epidermis, 2 layers of palisade beneath the lower epidermis and 2-3 layers of sponge (Fig. 4a).

Stem: The epidermal tissue consists of a single row of rectangular or orbicular cells. Some of the epidermal cells are covered with dense simple or branched hairs, the others are generally smooth. The collenchyma is generally located very close to the epidermis, with two or three to four rows. The cortex consists of usually parenchymatic oval cells, with 7 or 8 to 10 rows. The vascular bundles, interconnected with an intravascular sclerenchyma, formed one or two to three continuous rings in the stem. Secretory cells are located very close to the phloem tissue. Scleranchymatous cells are present in the phloem and xylem. There is also large pith in the stem centre (Fig. 4c).

Scorzonera pygmaea var. *nutans*

Leaf: It is ecvifacial type and mesophyll consists of 2-3 layers of palisade inside of upper epidermis, 2 layers of palisade beneath the lower epidermis and 2-3 layers of sponge (Fig. 4b).

Stem: The epidermal tissue consists of a single row of rectangular or orbicular cells. Some of the epidermal

Scorzonera pygmaea Sibth. & Sm. var. *nutans* (Czeczott) O. Koyuncu & Yaylacı stat. nov.

Basionym: *Scorzonera nutans* Czeczott, Feddes Report Beih. 107:202, t. 36 (1938).

Synonym: *Scorzonera pygmaea* Sibth. & Sm. subsp. *Nutans* (Czeczott) Chamberlain, Notes R.B.G. Edinb. 33: 433 (1975).

Syntypes: Turkey A4 Çankırı: Cacumine montis Kush Kayasy (jugum Ilgaz Da.), c. 2400 m, 26 vii 1925, Czeczott 439, 439 bis.

***Scorzonera pygmaea*:** Dwarf cushion-forming, ± subscaibigerous perennial, 1,5-11 cm; rootstock thick, cylindrical. Leaves entire, 1,2-7 x 0,2-0,3 cm linear to linear lanceolat, sparsely lanat, especially below, ± amplexicaul. Capitula 1 per stem, 13-17 mm long. Phyllaries pubescent, inner 9-13 mm, linear lanceolat; outer 3-5 mm long ovate lanceolat. Flowers pale yellow. Achenes 5-7,5 mm, cylindrical, ridged, otherwise smooth, glabrous; pappus pink tinged, hairs barbellate above, plumose below. Fl. 6-8, Fr. 7-8(-9).

cells are covered with dense simple or branched hairs, the others are generally smooth. The collenchyma is generally located very close to the epidermis, with two or three to four rows. The cortex consists of usually parenchymatic oval cells, with seven or eight to ten rows. The vascular bundles, interconnected with an intravascular sclerenchyma, formed one or two to three continuous rings in the stem. Secretory cells are located very close to the phloem tissue. Scleranchymatous cells are present in the phloem and xylem. There is also large pith in the stem centre (Fig. 4d).

Palynological characteristics

***Scorzonera pygmaea* var. *pygmaea*:** Pollen grains are radially symmetrical, isopolar, oblate-spheroidal. The size (polar axis x equatorial diameter, excluding spines) ranges from 39.28 (42-38) × 42.57 (48-39) μm in Wodhouse and 40.28 (42-38) × 41.57 (45-39) μm in Erdtman. It has echinolophate, trizonocolporate pollen with 15 lacune (6 abporal, 3 poral and 6 paraporal). The spines are convex-conic with a broadened base and a tapered apical portion. The length of the spine is 3.9 (3.2-4.3) μm and its width at the base is 3.0 (2.8-3.3) μm. The base of the spines has irregularly 1-2 seriate perforations with small holes (0.3 μm) and μm² 3-4 perforates. Exine 4.6 (4.3-5.2) μm. Sexine is much thicker than nexine. Intine 1.25 (1.0-2.0) μm (Figs. 5a,b; 6a,b; Tables 2; 3).

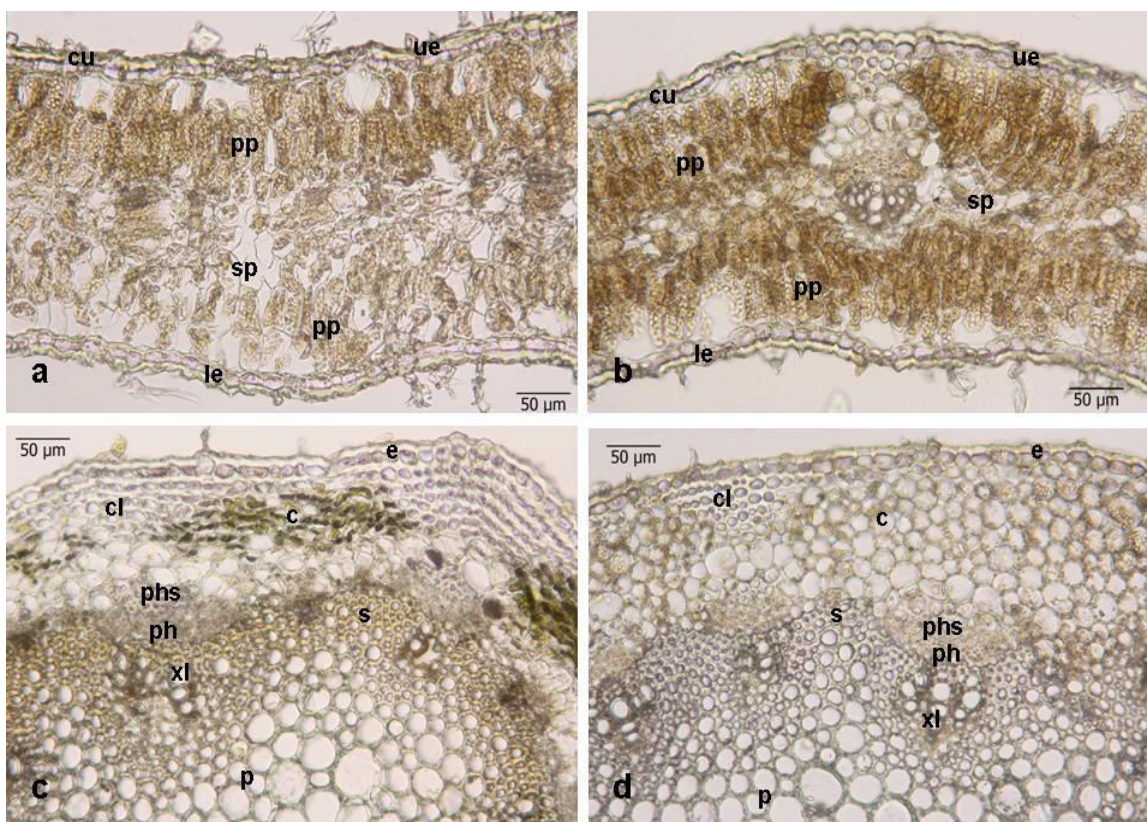


Fig. 4. Cross sections of leaf: (a) *Scorzonera pygmaea* var. *pygmaea* (b) *Scorzonera pygmaea* var. *nutans*. Cross sections of stem: (c) *Scorzonera pygmaea* var. *pygmaea* (d) *Scorzonera pygmaea* var. *nutans*. Abbreviations: (c) cortex, (cu) cuticula, (cl) collenchyma, (e) epidermis, (le) lower epidermis, (p) parenchyma, (ph) phloem, (phs) phloem sclerenchyma, (pp) palisade parenchyma, (s) sclerenchyma, (sp) sponge parenchyma, (ue) upper epidermis, (xl) xylem.

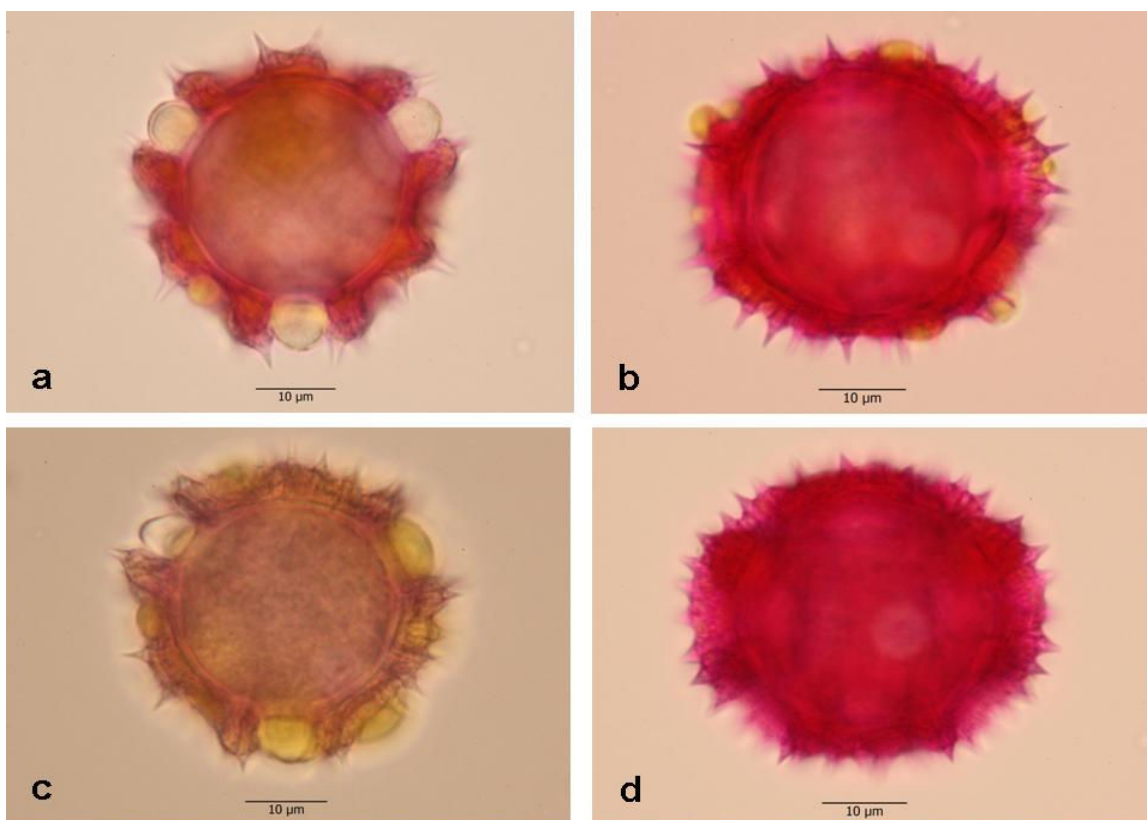


Fig. 5. Light micrographs of pollen grains. *Scorzonera pygmaea* var. *pygmaea*: (a) polar view, (b) equatorial view, *Scorzonera pygmaea* var. *nutans*: (c) polar view, (d) equatorial view.

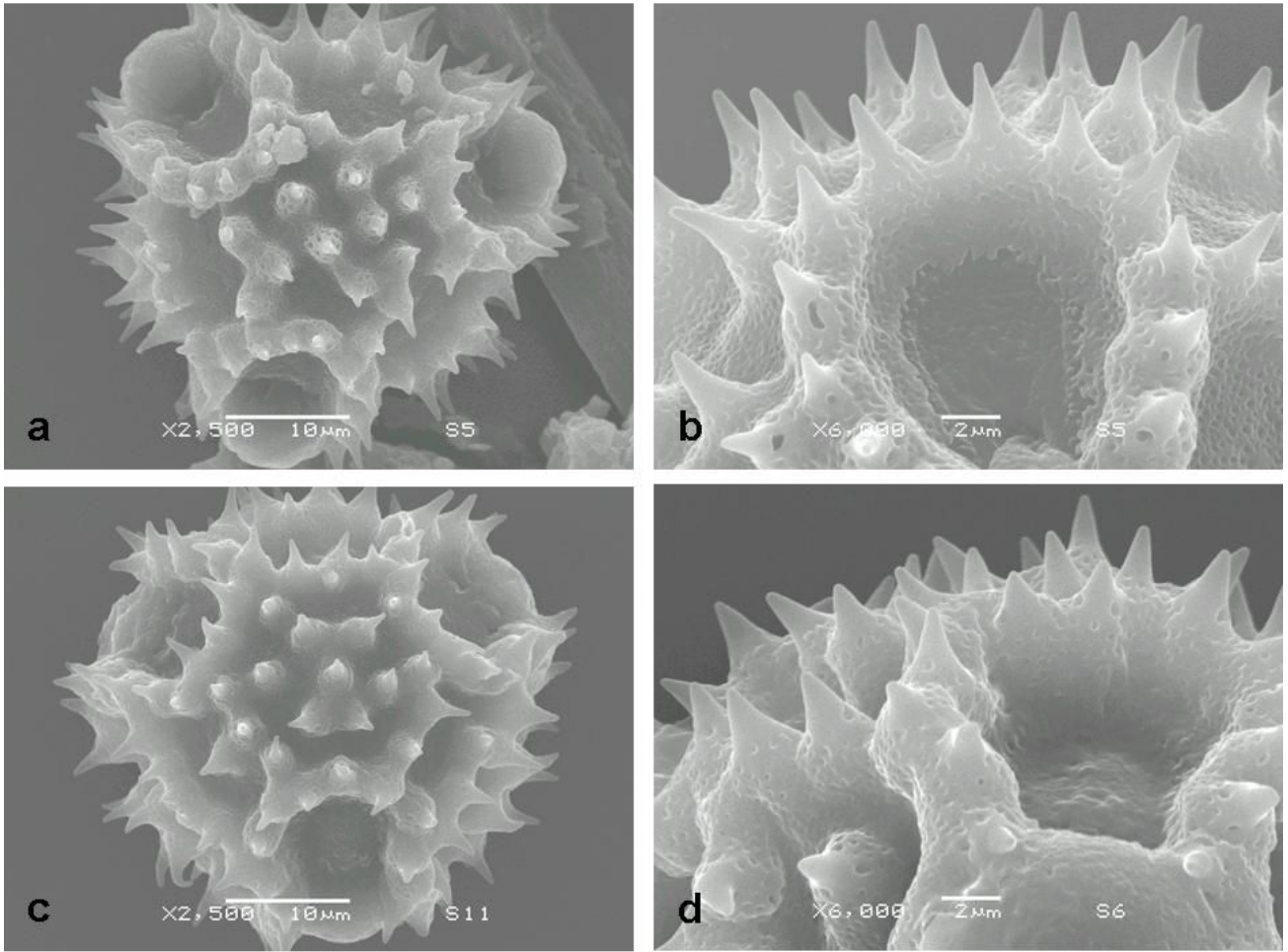


Fig. 6. Scanning electron micrographs (SEM) of pollen grains. *Scorzonera pygmaea* var. *pygmaea*: (a) overview, (b) ornamentation. *Scorzonera pygmaea* var. *nutans*: (c) overview, (d) ornamentation.

***Scorzonera pygmaea* var. *nutans*:** Pollen grains radially symmetrical, isopolar, oblate-spheroidal. The size (polar axis \times equatorial diameter, excluding spines) ranges from 40.30 (45–38) \times 43 (46–39) μm in Wodhouse and 39.25 (43–33) \times 44.70 (48–41) μm in Erdtman. It has echinolophate, trizonocolporate pollen with 15 lacune (6 abporal, 3 poral and 6 paraporal). The spines are convex-conic with a broadened base and a tapered apical portion. The length of the spine is 2.9 (2.5–3.4) μm and its width at the base is 3.0 (2.8–3.3) μm . The base of the spines has irregularly 1–2 seriate perforations with small holes (0.3 μm) and μm^2 3–4 perforates. Exine 3.6 (3.1–4.2) μm . Sexine is much thicker than nexine. (Figs. 5c,d; 6c,d; Table 2; 3).

Distribution:

Scorzonera pygmaea var. *pygmaea*

It is endemic to N.W. & S.W. Anatolia. A2(A) Bursa: Bithynian Olypus (Ulu Da.), 19 viii 1850, Clementi! İbid., 2300–2500 m; Davis 14846! İbid., 2400 m; Sorger 68-54-23!; Bursa Uludağ P. Quezel, 1968; Bursa: Uludağ, zirve yolu, N 40° 05' 36"-E 29° 10' 34", 2245m, 01 vii 2009, OUFE; A4 Ankara: Çubuk, Karagöl, ca. 1700 m a.s.l., 5.vi.1974, S. Erik 531; C3 Isparta: Dedegöl Da., 1600–2400m, Sorger 70-46-24!; Çankırı: Ilgaz Dağı, TRT vericisi civarı, N 41° 07' 46"-E 33° 40' 24", 2010m, 12 vii 2009, OUFE 16465; B3 Eskişehir: Arayit Dağı

(Sivrihisar), zirve civarı, N 39° 18' 07"-E 31° 44' 57", 1817m, 09 vii 2009, OUFE 16466; C5 Konya: Ereğli, Aydos Dağı, Kayasaray, Sayıntaş mevkii, 2500 m a.s.l., 15.vii.1977, S. Erik 2602, (Chamberlain, 1975; Duran & Hamzaoglu, 2004) (Fig. 3).

Scorzonera pygmaea var. *nutans*

It is endemic to N. Anatolia. A2 Bursa: Uludağ, 2400–2500 m a.s.l., 28.vii.2001, Y. Menemen 512 & E. Hamzaoglu; Bursa: Uludağ, zirve yolu, N 40° 05' 52"-E 29° 11' 18", 2280m, 01 vii 2009, OUFE; A4 Zonguldak: Keltepe above Karabük, 1950 m, Davis 38914!; Kastamonu: Ilgaz Da., 2200 m, D. 21577!; Ilgaz Dağı, Küçükhacet tepesi, ca. 2400 m a.s.l., 29. vii. 1982, Y. Akman 12178, E. Yurdakulol & M. Demirörs; Karabük: Keltepe, ca. 1950 m a.s.l., 12. vii. 1984, M. Demirörs 1263; Çankırı: Ilgaz Dağı, TRT vericisi civarı, 2000 m a.s.l., 21. vii. 2001, A. Duran 5818 & Y. Menemen; Çankırı: Ilgaz Dağı, TRT vericisi civarı, N 41° 03' 07"-E 33° 42' 58", 2070m, 12 vii 2009, OUFE 16467; A5 Kastamonu: Ilgaz Da., 2000 m. Darrah 28!; B3 Eskişehir: Arayit Dağı (Sivrihisar), zirve civarı, N 39° 18' 11"-E 31° 45' 02", 1830m, 09 vii 2009, OUFE 16468; (Chamberlain, 1975; Duran & Hamzaoglu, 2004) (Fig. 3).

We observed together in at least three different localities (Fig. 7).

Table 2. Palynological comparison of *Scorzonera pygmaea* var. *pygmaea* and var. *nutans*.

Characters	<i>S. pygmaea</i> var. <i>pygmaea</i>	<i>S. pygmaea</i> var. <i>nutans</i>
Shape in polar view	Semiangular	Semiangular
Spine shape	Convex-conic	Convex-conic
Pollen shape	Oblate-spheroidal	Oblate-spheroidal
Lachinae number	15 (6 3 6)	15 (6 3 6)
Aperture type	Nonlacunate	Nonlacunate
Pollen Class	Trizonocolporate	Trizonocolporate
Sculpture type	Echinolophate	Echinolophate
Ornamentation	Spiniate-microperforate-rugulate	Spiniate-microperforate-rugulate

Table 3. Measurements of pollen grains of *Scorzonera pygmaea* var. *pygmaea* and var. *nutans*.

	P			E			P/E	L			t			Plg		
	M	S	Var.	M	S	Var.		M	S	Var.	M	S	Var.	M	S	Var.
var. <i>pygmaea</i> (W)	39,28	1,6	42-38	42,57	2,76	48-39	0,92	29,57	1,72	32-27	11,55	0,88	13--10	11,43	0,97	13--10
var. <i>pygmaea</i> (E)	40,28	1,38	42-38	41,57	1,99	45-39	0,97	26,21	1,58	29-24	6,87	0,83	8--6	8,37	2,56	11--5
var. <i>nutans</i> (W)	40,3	2	45-38	43	1,76	46-39	0,94	30,4	1,84	34-28	11,42	1,62	15--9	12,5	3,37	18--9
var. <i>nutans</i> (E)	39,25	2,7	43-33	44,7	2,35	48-41	0,88	28,45	2,62	34-25	12,23	2,51	17--10	9,5	1,83	2--6

	Plt			Clg			Clt			Exine			Intine		
	M	S	Var.	M	S	Var.	M	S	Var.	M	S	Var.	M	S	Var.
var. <i>pygmaea</i> (W)	6,71	0,75	8--6	21,85	1,68	24-19	7,86	1,07	9--6	8,5	1,07	10--7	1,25	0,38	2--1
var. <i>pygmaea</i> (E)	7,31	1,46	10-5,5	25,28	2,43	28-22	6,86	0,94	8,5-6	9,57	1,4	11--7			
var. <i>nutans</i> (W)	5,4	0,7	6--4	25,3	3,53	30-19	6,3	1,42	8--4	6,5	1,08	8--5	1	0,23	1,5-0,5
var. <i>nutans</i> (E)	7,62	0,97	10-5,5	28,1	3,48	33-22	9,18	1,6	13--8	7	1,12	9-5,5			

Abbreviations: N: Non acetolysed (LM), A: Acetolysed (LM), P: Polar axis, E: Equatorial axis, L: Equatorial diameter, clg: Length of the colpus, clt: Width of the colpus, plg: Length of the porus, plt: Width of the porus, t: Apocolpium, M: Mean, S: Standard deviations, Var: Variation.

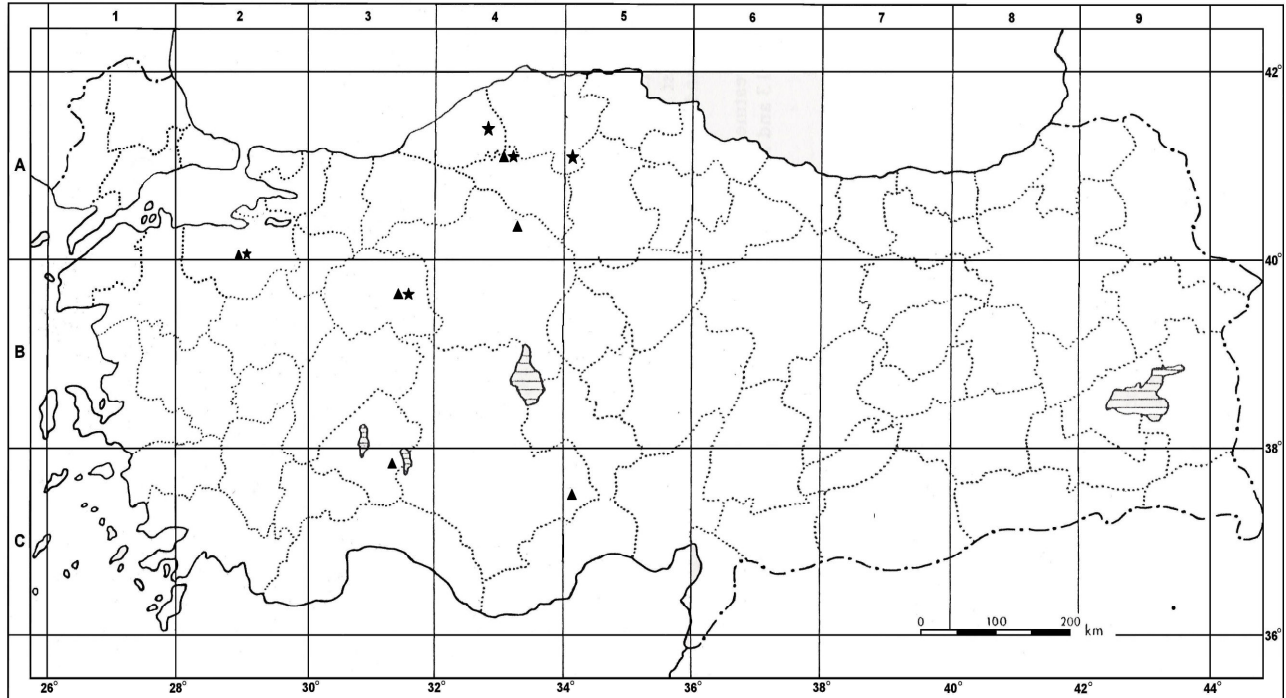


Fig. 7. Distribution map of *Scorzonera pygmaea* var. *pygmaea* (▲) and var. *nutans* (★) in Turkey.

Conservation status: *S. pygmaea* var. *pygmaea* and var. *nutans* are known at least from 5 localities and its populations are in good condition. However, its can be threatened within the next 10 years. Therefore be considered taxa Near Threatened 'NT' category (Anon., 2001).

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