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چکیده. دانه‌های گرده از ۳۴ جمعیت متعلق به ۱۶ گونه از زبان در قفاهای ایران توسط میکروسکوپ نوری و میکروسکوپ الکترونی نگاره مورد بررسی قرار گرفت. چهار ویژگی طول محور قطبی (P)، طول محور استوایی(E)، نسبت طول محور قطبی به محور استوایی(P/E) اندازه‌گیری شدند. دانه‌های گرده پخت یا تقریبا پخت بوده کوتاه‌ترین محور قطبی متعلق به *C. tehranica* (Boiss.) Rech.f. بوده و بلند ترین آن به *C. trigonelloides* (Boiss.) Munz (۲۸/۱۵–۳۷/۳ میکرومتر) تعلق داشت. همچنین کوتاه ترین محور استوایی به *C. stocksiana* (Boiss.) Nevski. و بلندترین آن به *C. orientalis* Schrödinger (۱۷/۵–۲۵/۲ میکرومتر) تعلق داشت. بر اساس تزیینات اگزین که با میکروسکوپ الکترونی نگاره مشاهده شده دو نوع تیپ دانه گرده مشخص شده است. تیپ ۱: اگزین به طور مشخص در قطبین ضخیم و برامده، شیار ها پهن و تزیینات با خارچه‌های متراکم. تیپ ۲: اگزین در قطبین نوک کند، شیارها باریک و تزیینات خارچه های پراکنده. تصاویر تمام گونه‌ها و ویژگی‌های ساختار دانه گرده ارائه شده است. نتایج ما نشان داد که شکل دانه گرده و تزیینات آن ویژگی‌های متمایز کننده‌ای برای گونه‌ها هستند. اگر چه برای حل پیچیدگی‌های جنس کافی نیستند اما نتایج این مطالعه قرار گیری گونه‌های *Aconitella* در *Consolida* را تایید کرد زیرا هر دو تیپ دانه گرده در گونه های *Aconitella* دیده می شود.

واژه‌های کلیدی. اگزین، ایران، خارچه، دانه گرده، میکروسکوپ الکترونی نگاره

Palynological study of the genus *Consolida* (Ranunculaceae) in Iran

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Abstract. The pollen grains of 34 populations, representing 16 species of *Consolida* (DC.) Gray, have been examined by LM and SEM. The polar axis (P), equatorial diameter (E), P/E ratio and exine patterns were measured. The pollen grains were found out to be 3-zonocolpate, euprolate to subprolate. The shortest polar axis to belong to *C. tehranica* (Boiss.) Rech.f. and the longest to belong to *C. trigonelloides* (Boiss.) Munz (28.15-37.3 µm); the shortest equatorial axis to belong to *C. stocksiana* (Boiss.) Nevski. and the longest to *C. orientalis* Schrödinger (17.5-25.2 µm). Based on exine ornamentation observed under SEM, two types of pollen grains were recognized. Type I, exine distinctly thickened at poles with broad colpi and stronglymicro-echinate sculpturing, and type II, with exine obtuse at poles, narrow colpi and weekly micro-echinate sculpturing. Pictures of all species and characteristics of pollen grain structure were presented. Our results showed that pollen shape and sculpturing were diagnostic characters for distinguishing the species. Although they did not suffice enough to resolve taxonomic conflicts in the genus, our results confirmed embed of *Aconitella* in *Consolida* due to the occurrence of *Aconitella* species in two pollen groups.

Keywords. exine, Iran, micro-echinate, pollen, SEM

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INTRODUCTION

The genus *Consolida* (DC.) Gray (Ranunculaceae) belongs to tribe Delphinieae. It comprises approximately 52 species, including the members of the genus *Aconitella* Spach. Iran is one of the richest countries for the genus in South-West Asia, since it has 24 species (Iranshahr *et al.*, 1992). *Consolida* has been separated from *Delphinium* by De Candolle based on single spurred petals, one follicle and annual life cycle and has occurred in separate section. Finally, it introduced as a separate genus by Gray in 1821 (Triffonova, 1990). Based on phylogenetic studies of Jabbour & Renner (2011), *Aconitella* is part of *Consolida*, both being embedded in *Delphinium*. The Jabbour & Renner (2011) results showed that *Consolida* diverged from *Delphinium* relatives in the Early to Middle Miocene, a period of increasing aridity, caused primarily by decrease in sea level in the Mediterranean (Rögl, 1999; Peryt, 2006; de Leeuw *et al.*, 2010) and desertification in Asia (Guo *et al.*, 2002). Investigations of pollen morphology in the Ranunculaceae have been essential to aid the classification within this family. Ranunculaceae is a europalynous family and the pollen grains include representatives of a number of classes, most of which are tricolpate, and pantocolpate or pantoporate. Pollen grain ornamentations show a variety of forms, including echinate and reticulate (Erdtman, 1952; Clarke *et al.*, 1991). However, the pollen morphology of the genus *Consolida* is poorly known, for only a limited number of previous studies have been conducted on it (Noor *et al.*, 2004; Oberschneider, 1998). Only brief notes with no description and a very limited number of taxa in *Consolida* have been studied by Erthman *et al.* (1963), Petrov & Borrisova-Ivanova (1980), Moor *et al*. (1991), and Clark *et al*. (1991).

One of the pollen types of pollen grains in Ranunculaceae family is *Consolida ambigua*, in which *Consolida* and *Delphinium* species occur (Clark *et al*., 1991). Pollen grains in this type are 3-zonocolpate, with weakly micro-echinate ornamentations. The objectives of this paper are to provide a detailed account of the pollen morphology of *Consolida* as a whole by light microscopy (LM) and scanning electron microscopy (SEM), and to determine the extent to which these palynological data can be used as a taxonomic character in the genus.

MATERIAL AND METHODS

The present study was carried out on the 17 species as mentioned in Table 1. Pollen samples were obtained from the herbarium of Alzahra University (ALUH) and herbarium of Research Institute of Forest and Rangelands (TARI). For scanning electron microscopy, pollen grains were prepared from herbarium material with no special treatment. Anthers were broken to release the pollen directly onto aluminum stubs, sputtered with gold, and then observed and photographed using a Hitachi S-800 SEM unit. The values of P (polar axis length) and E (equatorial diameter) were measured, and means were calculated based on the examination of 20 pollen grains. For LM studies, pollen samples were stored in Farmer's solution, then mounted in glycerol jelly on glass slides and studied by means of an Olympus Bx51 microscope and photographed by a digital camera.
Measurement of grains was based on approximately 25-35 grains per sample and each sample was measured using Image Tools V.3 software (Donald *et al.*, 2007). Descriptive terminology follows Erdtman (1966) and Clarke *et al.* (1991).

RESULTS AND DISCUSSION

Representative pollen grains are shown in Figures 1 to 4; size and shape measurements are summarizedin Table 2.

The grains are euprolate to subprolate; the shortest polar axis belongs to *C. tehranica* (Boiss.) Rech.f. (Fig. 2. a); the longest belongs to *C. trigonelloides* (Boiss.) Munz (28.15-37.3 µm) (Fig. 2.d, Fig. 3.a, Table 2); the shortest equatorial axis belongs to *C. stocksiana* (Boiss.) Nevski (Fig. 2.b, 3.c) and the longest equatorial axis belongs to *C. orientalis* (Gray) Schrődinger (17.5-25.2 µm) (Table 2). The pollen grains are also trizonocolpate, the colpi long, broad or narrow, sunken, margins indistinct, ends acute or linear, membranes coarsely granular or indistinct, exine distinctly thickened at poles or obtuse, weekly or strongly micro-echinate or rough and punctate/perforate. Based on (Clarck *et al.*, 1991), the pollen grains in the Ranunculaceae family could occur in 17 types, and *Consolida* species in *Consolida ambigua* type. One of the characters of this type is distinctly thickened poles, while only some of the studied species had this character (*C. anthoroidea* (Boiss.) Schrodinger, *C. paradoxa* Nevski, *C. regalis* Gray, *C. stocksiana* Nevski, *C. rugulosa* Schrödinger, *C. orientalis* and *C. ambigua* (L.) Ball. & Heywood) (Fig. 1).

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Table 1. List of species studied, localities and voucher specimens.

Species	Locality	voucher specimen	Collector & No.
C. camptocarpa (Fisch. & C.A.Mey.) Nevski	Khorassan: Jajarm road	ALUH	Poorhabibian 1599
C. camptocarpa (Fisch. & C.A.Mey.) Nevski	Semnan: 58 km of Shahrud to Sabzevar	ALUH	Poorhabibian 35379
C. camptocarpa (Fisch. & C.A.Mey.) Nevski	Khorassan: Sarakhs, 12 km to Mozduran	ALUH	Poorhabibian 1603
C. leptocarpa Nevski	Golestan: Golestan national park, Mirzabailoo	ALUH	Poorhabibian 1590
C. leptocarpa Nevski	Khorassan: Sarakhs road	ALUH	Poorhabibian 1605
C. leptocarpa Nevski	Khorassan: Sarakhs, 14 km to Mozduran	ALUH	Poorhabibian 1600
C. persica (Boiss.) Grossh.	Hamedan: Khan Abad	ALUH	Poorhabibian 1555
C. persica (Boiss.) Grossh.	Tehran: Firuzkuh	ALUH	Poorhabibian 1556
C. persica (Boiss.) Grossh.	Azarbayejan: Tabgriz, Ahar road	ALUH	Poorhabibian 1606
C. rugulosa Schrödinger	Golestan: Golestan national park, Mirzabailoo	ALUH	Poorhabibian 1597
C. rugulosa Schrödinger	Khorassan: Mashhad	ALUH	Poorhabibian 1557
C. rugulosa Schrödinger	Hamedan: Khan Abad	ALUH	Poorhabibian 1558
C. paradoxa Nevski	Khorassan: Neyshabur, Sharif Abad village	ALUH	Poorhabibian 1598
C. paradoxa Nevski	Khorassan: Ferdowsi University Campus	ALUH	Poorhabibian 18570
C. anthoroidea (Boiss.) Schrödinger	Hamedan: Almaghlagh village	ALUH	Poorhabibian 1586
C. anthoroidea (Boiss.) Schrödinger	Hamedan: Nahavand road, Garo Mt.	ALUH	Pakravan 1595
C. anthoroidea (Boiss.) Schrödinger	Markazi: Kuhe Chepeghli	ALUH	Mahdavii 2783
C. tehranica (Boiss.) Rech.f.	Tehran: Between Karaj and Eshtehard	TARI	Assadi & Maassoumi 1701
C. tehranica (Boiss.) Rech.f.	Mazandaran: Pol Sefid	HNBG	Zarre & Amini 5077
C. stocksiana Nevski	Khorassan: Neyshabur	ALUH	Poorhabibian 1598a
C. hohenackeri (Boiss.) Grossh.	Hamedan: Kuhe Garo	ALUH	Poorhabibian 1587
C. hohenackeri (Boiss.) Grossh.	Fars: Bamo national park	TARI	Mozaffarian 71498
C. aucheri (Boiss.) Iranshahr	Khorassan: Sarakhs, 14 km to Mozduran	ALUH	Poorhabibian 1600a
C. ambigua (L.) Ball & Heywood	Kermanshah: Ghasreshirin	TARI	Seraj 24663
C. ambigua (L.) Ball & Heywood	Tehran: Rudehen	ALUH	Poorhabibian 1580
C. orientalis (Gray) Schrödinger	Mazandaran: Sari	ALUH	Poorhabibian 27543
C. orientalis (Gray) Schrödinger	Mazandaran: Nowshahr	HNBG	Zarre & Amini 5075
C. orientalis(Gray) Schrödinger	Mazandaran: Polsefis	ALUH	Zarre & Amini 5086
C. oliveriana (DC.) Schrödinger	Kermanshah: 31 km to Ghasreshirin	TARI	Mozaffarian 24900
C. oliveriana (DC.) Schrödinger	Hamedan: Abbas Abad	ALUH	Pakravan 45532
C. flava (DC.) Schrödinger ex Hand.-Mazz.	Khuzestan: Ramhormoz	TARI	Mozaffarian 87128
C. flava (DC.) Schrödinger ex Hand.-Mazz.	Khuzestan: Behbahan	TARI	Mozaffarian 87148
C. trigonelloides (Boiss.) Schrödinger	Fars: Abadeh	ALUH	Pakravan 6709
C. oligantha Schrödinger	Kermanshah: Harsin	TARI	Mozaffarian 1914
C. regalis Gray	Azarbayejan: Ajabshir, Khanian village	ALUH	Poorhabibian 1607
C. regalis Gray	Azarbayejan: 35 km to Tabriz, Ahar road	ALUH	Poorhabibian 1606
C. regalis Gray	Golestan: Near Katul	ALUH	Pakravan 1763

Abbreviations: No. = herbarium number, ALUH = Alzahra University Herbarium; TARI= Herbarium of Research Institute of Forests and Rangelands.

Table 2. Pollen morphological data of *Consolida* species.

Species	Pollar axis length (µm)	Equatorial axis length (µm)	P/E	Total shape	Colpus shape	Thickened poles
C. ambigua	26, (28.85)32	20.02, (22.01),24	1.20	subprolate	broad	+
C. anthoroidea	25.97, (29.01), 33	20, (22.57),25.02	1.13	subprolate	broad	+
C. aucheri	29.70,(32.90),37.30	19.4,(19.60),20	1.67	euprolate	narrow	-
C. camptocarpa	30.4, (31.72), 34	16.60, (21.6)24.8	1.46	euprolate	narrow	-
C. flava	30.4,(31.72),34	17.4,(21.1), 24.1	1.59	Euprolate	narrow	-
C. hohenacker	30. 01, (30. 3), 31.3	21.02,(23.01)21	1.31	subprolate	narrow	-
C. leptocarpa	29.8,(31.7),34.40	21.80, (22.40), 23	1.42	euprolate	broad	+
C. oligantha	27.1, (30. 2), 35.70	21.05, (22.40), 23.02	1.34	euprolate	narrow	-
C. oliveriana	25.1, (32.90), 40.50	17.05, 21.40),26.40	1.5	euprolate	narrow	-
C. orientalis	29.70,(29.90),30.20	22.80,(25.23),26.31	1.18	euprolate	broad	+
C. paradoxa	26.02,(27.95),30.40	19.05,(20.80),23.40	1.34	euprolate	broad	+
C. persica	33.03,(36.97),43.01	18.04,(19.40),21.03	1.90	euprolate	narrow	-
C. regalis	29.02,(32),35. 05	20. 03,(20.11),23. 05	1.59	euprolate	broad	+
C. rugulosa	26. 05,(30.9),33.80	16,(20.80),27	1.48	euprolate	broad	+
C. stocksiana	27,(29.70),31.05	16. 05,(17.5),19.03	1.69	euprolate	broad	+
C. tehranica.	27.40,(28),28.70	21.60,(22.10), 23.05	1.27	subprolate	narrow	-
C. trigonelloides	31.50, (37.30), 47.10	21,(21.85), 23.10	1.70	euprolate	broad	+

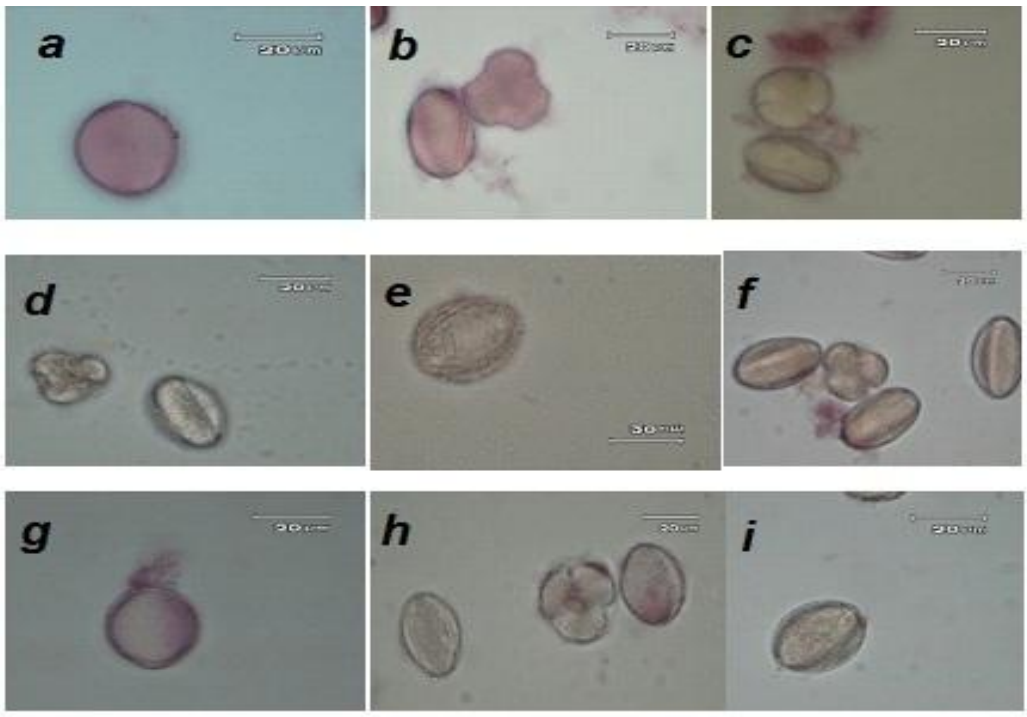

Fig. 1. LM micrographs of pollen grains in *Consolida* species: **a:** *C. paradoxa*; **b:** *C. regalis*; **c:** *C. ambigua*; **d:** *C. aucheri*; **e:** *C. oliveriana*; **f:** *C. persica*; **g:** *C. orientalis*; **h:** *C. hohenackeri*; **i:** *C. anthoroidea*

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Fig. 2. LM micrographs of pollen grains in *Consolida* species. **a:** *C. tehranica*; **b:** *C. stocksiana*; **c:** *C. flava*; **d:** *C. trigonelloides*; **e:** *C. leptocarpa*; **f:** *C. rugulosa*.

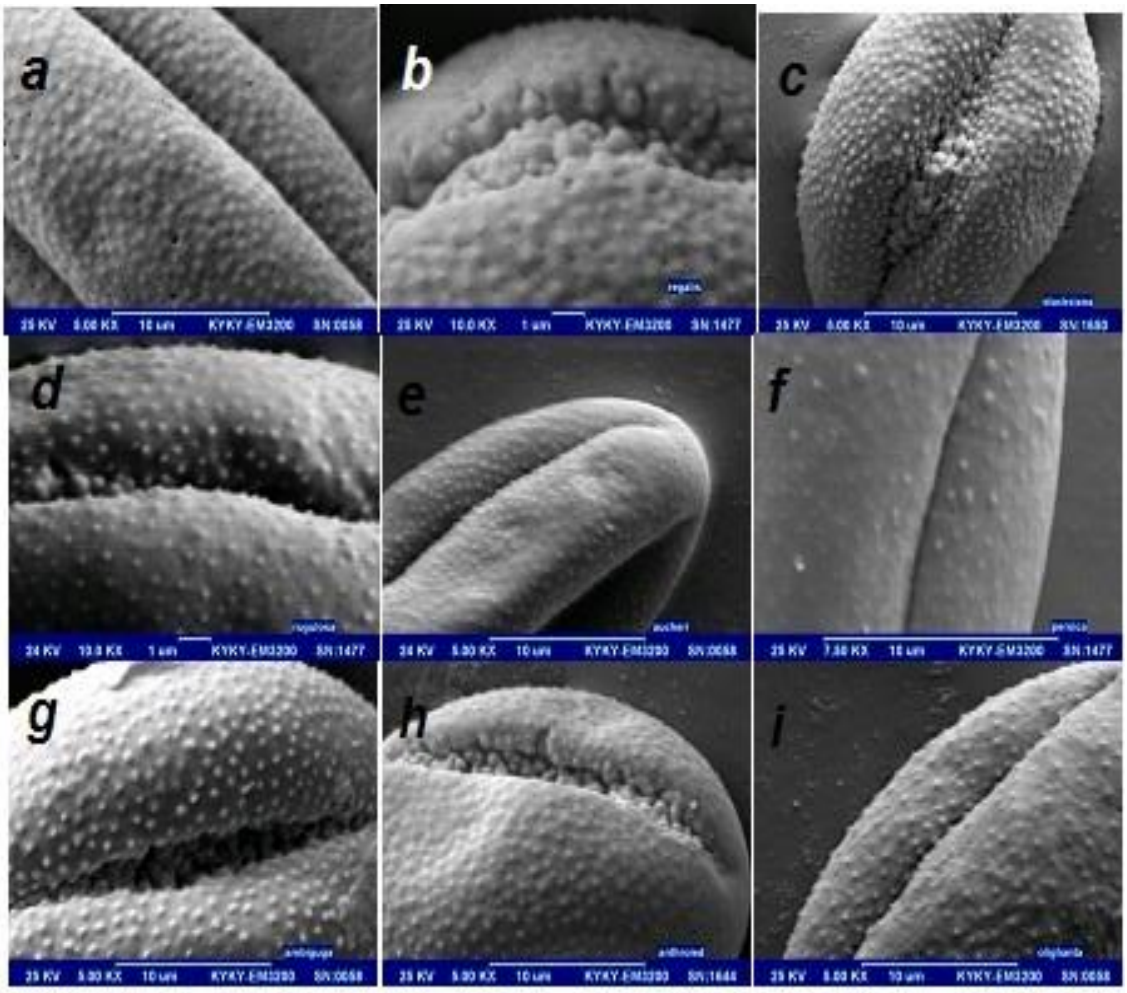

Fig. 3. SEM micrographs of pollen grains in *Consolida* species. **a:** *C. trigonelloides*; **b:** *C. regalis*; **c:** *C. stocksiana*; **d:** *C. rugulosa*; **e:** *C. aucheri*; **f:** *C. persica*; **g:** *C. ambigua*; **h:** *C. anthoroidea*; **i:** *C. oligantha*.

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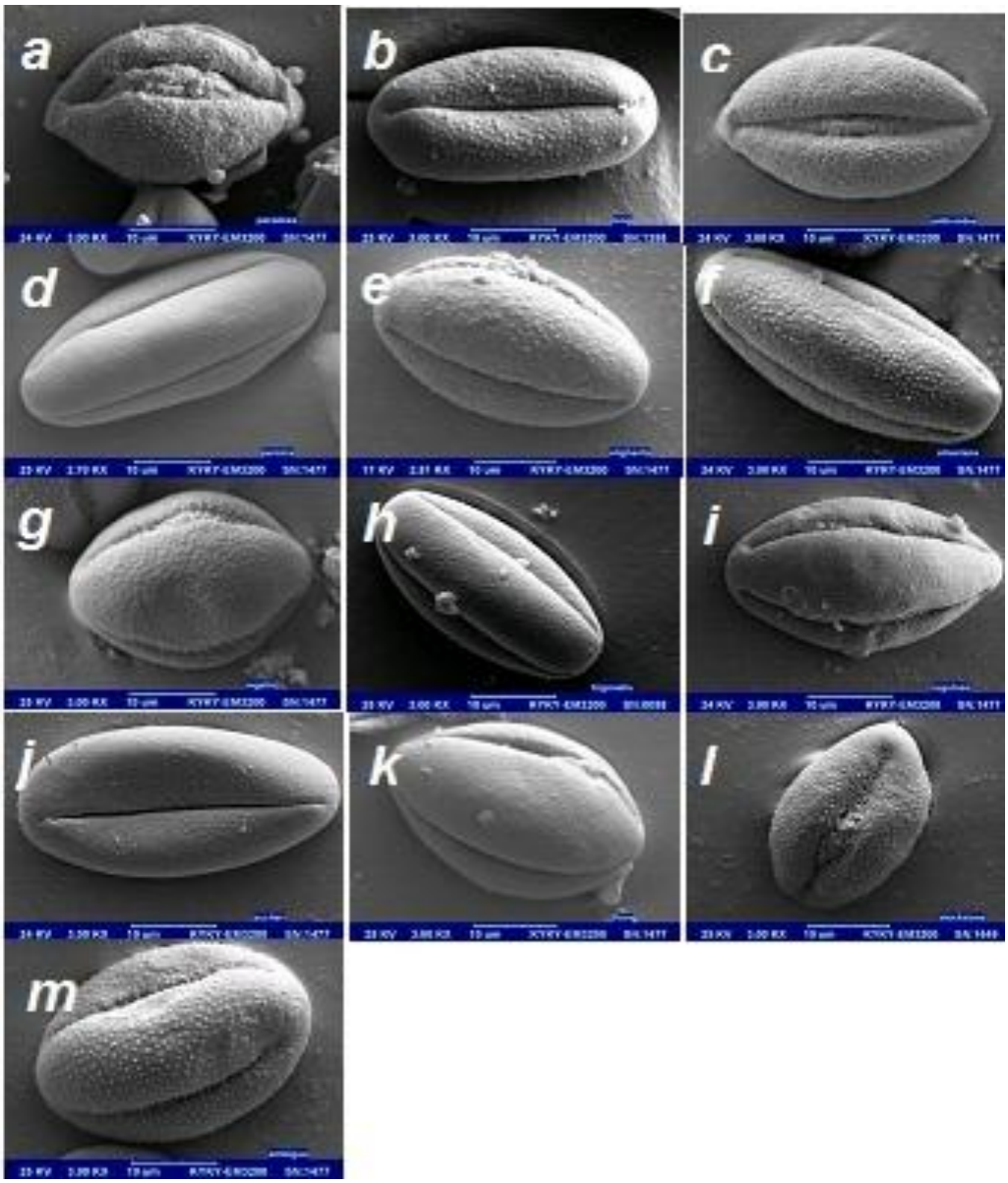

Fig.4. 2. SEM micrographs of pollen grains in *Consolida* species. **a:** C. *paradoxa*; **b:** *C. Camptocarpa*; **c:** *C. anthoroidea*; **d:** *C. persica*; **e:** *C. oligantha*; **f:** *C. oliveriana*; **g:** *C. regalis*; **h:** *C. trigonelloides*; **i:** *C. rugulosa*; **j:** *C. aucheri*; **k:** *C. flava*; **l:** *C. stocksiana*; **m:** *C. ambigua.*

Another character described by Clarck *et al.* (1991) is broad colpus, while some of the species had narrow colpi with sunken margins. Based on some of the pollen characters, Iranian species of the *Consolida* were close to the *Adonis annua* type, because of the narrowness of the colpus. Obtuse poles is a character that is not found in *Adonis annua* type but occurs in *Caltha palustris* type. (Faegri & Iversen, 1975) and (Moore & Webb, 1978) could not differentiate pollen of the genera *Aconitum* L., *Adonis* L., *Caltha* L. and *Consolida*. Therefore, not all of the Iranian species of the *Consolida* could occur in one type (*Consolida ambigua* type) but could be divided into two groups. First group species have broad colpi with coarsely granular membranes and thickened exine at poles (*Consolida ambigua* type). Second group species have narrow colpi and obtuse poles, not having been recorded previously from pollen grains of Ranunculaceae. The exine ornamentations are very variable. The scabrate, weakly or strongly microechinate forms could be found in various species. The perforated exine has been observed in *C. oligantha* Schrődinger, *C. persica* (Boiss.) Grossh., *C. regalis* Gray, *C. trigonelloides* (Boiss.) Munz and *C. oliveriana* (DC.) Schrődinger (Fig. 4).

According to SEM images, pollen shape and sculpturing are diagnostic characters to distinguish the species, and maybe essential to draw significant conclusions on the relative closeness and distance of the various taxa. However, they are not enough to resolve taxonomic conflicts in the genus (not as Hasani *et al.* (2011) have recorded). Our results confirmed the embeded of *Aconitella* in *Consolida* as proposed before (Jabbour & Renner, 2011, 2012). Because the *Aconitella* species are occurred in two pollen groups. When the variation in pollen morphology in *Consolida* is compared with in certain gross morphological characters, such as tepal, leaf blade and petiole morphology, the existence of various type of pollen may be part of an inherent variability.

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