Article

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First report of *Tripleurospermum rosellum* (*Asteraceae*) from Bulgaria and its distinction from the allied *T. oreades* and *T. caucasicum*

Abstract

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Tripleurospermum rosellum is here reported as native to Bulgarian flora for the first time based on materials gathered in the East Rhodope Mts. The revision of herbarium materials revealed that the taxon has been collected from several different localities in the same region of the country over the past 80 years. A detailed presentation on its morphology, distribution and habitat characteristics in Bulgaria are presented. The Greek collections of *Tripleurospermum oreades* are to be referred to *T. rosellum*. The species is also confirmed for the mainland of European Turkey.

Key words: new record, misidentification, revision, the Balkans, ruderal species, East-Mediterranean.

Introduction

Tripleurospermum Sch.Bip. (*Asteraceae*) is a monophyletic lineage within *Anthemideae* tribe (Inceer & al. 2018). It is a critical genus of about 40 species, distributed almost exclusively in the Northern hemisphere with exception of *Tripleurospermum inodorum* (L.) Sch. Bip, being distributed globally (POWO 2021). With nearly 30 species, half of which were reported endemic, Turkey has been considered its main center of diversity (Inceer & Ozcan 2021).

In Bulgaria, the most recent treatment of the genus (Kuzmanov 2012) includes *T. inodorum*, *T. tenuifolium* (Kit.) Freyn., *T. caucasicum* (Willd.) Hayek, and *T. disciforme* (C. A. Mey.) Sch. Bip.. Notably, Kuzmanov (2012) did not discuss the earlier described *T. anchialense* M. Král (Kral 1990).

During a phytosociological study performed by the first author in the period 2018–2020, in the East Rhodope Mts., Bulgaria, a representative of the genus *Tripleurospermum* was registered. It drew attention due to its perennial habit, low, ascending, unbranched stems, each bearing a single capitulum, and unusually early flowering – late April. The species has been collected from several different localities in different developmental stages during this period. Our study showed that the collected specimens do not correspond

to any of the taxa within *Tripleurospermum* reported from Bulgaria. Further comparison with herbarium collections and a crosscheck with relevant literature sources proved that its correct taxonomic identity is *Tripleurospermum rosellum* var. *album* E. Hossain.

The similar *T. oreades* (Boiss.) Rech. f. has been reported from a single site in Northeast Greece, which represents the sole European record of that taxon (Strid & Lassen 2000). Its close proximity (40 km in SW) to the sites of *T. rosellum* in Bulgaria impelled us to compare collections from both sides of the Greek-Bulgarian border. Examination of representative material conclude that all of it belongs to *T. rosellum.*

The aim of the current paper is to report *T. rosellum* as a member of the Bulgarian flora and to confirm its occurrence in Northeast Greece and continental European Turkey. Our conclusions are supported by a detailed description of its morphology (incl. photographs and SEM micrographs), distributional notes and habitat indications.

Materials and Methods

The specimens collected by us were deposited at SO and SOM (herbarium acronyms follow Thiers 2022). Identification is based on the treatments of Boissier (1875) and Hossain (1975), further corroborated with the aid of additional relevant literature (Hayek 1931; Kay 1976; Pobedimova 2000; Inceer & al. 2012; Inceer & Ozcan 2021), and comparison with specimen images, including types, kept at BR, K, E, WU, P, JE, and G (G–BOIS), and with photographs of live plants (Dimopoulos & al. 2021; Danin & Fragman-Sapir 2021). Herbarium collections of *Tripleurospermum*, *Matricaria* and *Chamaemelum* kept in Bulgarian herbaria (SO, SOM and SOA) were revised. The list of examined specimens is presented in Annex 1. Cauline leaf and a capitulum with not fully matured fruits of *T. oreades* collected in Greece were studied and compared with Bulgarian samples. Our key and morphological descriptive data of the discussed taxa is based on the cited specimens. Cypsela morphology was studied by SEM, selected micrographs being shown in Fig. 1, additional micrographs with higher resolution being enclosed in Annex 2. The distribution of the taxon in Bulgaria is mapped at a scale 1:300 000. Habitat characteristics and species lists at the studied sites are based on our personal observations. Climatic characteristics of the investigated region are presented according to numerical weather prediction model (Climate-Data 2021).

Results and discussion

Tripleurospermum rosellum var. ***album*** E. Hossain in Notes Roy. Bot. Gard. Edinburgh 32: 254 (1973), (Fig. 1, Annex2: Fig. 1s).

Caespitose perennial. Rhizome short, branched. Stems 1–20, ascending, narrowly ribbed, 10−30(–40) cm tall, unbranched or rarely with 1–3 branchlets, each bearing single capitulum (Fig. 1A), glabrous or with scattered trichomes. Leaves, 1–2 pinnatisect, alternate, sessile, the basal ones crowded, the middle cauline leaves sparse, segments linear-filiform, mucronate, with sparse trichomes or glabrous. Capitula radiate, 25–35 mm in dia. (ligules included). Receptacle hemispherical or broadly ovoid-conical (Fig. 1B).

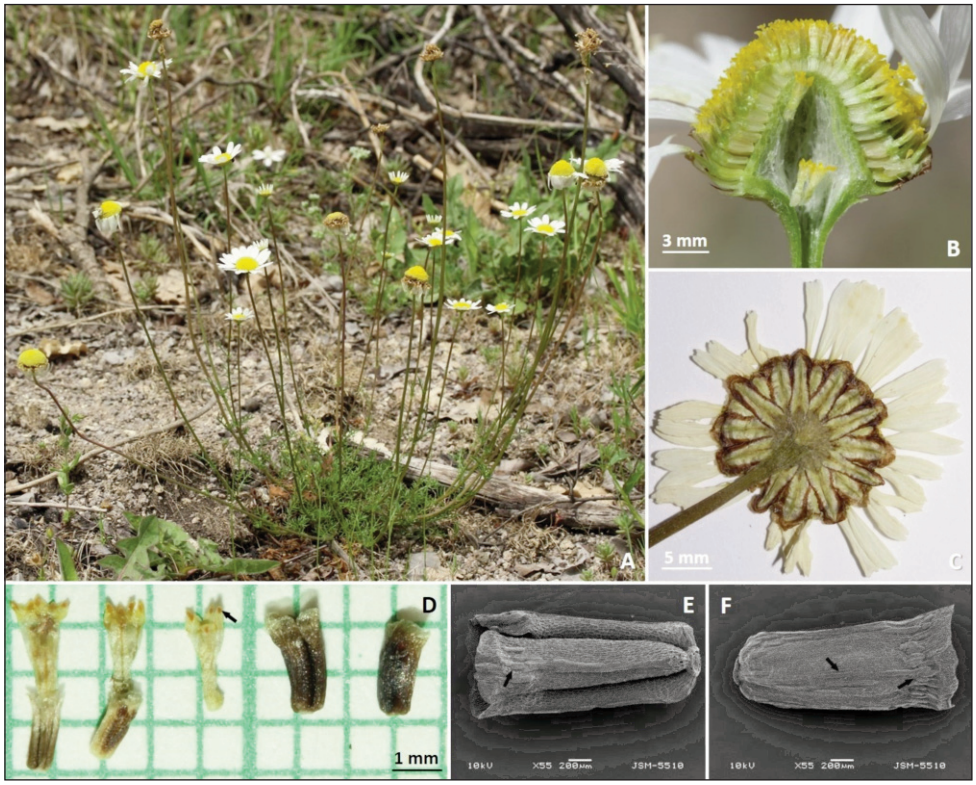

Fig. 1. Habitus (A), capitulum cross-section (B), involucral bracts (C), cypselae in different development stages and disk flowers (D) with black arrow pointing the glandules on the tips of corolla lobs (all extracted from exsiccates), fruit SEM micrographs with black arrows pointing the characteristic mucilaginous cells on adaxial (E) and abaxial (F) surface of *Tripleurospermum rosellum* from East Rhodope Mts., Bulgaria. Photos for the Fig. 1A, B, C and D made by Georgi Kunev.

Involucral bracts (Fig. 1C) entire, arranged in up to five rows, the outer ones shorter than the inner, oblong-triangular to subspatulate, glabrous or with sparse trichomes, weakly keeled, their margins more or less widely membranous, weakly undulate, pale brown or brown. Inner involucral bracts obovate, subglabrous or glabrous, their margins pale brown, entire, lacerate or apically bilobed. Ligules 8−13 x 2.2−2.8 mm, white. Disc flowers 1.8–2.3 mm long, corolla lobes and throat yellow, with a single verruciform apical gland, corolla tube whitish (Fig. 1D). Cypselae 1.8−2.2 × 0.7−1.1 mm, distally wider – 0.8−1.1 mm, occasionally slightly constricted just below the corona, base truncate, 0.7−0.8 mm wide (Fig. 1E-F, Annex 2). Immature fruits whitish, gradually and irregularly darkening to dark brown or blackish during maturation (Fig. 1D). Abaxial surface reticulate, with two yellowish, oval glands at the base of the corona. Adaxially 3-ribbed, the lateral ribs narrower than the median one. Cypselae mucilaginous (Annex 2, Fig.1s D). Corona (pappus) 0.4–0.7 mm long, 1/3–1/4 as long as the cypsela body, translucent, whitish or brownish, usually

with one median lobe, seldom trilobed or entire (not lobed). Median lobe obtuse, free (divided to its base), broadly obovate, its margins irregularly undulate. Flowering period: April and May. 2*n*=2x=18, 4x=36 [samples originates from Bolu, NW and Rize, NE Turkey respectively] (Inceer & Beyazoglu 2004; Inceer & Hayirlioglu-Ayaz 2010).

Distinction of morphologically similar taxa

Tripleurospermum rosellum is morphologically similar to *T. oreades* and *T. caucasicum* (Enayet Hossain 1975), both of which have recently been considered as conspecific (POWO 2021; Greuter 2021), and with controversial taxonomic value. We here accept their specific distinctness, using several characters for their discrimination (Table 1).

The Greek and Bulgarian material studied possess glands at the tip of disk corolla lobes; the corona of cypselae is usually lobed, with obtuse, obovate lobes; the median cypsela rib is distinctly broader than the lateral ones; all these features are typically present in *T. rosellum*. Therefore, we conclude that the Bulgarian and Greek collections initially reported as *T. oreades* (Strid & Lassen 2000) are better treated as *T. rosellum* var. *album*.

To our knowledge, the only references for the occurrence of this taxon in European Turkey are from Gökçeada island (Seçmen & Leblebici 1978; Inceer & Hayırlıoğlu-Ayaz (2014). Here, we confirm the presence of the species on the mainland of European Turkey based on material collected in the Istanbul area (*B. Davidoff* , SOM 79928, sub *Chamaemelum inodorum* (L.) Vis, Annex 1).

In Bulgaria, *T. rosellum* has been misidentified as *T. inodorum*, *T. tenuifolium* or *T. caucasucum* (Annex 1), so here we present an updated key to ease the identification of the representatives of the genus in Bulgaria.

Key to the Bulgarian species of *Tripleurospermum*

1 Capitula discoid ..... ***T. disciforme***
1*. Capitula radiate **2**
2 Plants annual or biennial **3**
2* Plants perennial **4**
3 Plants annual; cypselae obpyramidal, abaxially rugose, corona short ..... ***T. inodorum***
3* Plants biennial; cypselae obovoid-oblong, abaxially smooth or rugulose, corona absent ..... ***T. tenuifolium***
4 Stem branched; corona of cypselae short, truncate; species of saline costal habitats ..... ***T. anchialense***
4* Stem simple; corona of cypselae large, lobed **5**
5 Capitula up to 35 mm in dia.; lobs of disc flowers glandular; corona lobs obtuse-obovate, cypselae mucilaginous; species of ruderal or semi-ruderal habitats, at up to 400 m of elevation ..... ***T. rosellum***
5* Capitula usually larger than 35 mm in dia.; lobs of disc flowers eglandular; corona lobs acute, cypselae devoid of mucilage; species of subalpine or alpine meadows, above 1500 m of elevation ..... ***T. caucasicum***

Distribution and habitat in Bulgaria

Tripleurospermum rosellum has not been reported previously from the territory of Bulgaria (Kuzmanov 2012; Stoyanov & al. 2021). The revision of herbarium materials of

Table. 1. Comparative characters of three morphologically related species of *Tripleurospermum*, based on personal observations or extracted from the following references: (Boiss 1875; Enayet Hossain 1975; Pobedimova 2000; Kuzmanov 2012; Inceer & al. 2012; Dimopoulos & al. 2021; Danin & Fragman-Sapir 2021; GBIF 2022).

Character/species	*T. rosellum*	*T. oreades*	*T. caucasicum*
Habitus	Caespitose perennial	Caespitose perennial	Rhizomatous perennial
Stem leaf arrangement	Stem usually leafy in lower half only	Stems usually leafy for more than 1/2 of its length	Stem usually leafy in lower half only
Indumentum	Plants glabrous or subglabrous	Usually apressed-pubescent	Glabrous or subglabrous
Capitula size	Capitula medium-sized, up to 35 mm in dia.	Capitula medium-sized, up to 35 mm in dia.	Capitula usually large, 35 – 50 mm in dia.
Outher involucral bracts	Oblong-triangular or subspatulate, margins broad, usually pale brown	Acutely triangular, margins moderately broad, brown	Oblong-triangular, margins broad, dark brown or blackish
Ligules	White or pale pink	White	White
Lobes of disc flowers	Glandular-tipped	Eglandular	Eglandular
Corona of cypselae/cypsela body length ratio	1/3 – 1/4	1/3 – 1/4	1/2 – 1/3
Corona morphology	Lobate, lobes obtuse, obovate, white-translucent	Lobulate, lobes obtuse-triangular, white-translucent	Deeply 3-lobed, lobes acute; margins toothed, brown, entirely or distally
Ribs	Median rib broader than lateral ones	Ribs more or less equal in width	Ribs more or less equal in width
Mucilaginous cells	Present	Present	Absent

the genera *Tripleurospermum*, *Matricaria*, and *Chamaemelum* kept in Bulgarian herbaria (SO, SOM and SOA) has shown that the taxon has been collected repeatedly in the past 80 years, but was apparently misidentified (Annex 1).

In the country, the species so far has been recorded only in the East Rhodope Mts. (Fig. 2), a region with established phytogeographical connections with the eastern Mediterranean and Aegean areas through the valleys of the Arda and Maritza rivers.

The region is influenced by transitional Continental-Mediterranean climate (Velev 2002). Mean annual temperatures for the different localities ranges from 12.0°C to 13.3°C, while the average temperature for January exceeds 0°C in all know sites of the taxon. Annual precipitation ranges from 640 to 900 mm, with maximum precipitation in December.

In all Bulgarian sites, the species displays ruderal behavior. It was found on eroded slopes and bare ground, on roadsides, field margins, in overgrazed pastures, in grassland or scrub communities of low density, and open thermophilous woodlands mostly of deciduous oaks or pine groves, in xeric to xero-mesic conditions, at up to 400 m of elevation. It was most often observed in sunny locations or in the sunniest spots of scrub or woodland.

The substrates were mostly siliceous, on ground trampled by sheep herds or compacted by vehicle traffic.

T. rosellum var. *album* typically participates in communities with numerous annual species; such as *Myosotis ramosissima* Rochel, *Aphanes arvensis* L., *Crepis sancta* (L.) Bornm., *Vicia sativa* L., *Helianthemum salicifolium* (L.) Mill., *Ornithopus compressus* L., *Psilurus incurvus* (Gouan) Schinz & Thell., *Moenchia mantica* (L.) Bartl., and *Geranium columbinum* L. Among the associated perennial grasses are: *Poa bulbosa* L., *Chrysopogon gryllus* (L.) Trin., *Achnatherum bromoides* (L.) P. Beauv., *Bothriochloa ischaemum* (L.)

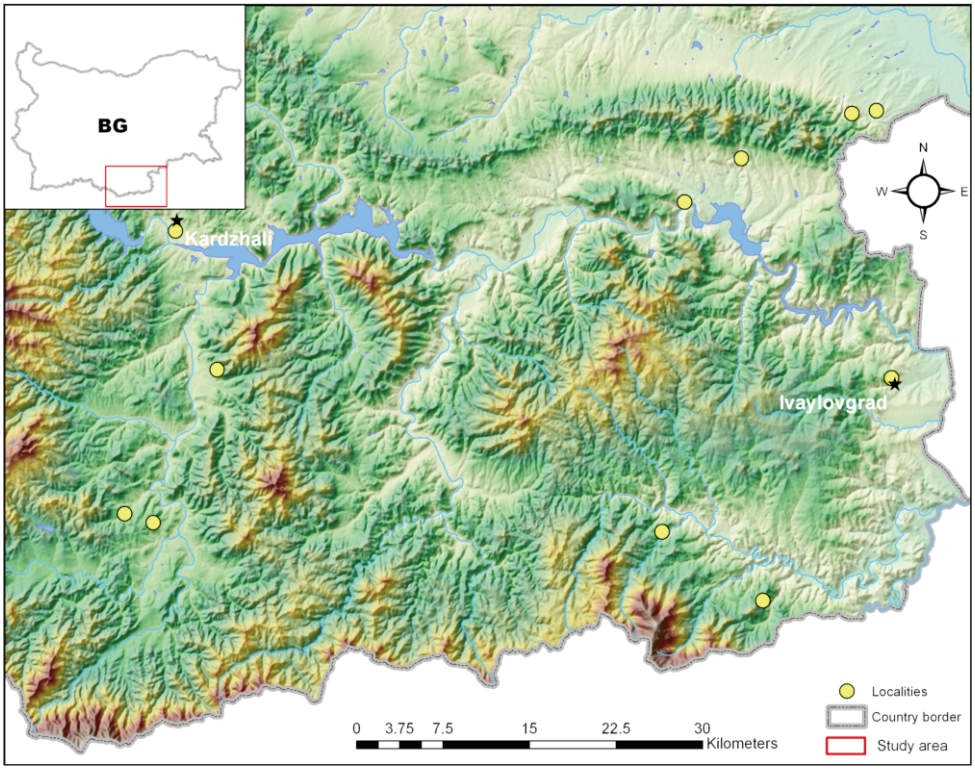

Fig. 2. Distribution of *Tripleurospermum rosellum* in Bulgaria. Map design by Gabriela Petrova.

Keng, *Avenula pubescens* (Huds.) Dumort., and *Agrostis castellana* Boiss. & Reut.. On rare occasions, the species has been observed in open scrub communities or sparse woodlands with *Juniperus communis* L., *J. deltoides* R. P. Adams, *Genista rumelica* Velen., *Cistus creticus* L., *Quercus frainetto* Ten., *Q. dalechampii* Ten. *Q. pubescens* Willd. and *Pinus nigra* J. F. Arnold.

In Bulgaria, *T. rosellum* could be observed only for a short period between end of April and mid-May. For this reason and because of its superficial resemblance with other *Anthemideae* (e.g. *Anthemis cretica* L., *Anthemis rumelica* (Velen.) Stoj. & Acht.), the species was probably overlooked until now.

Conclusion

Tripleurospermum rosellum is here added to the Bulgarian flora as a native species. Particularly, in Greece, it is replacing *T. oreades* with which it had been confused. In Bulgaria it is represented only by its white-ligulate variety. In the country, it is documented up to now only from the East Rhodope Mts. Since the taxon was registered in about ten relatively distant localities with several tens to several hundred individuals and it is more or less ruderal with the ability to produce abundant number of seeds, we consider that at present it does not require any conservation measures. Based on the revision of herbarium collections, the taxon is confirmed also for the territory of Northeast Greece and continental European Turkey. We assume that it is more widespread on the territory of Bulgaria, but was probably overlooked, due to its short flowering period, misinterpretation, and gaps in floristic sampling.

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Annex 1.
List of examined specimens

Tripleurospermum rosellum (Boiss. & Orph.) Hayek
Bulgaria:
1. In graminosis prope urbem Mastanli (Momchilgrad) in m. Rhodope orientalis, 6.05.1938, N. Stojanoff (**SO** 75602, sub *Matricaria inodora* L.);
2. East Rhodope Mts., in the shrubs on the slopes above Mezek village, 5.05.1940, *B. Kitanov* (**SO** 75629, sub *Matricaria trichophylla* Boiss.);
3. In abandoned fields, close to Borislavtsi village, Haskovo Province, East Rhodope Mts., 16.05.1980, *D. Delipavlov* (**SOA** 38461, 38462 sub *Matricaria perforata* Mérat);
4. In the field of the "Complex experimental station" Kardzhali, 25.04.1980, *D. Delipavlov* (**SOA** 43259, 43260 sub *Matricaria perforata* Mérat);
5. West side of the main ridge, southeast exposition, Ivaylovgrad Municipality, 13.04.1988, *M. Genovski* (**SOM** 154996, sub *Tripleurospermum* af. *caucasicum* (Willd.) Poir.);
6. East Rhodope Mts., N from the entrance of the Thracian Tumulus at Mezek, at a side of a dirt road, 165 m, 41.73677°N, 26.10172°E, 12.05.2019, *G. Kunev* (**SO** 108125, **SOM** 177475);
7. East Rhodope Mts., N from Malki Voden, on a dirt road through a forest of *Quercus frainetto*, 280 m, 41.69895°N, 25.96137°E, 17.05.2020, *G. Kunev* (**SOM** 177476);
8. East Rhodope Mts., E from Kazak, Haskovo Province, in pasture at the side of the road, 400 m, 41.41160°N, 25.88371°E, 4.05.2021, *G. Kunev* (**SO** 108123);
9. East Rhodope Mts., E from Karchovsko, Kardzhali Province, eroded place at the side of a dirt road, 415 m, 41.41207°N, 25.35591°E, 13.05.2019, *G. Kunev* (**SO** 108124);
10. East Rhodope Mts., W from Borislavtsi, Haskovo Province, side slope of road embankment, 213 m, 41.66493°N, 25.90287°E, 12.05.2019, *G. Kunev* (**SO** 108126);
Turkey:
11. In campis sirca Constantinopolem: ad stationem Bijuk–Han, 2.05.1913, *D. Davidfoff* (**SOM** 79928, sub *Chamaemelum inodorum* (L.) Vis.);
Greece:
12. In monte Malevô Laconiae prope Hagios Petros, rarissime, alt. 3000' (915m), April-May 1857, *Theodoros G. Orphanides* 778 (isotype, **BR** 0000005533459, sub *Chamaemelum oreades* Boiss.,

photo!), also isotypes examined as photos!: **K** 000929379, 000929380, 000929381; **E** 00385814; **WU** 0076323; **P** 03723155; **JE** 00017345; **G** 00764361, 00764362;

13. Thrace, Nomos of Evros, Eparchia of Souflion: 17.5 km from Dadia along the road to Loutros, serpentine, open, gravelly patches and small stream in deciduous oak woodland, 400 m, 41°06'N, 26°06'E, 7.5.2000, *Strid* & *Lassen* 50427 (collection represented in **B**, **G**, **LD**, herb. *A. Strid*, herb. *Kit Tan*, sub *Tripleurospermum oreades* (Boiss.) Rech. f.).

Tripleurospermum oreades (Boiss.) Rech.f.

Turkey:

14. In Monte Tauro, Aestate 1836, *Th. Kotchy* 296 (isolectotype, **G** 00764344, photo!); also isolectotypes examined as photos!: **K** 000929384, 00092935, 000929386

Annex 2.

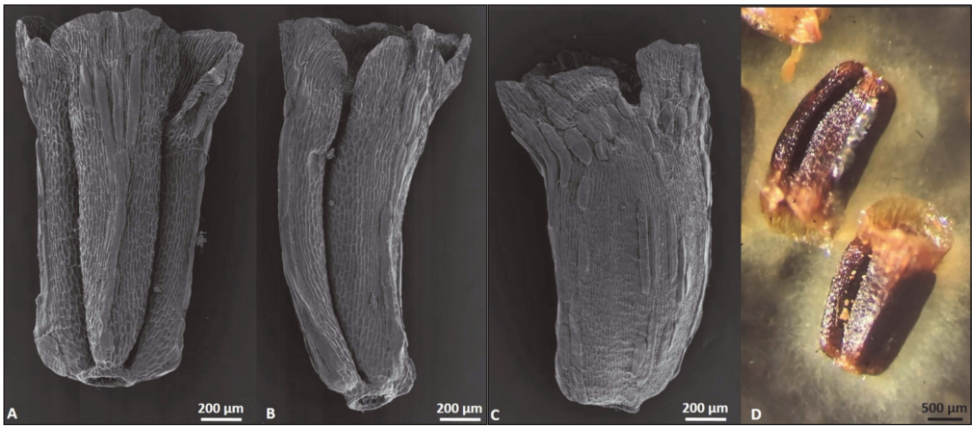

Fig. 1s. SEM morphographs (A, B and C) and photographs (D) of cypselae of *Tripleurospermum rosellum* from East Rhodope Mts., Bulgaria. Features as lobate corona with obtuse-obovate lobs, truncate base, broad median rib, reticulate surface, and mucilaginous cells are all well represented on the morphographs. Mucilage envelope stained with iodine in pale orange (D), photographed under stereomicroscope (×4).