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Biosystematics of *Tanacetum tenuissimum*, a new record from NW of Iran

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Tanacetum tenuissimum is reported as a new record from E Azerbaijan province (NW of Iran). The species by having some characters such as plant height (15–40 cm), length of pappus (0.2–0.4 mm), achene length (1.8–3.2 mm) and ribs (6–10), margin of pappus (crenulate), length and width of capitula (4.5 × 8 mm), and basal leaves (5 × 1 cm) are distinguishable from its close relative *T. tabrisianum*. In addition, the survey of light and scanning electron microscopy showed pollen grains are tricolporate and echinate (Anthemis-type). Based on the P/E ratio (Polar axis/Equatorial diameter), shape and percentage of the pollen grains were found to be oblate-spheroidal (75%), suboblate (15%), and spheroidal (10%). Distribution map of the new record species and two relatives (*T. canescens* and *T. tabrisianum*) in the world and Iran, morphological and micromorphological characteristics of pollen grains and identification key are presented herewith.

Keywords: *Compositae*, new record, pollen micromorphology, *Tanacetum***مطالعه بیوسیستماتیکی *Tanacetum tenuissimum* گزارش جدید از شمال غرب ایران**

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نیره اولنج✉: استادیار گروه زیست‌شناسی، دانشکده علوم پایه، دانشگاه ملایر، ملایر، ایران (n.olanj@malayeru.ac.ir)**علی سنبلی**: دانشیار پژوهشکده گیاهان و مواد اولیه دارویی، دانشگاه شهید بهشتی، تهران، ایران**خلاصه**

گونه *Tanacetum tenuissimum* (Trautv.) Grossh (تیره کاسنی) به عنوان گزارش جدید از شمال غرب ایران و استان آذربایجان شرقی گزارش می‌شود. این گونه، با داشتن صفاتی از قبیل طول گیاه (۱۵–۴۰ سانتی‌متر)، طول جقه (۰/۲–۰/۴ میلی‌متر)، طول فندقه (۱/۳–۸/۲ میلی‌متر)، تعداد رگه‌های فندقه (۶–۱۰ عدد)، شکل حاشیه جقه (کنگره‌ای)، طول و عرض کپه (۸ × ۴/۵ میلی‌متر) و طول و عرض برگ‌های قاعده‌ای (۱ × ۵ سانتی‌متر) از *T. tabrisianum* که نزدیک‌ترین گونه خویشاوند است، قابل تشخیص می‌باشد. همچنین، بررسی ریزریخت‌شناسی دانه گرده به کمک میکروسکوپ نوری و الکترونی نشان داد دانه‌های گرده از نوع سه شیار-روزنه‌ای و خاردار (تیپ آنتمیس) هستند و براساس نسبت طول قطبی (P) به قطر استوایی (E)، شکل ۷۵٪ آن‌ها بیضی-دایره‌ای، ۱۵٪ تقریباً بیضی و ۱۰٪ دایره‌ای است. نقشه پراکندگی این گونه به همراه دو گونه خویشاوند خود *T. canescens* و *T. tabrisianum* در دنیا و ایران، شرح کامل ریخت‌شناسی گیاه، ریزریخت‌شناسی دانه گرده و کلید شناسایی برای سه گونه ذکر شده، ارائه گردیده است.

واژه‌های کلیدی: تیره کاسنی، ریزریخت‌شناسی دانه گرده، گزارش جدید، *Tanacetum*

Introduction

Tanacetum L. (*Asteraceae*) with about 160 species is the 3rd largest genus in the tribe *Anthemideae* (after *Anthemis* and *Artemisia*), which is widely-distributed in the Mediterranean region, Asia (central, NW, and NE), and some areas of N America (Oberprieler *et al.* 2009, Moradi Behjouet *al.* 2016). Podlech (1986) in the Flora Iranica introduced 18 sections and altogether 25 species from the genus *Tanacetum* growing in Iran and is treated *T. tabrisianum* (Boiss.) Sosn. & Takht. as a synonym of *T. canescens* DC. Tzvelev (1995) in the Flora of the U.S.S.R., divided *Tanacetum* into four sections and included *T. tenuissimum* (Trautv.) Grossh. and *T. tabrisianum* as distinct taxa in *Xanthoglossa* section. Mozaffarian (2008) in his treatment for Flora of Iran is resurrected *T. tabrisianum* as a separate species.

During a field excursion in the northwestern parts of Iran (E Azerbaijan province) in 2012, an interesting *Tanacetum* species was found. A more close examination of the sample, revealed it to be *T. tenuissimum*, which is a new record for the flora of Iran. Here, the new record species is reported and illustrated, and its pollen micromorphology and karyological features are also presented. According to the recent findings including the recorded species here, the number of *Tanacetum* species in Iran is increased to 37, of which 16 are endemics (Mozaffarian 2005, Djavadi 2008, Sonboli *et al.* 2010a,b & 2011, Kazemi *et al.* 2014a, b). Variation in chromosome number is occurred on the basis of the polyploidy and aneuploidy (Kadereit & Jeffrey 2007) and basic chromosome number of the tribe *Anthemideae* is mainly $X=9$ (Sample & Watanabe 2009). Chromosome count investigations have been conducted on some species of the tribe *Anthemideae* (Inceer *et al.* 2007 & 2012, Li *et al.* 2008, Chehregani & Mehanfar 2008, Chehregani & Hajisadeghian 2009, Chehregani *et al.* 2011, Olanj *et al.* 2013, Baltisberger & Widmer 2016) and the chromosome number of some species of the *Xanthoglossa* section of *Tanacetum* has been reported as follows: *T. canescense*, *T. sonbolii*, *T. heimerlii* (Olanj *et al.* 2013 & 2015); *T. stapfianum*, *T. lingulatum* (Sadeghian *et al.* 2019)

diploid; *T. aureum* and *T. tenuissimum* ($2n=4x=36$, Figs 4 E & F) tetraploid (Olanj *et al.* 2015); *T. pinnatum*, diploid and tetraploid (Chehregani & Mehanfar 2008, Olanj *et al.* 2015), *T. chiliophyllum* var. *oligocephalum*, diploid, triploid and tetraploid (Chehregani & Hajisadeghian 2009, Olanj *et al.* 2015); *T. tabrisianum*, hexaploid (Olanj *et al.* 2015), and *T. polycephalum* with seven different subspecies is diploid, tetraploid, aneuploid ($2n=2x=35$), hexaploid, octaploid and decaploid (Chehregani & Mehanfar 2008, Chehregani & Hajisadeghian 2009, Chehregani *et al.* 2011, Inceer *et al.* 2012, Ghasemkhani *et al.* 2013, Olanj *et al.* 2013 & 2015, Javadi 2017, Kurşat 2020).

Materials and Methods

The plant samples of the new record species were collected from Poldasht-Jolfa and Jolfa-Aras road, E Azerbaijan province (NW Iran) and deposited at the Medicinal Plants and Drugs Research Institute herbarium (MPH), Shahid Beheshti University of Tehran, Iran (1855).

For morphological study, three individuals (herbarium specimens) from each population of the studied taxa were selected and 30 qualitative and quantitative traits such as: plant height, length and width of cauline and basal leaves, indumentum of leaf and plant, petiole length of leaves, inflorescence, length of floret, length and width of capitula, achene and pappus characteristics, were recorded (Tzvelev 1995); and for palynological study, pollen grains were examined by Light Microscope (LM) and Scanning Electron Microscope (SEM). The samples were acetolysed using Erdtman method (1969). For LM, one part of the acetolysed material was transferred to fresh drop of glycerin on a glass slide and quantitative traits, including exine thickness (Ex), distance between spine (De), polar axis (P), equatorial diameter (E) and the ratio of the polar axis to the equatorial diameter (P/E ratio) were evaluated for 20 pollen grains using ocular micrometer. The pollen grain shape was described based on the ratio of the polar axis to the equatorial diameter (P/E ratio) and the ratio

explained by Olanj *et al.* (2017). For SEM, acetolysed pollen grains were transferred on a SEM stub and sputter coated with gold and they were photographed using Philips, XL-30 apparatus. The chromosome numbers were also obtained from mature achenes that were collected in the field, achenes were germinated at 24 °C on moist filter paper. Actively growing root tips, 1 cm in length, were excised from germinating seed and pretreated with 0.002 M 8-hydroxyquinoline for 3–3.5 h. The material was fixed with Carnoy for 24 h and stored in 70% ethanol. They were hydrolysed in 1 N HCL for 10 min at 60 °C, then meristematic regions were stained and squashed on slides with 2% aqueous aceto-orcein. The slides were prepared and best metaphase nuclei were then photographed using light microscope (Olympus BX-51) (Olanj *et al.* 2013).

Results and Discussion

The new record of *Tanacetum tenuissimum* reported from Jolfa, E Azerbaijan province (NW Iran). The identification is verified based on the species description for *T. tenuissimum* in the Flora of the U.S.S.R., and study of the herbarium sheet at W herbarium. Morphological features of this species is most similar to *T. tabrisianum* and *T. canescens* but differ in several characters including plant height, length and width of basal leaves, cauline leaves, inflorescence type, length and width of capitula, achene and pappus length, number of achene ribs and pappus margin shape (Table 1).

Tanacetum tenuissimum (Trautv.) Grossh. Oprod. Rast. Kavk. (1949) 462 (Figs 1–3).

Syn.: *Pyrethrum tenuissimum* Trautv. In: Tr. Peterb. Bot. Sada, IX, 1 (1884) 392; Grossh. Fl. Kavk. IV <129.—*P. dumosum* auct. Non Boiss.: Lipsky In: Tr. Peterb. Bot. Sada, XIII (1894) 306 and Fl. Kavk. (1899) 349; Sosn. In: Tr. Tifl. Bot. Sada, XVII (1915) 42.—*T. tabrisianum* auct. Non Boiss.: Sosn. In: Zhurn. Russk. Bot. Obshch. XIV (1929) 84.—*Chrysanthemum tenuissimum* (Trautv.)

Winkl. In: Tr. Peterb. Bot. Sada, XI (1889) 123; B. Fedtsch. Rast. Turk. 737.

Perennial herbs, stems 15–40 cm tall, erect or more or less prostrate, basally woody, branched from base. Indumentum densely gray-green pilose. Stem leaves few (mostly linear). Leaves grayish, hairy (densely pilose and sparsely glandular); basal and lower stem leaves: petiole 2.5–4 cm long with broad base; leaf blade narrowly elliptic, 5–10 × 0.5–10 mm; pinnatisect, broad linear, primary lateral segments 5–13 paired, pinnately lobed or mostly entire margin, ultimate segments and lobes 1.2–1.5 mm wide, blunt apex with short beak. Middle and upper stem leaves very small, 5–7 mm × 2.2–3.5 cm, sessile, usually pinnately lobed to entire. Receptacle slightly convex, usually 1.5 mm height, capitula solitary, numerous, peduncle long and not thickened. Involucre globose to broad cylindrical, (4.5–)6–8 mm long and 5.5–7.5 mm wide, sparsely hairy, phyllaries coriaceous to herbaceous, outer triangular-ovate, inner phyllaries linear-oblong, 2–3 times as long as outermost; all phyllaries bright (green) colored, membranous on margin, innermost with broad appendage. Marginal-florets bisexual, corolla tubular, some florets, more or less lingulate, disc florets 2–3 mm long. Achenes 1.8–2.3 mm long and 0.5–0.8 mm wide, 6–10 ribbed, pappus coroniform, 0.2–0.4 mm long, crenulate (Figs 1 & 2).

Flower and fruit time: June–July

Distribution and conservation status: General distribution: South Caucasus (SE Nakhichevan, Azerbaijan), Iran (NW); Iran: NW, E Azerbaijan, Poldasht, Jolfa-Poldasht road, km 55, 5 km after Arasdam, 840 m, Olanj, MPH-11855; between Jolfa and Aras, 10 km after Jolfa, 800 m, Sonboli, MPH-11856 (Fig. 3). It is suggested that, this new record could be placed under IUCN threat category “Least Concern” criterion.

Habitat: Mountains and stony slopes (Fig. 3).

Identification key of *Tanacetum tabrisianum*, *T. tenuissimum* and *T. canescens*

- 1. Stem with many leaves, similar to basal leaves, corymb inflorescence, achene ribs 10–12 *T. canescens*
- Stem leaves few, reduced, single inflorescence, achene ribs 6–10..... 2
- 2. Width of capitula 5.5–7.5 mm, achene length 1.8–2.3 mm, length of pappus 0.2–0.4 mm, pappus margin crenulated *T. tenuissimum*
- Width of capitula 9–12 mm, achene length 2.8–3.6 mm, length of pappus 0.3–0.6 mm, pappus margin serrate..... *T. tabrisianum*

Table 1. Diagnostic morphological characters of *Tanacetum tenuissimum* from close-relatives, *T. tabrisianum*, and *T. canescens*

Character	<i>T. canescens</i>	<i>T. tabrisianum</i>	<i>T. tenuissimum</i> (Jolfa)	<i>T. tenuissimum</i> (Flora of U.S.S.R.)
Plant height (cm)	Up to 45	30–35	27–40	15–40
Length and width of basal leaves (cm)	8–10	6–8 × 1.3	5–8 × 0.5–0.9	5(8) × 1
Cauline leaves	Stem with leaves, similar to basal leaves	Stem leaves few, reduced	Stem leaves few, reduced	Stem leaves few, reduced
Inflorescence	Corymb	Single	Single	Single
Length and width of capitula (mm)	6–8	5–8 × 9–12	6–8 × 6–7	4.5–5.5 × 5.5–7.5
Achene length (mm)	2.4–3.6	2.8–3.6	1.8–2.3	2–3.2
Achene ribs	10–12	7–10	6–8	6–10
Pappus length (mm)	0.08–0.12	0.3–0.6	0.2–0.4	0.2–0.4
Pappus margin	Serrate	Serrate	Crenulate	Crenulate



Fig. 1. Aerial parts of *Tanacetum tenuissimum* at fruiting stage in the natural habitat.

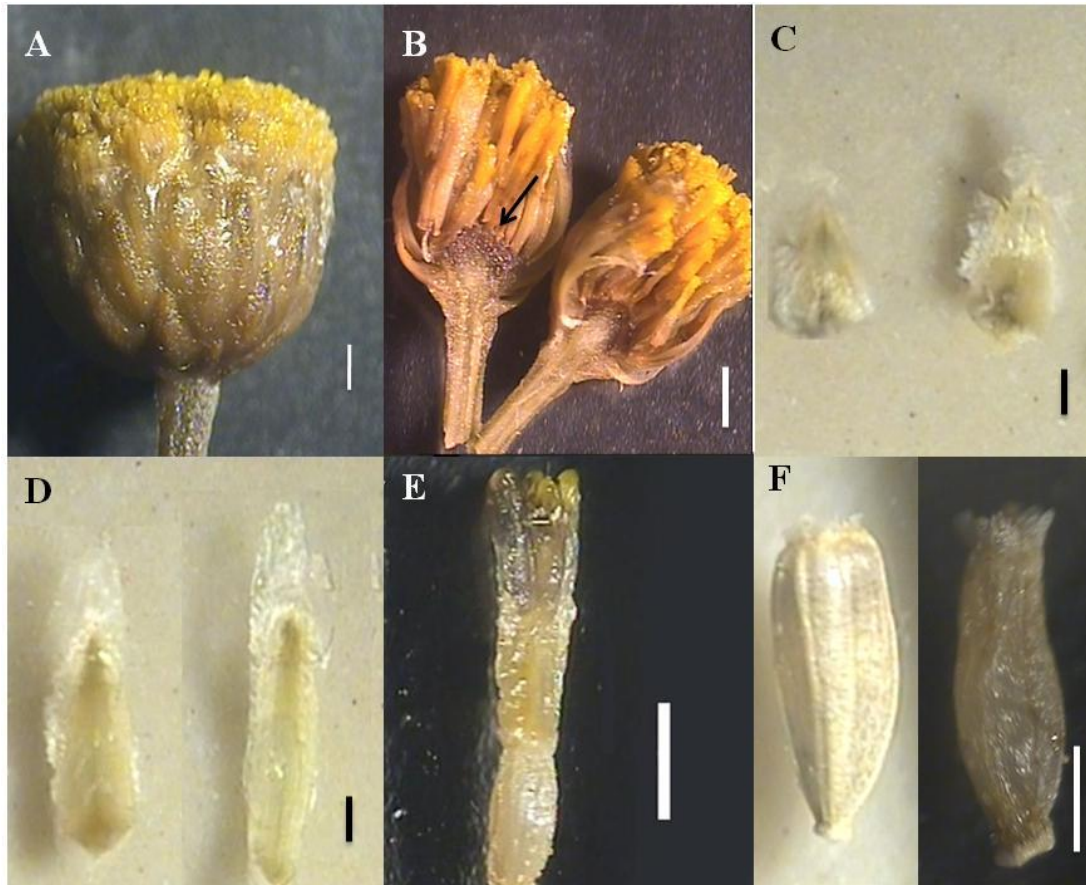


Fig. 2. *Tanacetum tenuissimum*: A. Capitulum, B. Receptacle, C. External involucre bracts, D. Internal involucre bracts, E. Tubular floret, F. Mature and immature achenes and coroniform pappus with crenulate margin (Bar = 1 mm).

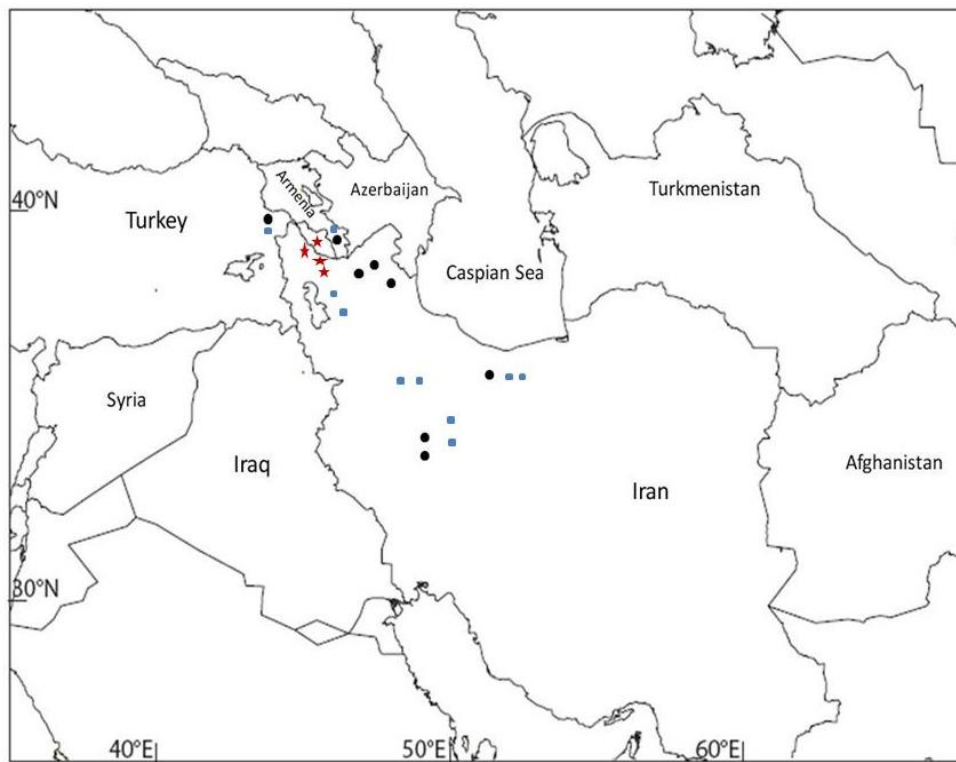


Fig. 3. Distribution map of *T. tenuissimum* (★), *T. tabrisianum* (●), and *T. canescens* (■).

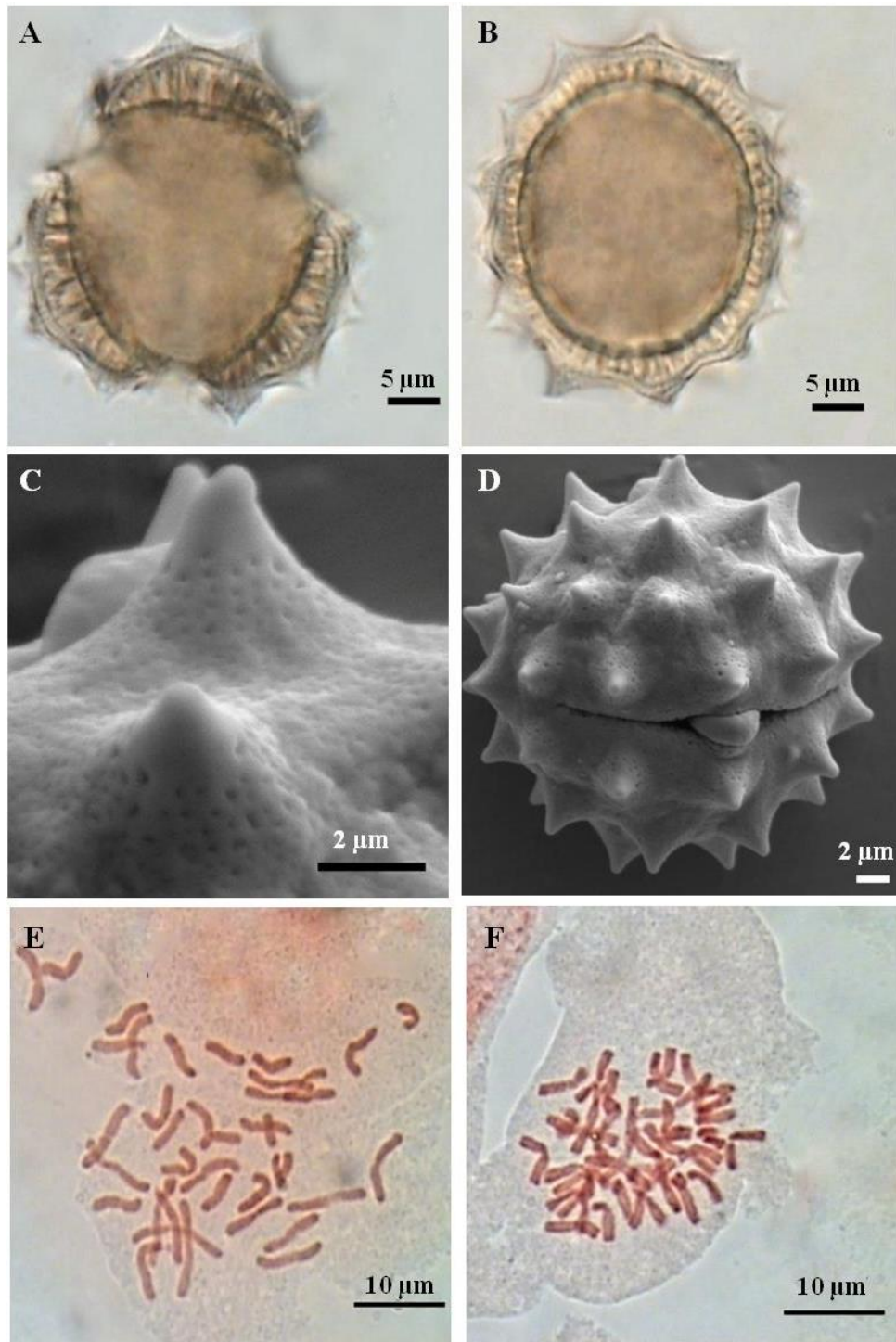


Fig. 4. *Tanacetum tenuissimum*: A-B. LM micrographs of pollen grains, C-D. SEM micrographs of pollen grains, E-F. Mitotic metaphase chromosome ($2n=4x=36$).

Generally, the Asteraceae pollen grain is tricolporate. Two types of pollen grain have been reported from the tribe *Anthemideae*: Anthemis-type and Artemisia-type (Oberprieler *et al.* 2009). Over the past decades, pollen micromorphology and exine ornamentation characters have provided considerable insight to the plant taxonomy especially among genus with high degrees of morphological variations (Martin 2001). The micromorphology study of *Anthemideae* pollen grain with focused on Iranian taxa (12 genera and 32 populations) was carried out using LM and SEM this investigation showed that tricolporate pollen grain, oval-spheroidal shape and Anthemis-type (based on the exine ornamentation) are generally among studied taxa (Olanj *et al.* 2017). As expected, the experiment to study pollen grain characters of *T. tenuissimum* using LM demonstrated that all pollen grains are tricolporate with variable shapes, including oval-spheroidal (75% of studied pollen), sub-oval (15% of studied pollen) and spheroidal (10% of studied pollen). Some quantitative characters were also measured including polar axis 15–17.5 μm (15.9 μm), equatorial diameter 16–18 μm (17.37 μm), the polar axis to the equatorial diameter 0.1–0.86 (0.91), exine thickness 1.2–4.0 μm (1.68 μm) and distance between spines 4–5 μm (4.19 μm) (Figs 4 A & B). Moreover, an investigation of exine sculpture using the SEM showed that *T. tenuissimum* pollen belonged to Anthemis type (spiny) (Figs 4 C & D). The obtained results agreed with Oberprieler *et al.* 2009 and our earlier findings (Olanj *et al.* 2017). Chromosome counting and ploidy level of *T. tenuissimum* were reported: $2n=4x=36$. As was mentioned in the method, the best metaphases stages were selected and photographed using the LM (Olympus BX-51) (Figs 4 E & F).

Discussion

Study of *Tanacetum* description in the flora Iranica (Podlech 1986), the Flora of the U.S.S.R. (Tzvelev 1995) and Flora of Iran (Mozaffarian 2008) revealed that, *T. canescens* and *T. tabrisianum* are most similar species to *T. tenuissimum*. As mentioned in the introduction, *T. tabrisianum* was a synonym of *T. canescens* in the Flora Iranica and was a close relative to *T. tenuissimum* in the Flora of the U.S.S.R. Although, *T. tabrisianum* and *T. tenuissimum* are more similar to each other than *T. canescens*, each of these three species morphologically are distinct (Table 1). Regarding distribution range, *T. canescens*, *T. tabrisianum*, and *T. tenuissimum* exists within the same geographic region. As shown in figure 3, the former two species distribute in Caucasus, Turkey, NW, and central of Iran, while the latter species occur in the south of Caucasus and now in NW of Iran.

Previous research (Olanj *et al.* 2017) using the LM and SEM has shown that Anthemis-type of pollen grain is predominant in the tribe *Anthemideae* (11 genera) except for *Artemisia* pollen grain (*Artemisia*-type) and common pollen grain shapes were oval-spherical, sub-oval, oval and oblong-spherical, respectively. The current study supports our previous research in this area. Our finding demonstrated that, pollen grain of *T. tenuissimum* was Anthemis-type and the common pollen shapes were oval-spheroidal (75%), sub-oval (15%), and spheroidal (10%).

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