Rostaniha 21(2): 278–291 (2020)-Research Article

رستنیها ۲۱(۲): ۲۹۱–۲۷۸ (۱۳۹۹)–مقاله پژوهشی

DOI: 10.22092/BOTANY.2021.352043.1223

Palynological study of some species of *Anthemis* genus and its systematic implications

Received: 29.09.2020 / Accepted: 12.12.2020

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Abstract

To clarify taxonomic complexity within the *Anthemis* species, pollen grains of 19 species belonging to three sections of the genus *Anthemis* sensu stricto and two species of the genus *Cota* from Iran were examined by using scanning electron microscopy. The pollen grains morphological characteristics are provided for this genus in detail. Pollen grains were trizonocolporate and echinate. The pollen grains shape was prolate and spheroidal with the mean polar axes 20–35 µm and the mean equatorial diameter 20.3–26.5 µm. Among the studied taxa, *A. microcephala* showed the smallest pollen grains, and *A. odontostephana* possessed the largest ones. The spines were commonly conical with a broad basis, ornamentation within spines was generally rugulate-perforate. Thirteen pollen quantitative and qualitative characters have been analysed statistically by employed UPGMA, PCA and MDS in PAST software. Numerical analysis showed that, features as pollen shape, exine ornamentation, spine length and density were valuable characters for separating the examined taxa. In the resulting clusters, three groups within the investigated species have been recognized. The results derived from the present study, therefore, proved to be concomitant with the previous study on taxonomic infrageneric classification. The palynological evidences confirmed the sectional divisions, and revealed that, *A. brachystephana* and *A. lorestanica* in *A.* sect. *Anthemis*, have close relationship with *A. odontostephana*. The palynological characters of sect. *Anthemis* were similar to *Tripleurospermum* and *Cota*. The analysis of the palynological data confirmed the conventional taxonomic classification of the genus *Anthemis* rather than the phylogenetic classification of the genus.

Keywords: *Asteraceae*, chamomile, *Cota*, micromorphology, pollen

مطالعه گرده‌شناسی برخی گونه‌های جنس *Anthemis* و اهمیت سیستماتیکی آن

دریافت: ۱۳۹۹/۰۷/۰۸ / پذیرش: ۱۳۹۹/۰۹/۲۲

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اِرنست ویتک: محقق گروه گیاه‌شناسی، موزه تاریخ طبیعی وین، وین، اتریش

چکیده

با هدف روشن نمودن پیچیدگی‌های آرایه‌شناختی جنس *Amthemis*، ریخت‌شناسی دانه گرده ۱۹ گونه متعلق به سه بخش از جنس *Anthemis* (با مفهوم محدود) و دو گونه از جنس *Cota* از ایران با استفاده از میکروسکوپ الکترونی نگاره (SEM) مورد بررسی قرار گرفتند. در این تحقیق، مشخصات دقیق ریخت‌شناسی دانه‌های گرده این جنس ارایه می‌شود. دانه‌های گرده از نوع سه شیار-منفذی میانی و خاردار بودند. شکل گرده‌ها پرولیت و گرد با طول محور قطبی ۳۵-۲۰ میکرومتر و طول محور استوایی ۲۶/۵-۲۰/۳ میکرومتر بود. کوچک‌ترین دانه گرده در *A. microcephala* و بزرگ‌ترین آن در *A. odontostephana* یافت شد. خارها به طور معمول هرمی با قاعده پهن و آرایش سطحی بین خارها عمدتا چروکیده-حفره‌دار بودند. سیزده صفت کمی و کیفی به کمک روش UPGMA، MDS و PCA در نرم‌افزار PAST مورد بررسی قرار گرفتند. آنالیز عددی نشان داد که در جدایی آرایه‌های بررسی شده، صفاتی مانند شکل دانه گرده، تزیینات اگزین، طول و تراکم خارها، مشخصه‌های با ارزشی هستند. داده‌های گرده‌شناسی تقسیم‌بندی موجود بخش‌های این جنس را تایید نمود، به طوری که آنالیز خوشه‌بندی گونه‌های بررسی شده را به سه گروه مجزا تقسیم‌بندی کرد و مشخص شد که *A. brachystephana* و *A. lorestanica* از *A.* sect. *Anthemis* قرابت زیادی با *A. odontostephana* داشتند. مشخصات دانه‌های گرده *A.* sect. *Anthemis* با جنس‌های *Tripleurospermum* و *Cota* مشابهت زیادی داشت و داده‌های گرده‌شناسی جنس *Anthemis* از آرایه‌شناختی مرسوم نسبت به طبقه‌بندی فیلوژنی بیشتر حمایت می‌کند.

واژه‌های کلیدی: بابونه، کاسنیان، دانه گرده، ریزریخت‌شناسی، *Cota*

Introduction

Anthemis L. is a genus in the subtribe *Anthemidinae*, tribe *Anthemideae* of the family *Asteraceae* (Bremer & Humphries 1993, Funk *et al*. 2009). This genus in the narrow sense, comprises of approximately 175 species (Jeffrey 2007), which are widely distributed throughout Europe, south-western Asia, northern and eastern Africa, naturalized in North America, Australia, New Zealand and South Africa (Oberprieler 1998). The center of diversity of the genus is in SW Asia (Presti *et al*. 2010).

The historical taxonomy of *Anthemis* is rather complicated (Presti *et al*. 2010). It was first time described by Linnaeus in Genera Plantarum (1737), and officially published in Species Plantarum (Linnaeus 1753). Soon some authors partitioned *Anthemis* into a number of different genera: *Anthemis*, *Anacyclus* L., *Chamaemelum* Mill., *Maruta* (Cass.) Gray, *Ormenis* Cass., *Cladanthus* Cass., *Lepidophorum* Neck., *Archanthemis* Lo Presti & Oberpr., and *Cota* J. Gay. However, some segregations have been merged *Lyonnetia* Cass., *Maruta*, and *Ammanthus* Boiss. & Heldr. again in the *Anthemis* genus (Greuter 1968, Jeffrey 2007). *Anthemis* is related with the genera *Matricaria* L., *Chamaemelum*, and *Tripleurospermum* Sch. Bip. (Ghafoor 2010, Dauti *et al*. 2014). Based on molecular phylogenetic studies (Oberprieler 2001), *A.* subgen. *Anthemis* is most closely related to *Tripleurospermum*.

Comprehensive revision of Iranian *Anthemis* species, after Boissier's Flora Orientalis in 1875, and Eig's studies in 1938 on the oriental species, was made by Iranshahr (1986) in Flora Iranica and Mozaffarian (2008) in Flora of Iran. They reported 38 species of *Anthemis* in the wide sense (incl. genus *Cota*) from Iran, of which 15 taxa are endemics. In this view, *Anthemis* s.l. was divided into *A.* sect. *Anthemis* (21 species with two varieties), *A.* sect. *Maruta* (Cass.) Griseb (four species), *A.* sect. *Ramata* (four species with one subspecies and one variety), and *A.* sect. *Odontostephana* (one species with two varieties). For the genus *Cota*, they recorded nine species along with three subspecies and four varieties.

Many reports have been published on the palynology of *Anthemideae* (Čigurjaeva & Tereškova 1983, De Leonardis *et al*. 1991, Martin *et al.* 2003, Hayat *et al.* 2010, Stanski *et al*. 2018). Pellicer *et al.* (2009) examined the seven genera of this tribe and confirmed the results of Stix (1960) and Martin *et al.* (2003), who reported two different pollen types: *Anthemis*-type (echinate) and *Artemisia*-type (microechinate) based on exine ornamentations. The *Anthemis* type that introduced by Stix (*l.c.*) was found to be rather uniformly sphaeroidal, trizonocolporate and echinate. Two separate comprehensive studies conducted by Ceter *et al.* (2013) and Özbek *et al*. (2016) on pollen grains of *Matricaria*, *Tripleurospemum*, and *Cota*, respectively. They found that, pollen shape and exine ornamentations showed diagnostic values.

Wodehouse (1926, 1935) studied pollen grain characteristics in *Anthemis cotula* and *A. nobilis* from 1926 to 1935. Afterwards, several results on pollen morphology of *Anthemis* s.l. have been published (Skvarla 1971, Dauti *et al*. 2001, Oberprieler & Vogt 2006, Punt & Hoen 2009, Altan & Akyalçin 2017, Olanj *et al.* 2017).

We hypothesized that, pollen morphology could clarify taxonomic problems within the *Anthemis* species in Iran. Therefore, pollen grain morphological characteristics of 21 taxa belonging to the *Anthemis* s.l. (ten out of 21 species endemic of Iran) have been analysed based on type and approved materials.

Materials and Methods

- Pollen morphological analysis

Pollen grains of 19 species of the genus *Anthemis* s.s. and two species of the genus *Cota* were studied by scanning electron microscopy (SEM).

Because of difficulties in recognizing *Anthemis* species, pollen materials were removed from type and approved herbarium samples, which were deposited in W, TARI and T herbaria (acronyms according to Thiers 2019). Voucher specimens for this analysis are listed in table 1 and details for the specimens of W herbarium can be found in JACQ (2019). Their names are accepted by POWO (2020) and Flann (2009). For scanning electron microscopy study (SEM), pollen grains were transferred into stubs and then coated with gold. This study is focused on 15 quantitative and qualitative characters (Table 2). Measurements were based on at least 20–30 developed grains per specimen for each morphological character. Micrographs were obtained using SEM JSM-6380A, JEOL at Shahid Beheshti University (Tehran, Iran). The terminology was obtained from Faegri & Iversen (1992), Hesse *et al.* (2009), Punt & Hoen (2009).

- Numerical analysis

For the multivariate analysis, 13 palynological characters were selected of the studied taxa (Table 2). For these average values for each species have been determined. After linear standardization by range of each variable of the original data set. Cluster analysis by using UPGMA were performed to check the similarity and dissimilarity between different taxa of the studied species. The un-weighted paired-group average algorithm (UPGMA) yielded the highest coefficient of cophenetic correlation. Principle Component Analysis (PCA) was employed for identification of valuable pollen morphological characters used for taxonomy (Podani 2001, Atazadeh *et al.* 2020). Non metric-MDS performed to calculated Euclidean and taxonomic distance between the studies species (Podani 2001).

All computations were made using the Past software Ver. 3.22.

Results

Details of the characteristic features of studied pollen grains are as follows and their traits are summarized in table 2:

- Size and shape

The pollen grains of the studied species were radially symmetrical and isopolar, spines, with the mean polar axes 20–35.2 µm and the mean equatorial diameter 20.1–26.5 µm. The size of pollen grains was different; their dimensions were smaller in *A. microcephala* and larger in *A. odontostephana*. Pollen grains showed circular outline in polar view, while in equatorial view, elliptic and circular outline were observed. According to the P/E ratio, two distinct pollen shapes could be recognized: prolate (P/E = 1.1–1.4) in *A. odontostephana*, *A. brachystephana*, and speroidal (P/E = 0.92–1.1) in rest studied species (Table 2 & Figs 1 and 2).

- Aperture

The pollen apertures of all examined species were trizonocolpororate. The colpi were usually wider at the pore and narrower near the poles (Figs 1 & 2). The mean of colpus length varied from 9.3 µm in *A. candidissima* to 24.2 µm in *A. odontostephana*. It is strongly correlated with length of polar axis. The mean of colpus width ranged from 0.3 µm in *A. gayana*, and *A. candidissima* to 2.2 µm in *A. mirheydari*. The mean of Mesocolpial area varied from 11.7 to 16.5 µm. The pore diameter ranged between 4.3–6.9 µm. The colpi were not fused at the poles, and the Apocolpium diameter ranged from 5 µm in *A. gilanica* and 10.5 µm in *A. odontostephana* (Table 2).

Table 1. Studied taxa along with their related data

Taxon	General distribution	Locality	Voucher No.
Sect. ***Anthemis***			
Anthemis atropatana Iranshahr	Caucasus, Iran	Azerbaijan prov.: Tabriz-Sufian	W 1984-0009117
A. austroiranica Rech.f., Aellen & Esfand.	Iran (endemic)	Semnan prov.: Shahrud-Bastam	W 1978-0003986
A. brachystephana Bornm. & Gauba	Iran (endemic)	Alborz prov.: Karaj-Saveh	W 1978-0007813
A. bushehrica Iranshahr	Iran (endemic)	Bushehr prov.: Borazjan, Chahkhani	W 1986-0005735
A. candidissima Willd. ex Spreng.	Turkey to C. Asia	W. Azerbaijan prov.: Piranshahr-Sardasht	W 1975-0005471
A. gayana Boiss.	Iran (endemic)	Esfahan prov.: Kolah Ghazi National Park	W 1984-0009305
A. gilanica Bornm. & Gauba	Iran (endemic)	Alborz prov.: Pashand	W 1956-0003900
A. gracilis Iranshahr	Iran (endemic)	Esfehan prov.: Shahreza-Semirom	W 1986-0005318
A. haussknechtii Boiss. & Reut.	Iran, Syria	Lorestan prov.: Chamchid	W 1956-0004837
		Lorestan prov.: Mirabad	W 1960-0015983
A. hyalina DC.	Europe, Lebanon to Iran, Saudi Arabia	E. Azerbaijan prov.: Azaran	W 1982-0007461
		Ardabil prov.: Khalkhal, Hashjin	T 2017-5890
A. leptophylla Eig.	Saudi Arabia, Iraq, Iran	Iraq: Kirkuk	W 1981-0009767
A. lorestanica Iranshahr	Iran (endemic)	Lorestan prov.	W 1986-0005734
A. mirheydari Iranshahr	Iran (endemic)	Hormozgan prov.: Bandar-abbas, Qotbabad	W 1975-0021811
A. moghanica Iranshahr	Iran (endemic)	Ardabil prov.: Moghan	W 1966-0020218
A. persica Boiss.	Iran (endemic)	Fars prov.: Takhte-Jamshid	W 1959-0022634
A. schizostephana Boiss. & Hausskn.	Iraq, Iran	Iraq: Kurdistan	W 1981-0009755
A. susiana Nábělek	Iraq, Iran	Khuzistan prov.: Ahvaz	W 2016-0012520
Sect. ***Murata*** (Gass.) Boiss.			
A. microcephala (Schrenk) B. Fedtsch.	Iran, Iraq, Afghanistan, C. Asia	Fars prov.: Kotal-Dokhtar	W 1974-0022247
Sect. ***Odontostephana*** Boiss.			
A. odontostephana Boiss.	Temperate and tropical Asia	Fars prov.: Takht-e Jamshid	W 2016-0012527
		Zanjan prov.: Damirlu	TARI 2015-141501
Cota J. Gay			
Cota austriaca (Jacq.) Sch. Bip.	Europe to W. Asia	Ardabil prov.: Meshkinshar, Ahar-chay	W 1984-0009110
C. wiedemanniana (Fisch. & C.A. Mey.) Holub	Anatolia, Transcaucasia, Iran	W Azerbaijan prov.: Urmieh-Qushchi	W 1984-000916

Table 2. Pollen grains morphological data of *Anthemis* and *Cota* species

Taxon	P (µm)	E (µm)	PE	SL (µm)	SWB (µm)	DBS (µm)	PNBS	NS	PW (µm)	CW (µm)	CL (µm)	MES (µm)	APO (µm)	PS	OIA
Anthemis atropatana (A1)	23.5±2	23.7±1.8	0.98	2.6±0.4	3.1±0.3	5.7±1	5–10	4–5	5.8±0.3	1.1–1.7	12.6–14.9	13.8±2	6.8±0.7	S	Ru. P
A. austroiranica (A2)	25±1.5	25.7±1.8	0.97	2.4±0.5	3.7±0.5	5.5±2	6–10	4–5	5.4±0.2	0.5-0.9	14±1	16±1.5	9±1.5	S	Ru. P
A. brachystephana (A3)	31.5±2.7	24.9±2	1.27	2.1±0.6	2.8±0.3	6.7±1	4–7	3–4	6±0.8	0.9±0.4	20.7±1.6	16±2.2	7.5±1.5	P	Ru. P
A. bushehrica (A4)	21.5±1.6	22.3±2	0.96	2.6±0.6	2.8±0.3	4±1.2	7–10	6–7	4.5±0.3	0.9±0.2	11.5±0.8	12.3±1	8.7±0.8	S	Ru. P
A. candidissima (A5)	22.7±2	23.5±1.9	0.97	3.3±0.4	3.6±0.4	5.3±1.2	8–10	5–6	5±0.5	0.3±0.3	9.3±1.5	14.5±1.5	8.6±1.6	S	Ru. P
A. gayana (A6)	28.3±3.5	25.4±3	1.12	3.3±0.5	3.3±0.2	6.5±1.2	6–10	3–4	6.8±0.3	1±0.2	14±2.2	14.7±2	10±1.5	P	Ru. P
A. gayana (A7)	21.4±2	21.8±1.9	0.98	3.5±1	3.5±0.4	5.3±1.1	8–10	5–7	5±0.5	0.2±0.2	11.5±1.3	11.8±1.5	8±1.5	S	Ru. P
A. gilanica (A8)	23±3.1	23.1±3	0.99	4.3±0.8	3.4±0.5	4.4±1.3	6–10	6–7	5±0.6	3±0.2	13.5±0.3	12.3±1.5	5±0.4	S	Ru. P
A. gracilis (A9)	24.9±2.3	25.1±2.5	0.99	3±0.2	3.4±0.2	6±0.5	5–7	4–5	5.8±0.7	1.6±0.6	17±0.3	15±2	9±0.7	S	Ru. P
A. haussknechtii (10)	24.4±2.2	26.5±1.5	0.92	2.9±0.2	2.8±0.3	7.6±1.2	5–9	5–7	5.3±0.3	0.5±0.3	16±0.5	13±1	7.7±0.6	S	Ru. P
A. haussknechtii (A11)	23.3±2.4	23.7±1.9	0.98	3±0.2	3±0.2	5.8±1	7–11	5–7	5±0.3	0.9±0.4	14±0.5	12.3±0.2	7.2±0.6	S	Ru. P
A. hyalina (A12)	23.1±1.9	23.5±2	0.98	2.5±0.1	3.4±0.5	5±0.6	5.–7	4–5	5.8±0.7	1.3±0.5	15.5±08	13.9±0.5	8.9±0.4	S	Ru. P
A. hyalina (A13)	23.5±2	23.6±2	1	2.5±0.4	3.1±0.5	6.8±1	6–10	4–5	6±0.5	0.9±0.3	14.5±0.4	13±0.9	9±0.9	S	Ru. P
A. leptophylla (A14)	22.3±1.6	23.2±1.9	0.97	2.9±0.3	2.9–3.8	5.5±1	9-15	5–7	6±0.6	1.3±0.5	14±1	13.6±0.6	8.3±0.7	S	Ru. P
A. lorestanica (A15)	27.6±3.3	22.9±2.2	1.21	2.1±0.4	2.5±0.6	6.9±0.7	2-8	3–4	6.6±0.6	1.3±0.2	20.5±1.3	14.8±0.8	7.5±1	P	Re. P
A. mirheydari (A 16)	25.1±2.1	25.6±1.9	0.98	2.3±0.2	2.8-3.3	6±0.4	9–11	4–5	6.9±0.3	2.2±0.3	18.8±1.4	14.7±0.6	8.5±0.6	S	Ru. P
A. moghanica (A 17)	23±1.5	23±1.6	1	3±0.2	2.8±0.4	5.5±1.4	7–10	5-7	5.1±0.5	1.3±0.5	12.6±0.6	12.8±0.5	8±1	S	Ru. P
A. persica (A18)	25.2±1.6	25.4±1.5	0.99	2.8±1	3.3±0.8	6.6±1.4	7–10	4–5	5.5±0.7	1.6±0.3	18.5±1.5	16.5±0.6	6.6±3	S	Ru. P
A. schizostephana (A19)	22.1±2	22.4±2	0.99	3±0.2	3.5±0.5	6.5±1.3	5–8	4-7	5.2±0.2	0.6±0.3	13.2–14	15.3±0.2	7.44±0.3	S	Ru. P
A. schizostephana (A20)	22.4±4	21.4±4	1.06	3.2±0.3	3.3±0.6	6.1±1.2	5-8	5–7	6.5±0.5	1.3±0.5	12.5±1.5	14±1.3	7.9±0.5	S	Ru. P
A. susiana (A21)	24.7±1.8	22.5±1.5	1.09	2.6±0.2	2.9±0.5	5.8±1.6	6-10	4–5	5.5±0.4	1.6±0.6	16±0.8	12.1±1	9.5±1	SP	Ru. P
Cota austriaca (C1)	25.5±2.8	25.4±3	1	2 ±0.4	2.3±0.4	4.8±0.6	5-8	4–5	4.3±0.5	1.6±0.5	15±2.2	14.8±1	9±2.2	S	Ru. P
C. wiedemanniana (C2)	24.2±2	24.7±2.2	0.98	2.5±0.4	2.9±0.5	6.5±0.5	8-12	5–7	5.3±0.5	0.3±0.2	12.5±2	14±1.5	9.5±1	S	Ru. P
A. microcephala (A22)	20 ±2.2	20.1 ± 1.9	1	2.9±0.2	3.5±0.5	6.6±1.4	4-8	5–7	4.8±0.9	1.1±0.2	12±1.3	12.5±0.8	7.7±1	S	Mic
A. odontostephana (A23)	35.2±3	24.1–26.6	1.39	1.9±0.2	2.6±0.5	7±1.5	5–8	3–4	6.9±0.8	0.8±0.4	24.2±2	15.3±1.5	10.5±1.1	P	Re. P

Abbreviations: P: polar axis, E: equatorial axis, SL: spine length, SWB: spine width at base, DBS: distance between spines, PNBS: perforation number at base spine, NS: number of spines, PW: pore width, CW: colpous width, CL: colpous length, MES: mesocolpium, APO: apocolpium, APT: aperture type trizonocolpororate EO: echinate ornam., PS: pollen shape (P: prolate and S: speroidal), OIA: ornamentation of interspinal area, Ru. P: rugulate-perforate, Mic.: microreticulate, Re. P: reticulate-perforate.

- Exine ornamentation

Exine sculpturing was echinate in all investigated species (Figs 1 & 2). The spines were commonly conical with a broadened base and a tapered apical portion (acuminate). Their mean length varied from 1.9 µm in *A. lorestanica* and *A. odontostephana* to 4.3 µm in *A. gilanica*, while their width was 2.3–3.7 µm. The mean distance between spines varied from 4 µm in *A. bushehrica* and *A. gilanica* to 7.6 µm in *A. lorestanica*. Pore at the base of the spines were distinct and larger than the others, their number is 4–12 (mainly 8). The number of spine in 100 µm^2 among in the studied pollens grains varied from 3 to 6. This character was correlated to distance between spines and spine wide at the base. The basic ornamentation of the inter-spinal area was rugulate-perforate while, *A. microcephala* with microreticulate, and *A. lorestanica* and *A. odontostephana* were recognizable with reticulate-perforate ornamentation (Table 2, Figs 1 & 2).

- Numerical analysis

The dendrogram constructed by UPGMA based on 13 palynological variables of 21 taxa (OTUs) is presented in figure 3. This dendrogram shows the similarities that exist among the studied taxa. Cluster analysis divided the taxa into three main groups with a 60% level of similarity; the first one comprised of members of *A.* sect. *Odontostephana* (A23) along with *A. brachystephana* (A3), *A. gayana* (A6), and *A. lorestanica* (A15) from *A*. sect. *Anthemis*. The second one comprises members of *A.* sect. *Anthemis*: *A. atropatana* (A1), *A. austroiranica* (A2), *A. bushehrica* (A4), *A. candidissima* (A5), *A. gayana* (A7), *A. gilanica* (A8), *A. gracilis* (A9), *A. haussknechtii* (A10, A11), *A. hyalina* DC. (A12, 13), *A. leptophylla* (A14), *A. mirheydari* (A 16), *A. moghanica* (A 17), *A. persica* (A18), *A. schizostephana* (A19, 20), *A. susiana* (A21), and two selected species from *Cota*, *C. austriaca* (C1), *C. wiedemanniana* (C2), and the third group *A.* sect. *Murata*: *A. microcephala* (A22).

The first two axes of the PCA explain 68.44% of the total variance, with first and second axes explaining 57.765% and 10.68% of the variance, respectively (Fig. 5). The most significant variables of the first principal component were: polar axis (P), Pollen shape (PSH), equatorial diameter (E), and colpus length (Clg). The most significant variables of the second axis were: ornamentation of the inter-spinal (OIA) and distance between spines (DS). The cluster analysis results were confirmed by Non-parametric MDS and PCA analysis (Figs 4 & 5). This separated the studied species into three groups, the first group showed that, *A. brachystephana* (A3) and *A. gayana* (A6), *A. lorestanica* (A15), and the species of *A.* sect. *Odontostephana* were closely associated. In the second group, the selected species of *Cota* were very similar to the species belonging to *A.* sect. *Anthemis*. *Anthemis microcephala* (A22) was distinct from the other two groups, and it was considered as an independent group.

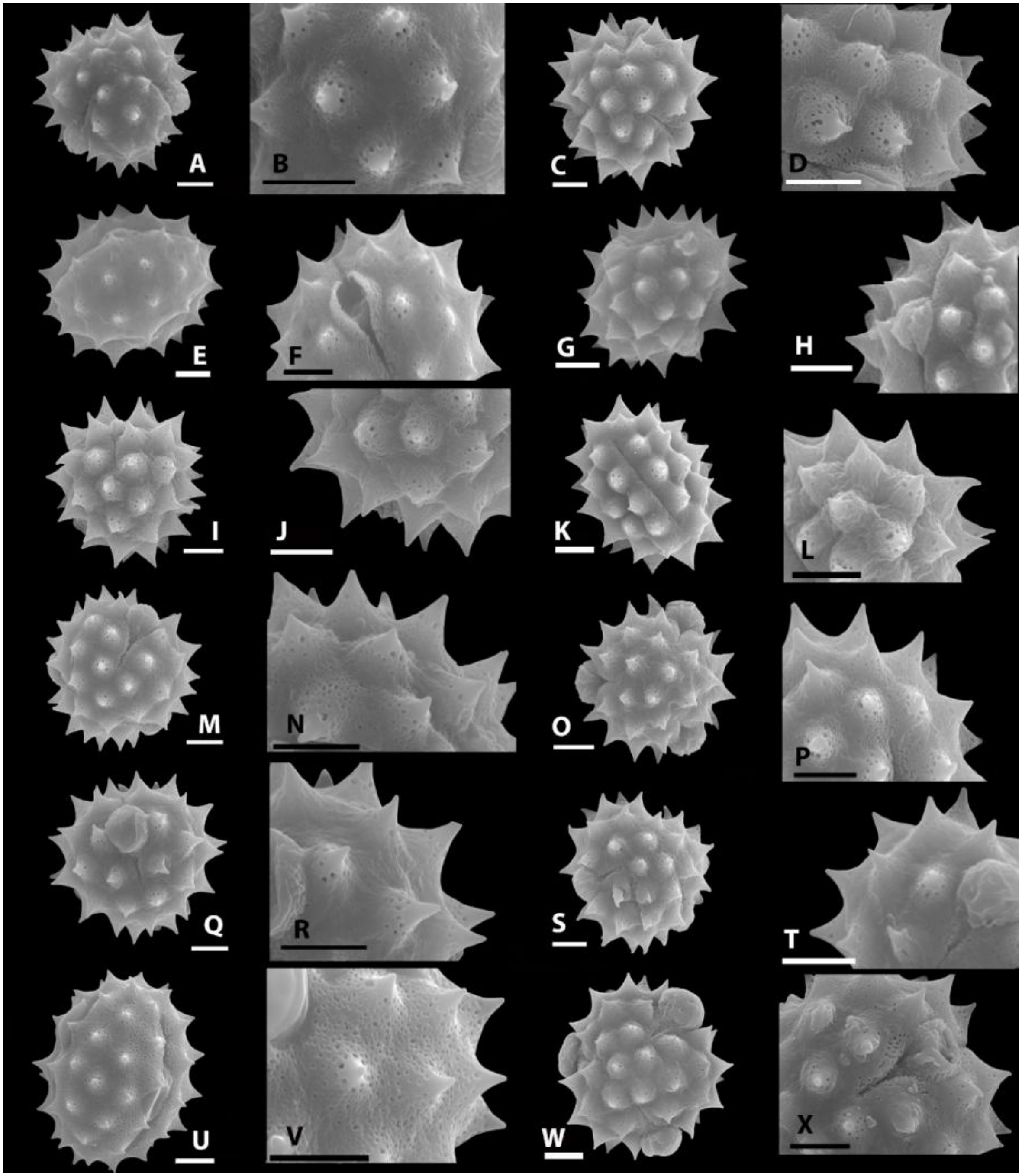

Fig. 1. Pollen grain morphology of *Anthemis*: A, B. *A. atropatana*, C, D. *A. austroiranica*, E, F. *A. brachystephana*, G, H. *A. bushehrica*, I, J. *A. candidissima*, K, L. *A. gilanica*, M, N. *A. gracilis*, O, P. *A. haussknechtii*, Q, R. *A. hyaline*, S, T. *A. leptophylla*, U, V. *A. lorestanica*, W, X. *A. mirheydari* (Bars = 5 µm).

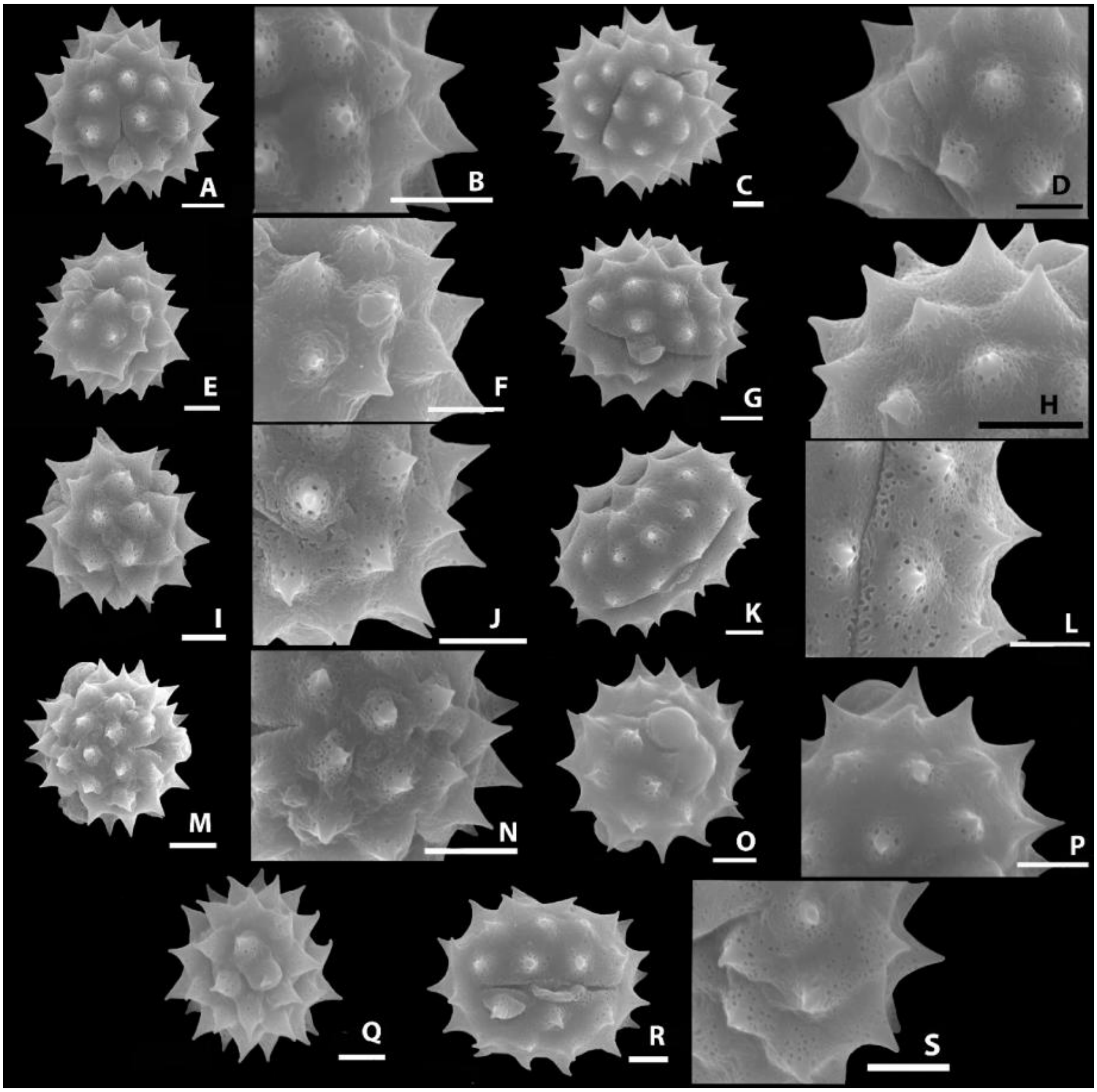

Fig. 2. Pollen grain morphology of *Anthemis*: A, B. *A. moghanica*, C, D. *A. persica*, E, F. *A. schizostephana*, G, H. *A. susiana*, I, J. *A. microcephala*, K, L. *A. odontostephana*, M-O. *A. gayana*, and *Cota*, P, Q. *C. austriaca*, R, S. *C. wiedemanniana* (Bars = 5 µm).

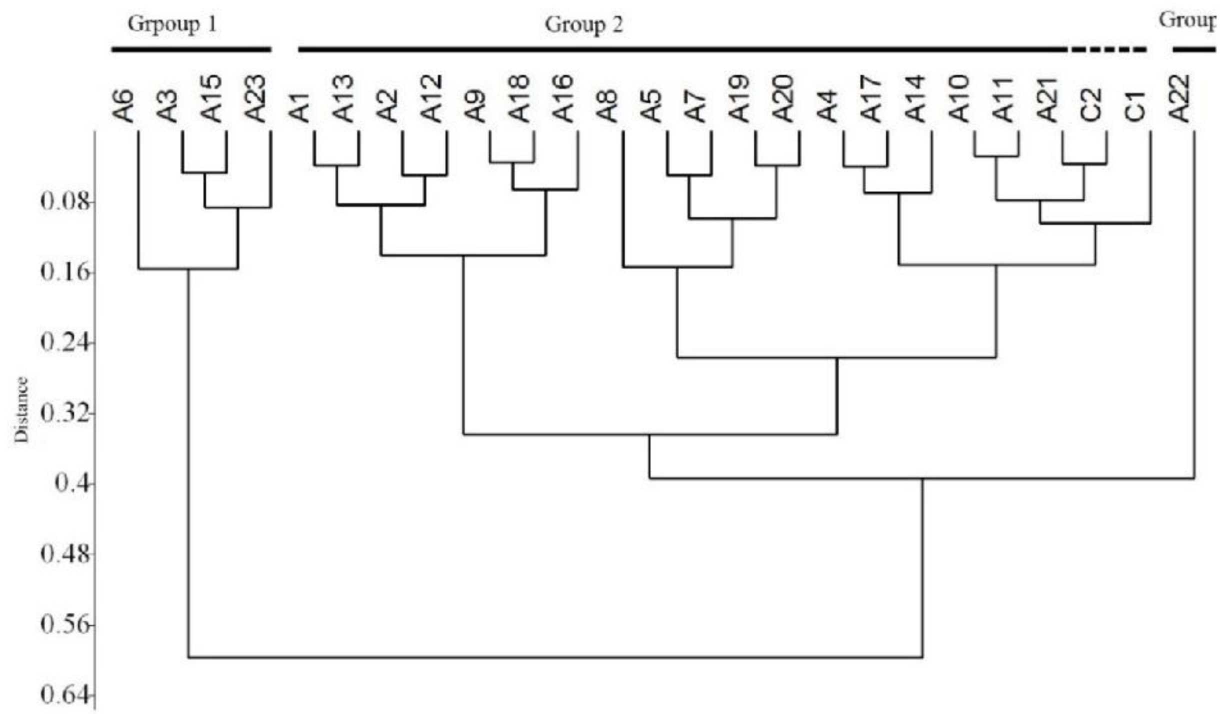

Fig. 3. Cluster analysis (UPGMA, Euclidean distance) performed with the pollen grain morphological characters and the species from the studied species. A1: *Anthemis atropatana*, A2: *A. austroiranica*, A3: *A. brachystephana*, A4: *A. bushehrica*, A5: *A. candidissima*, A6: *A. gayana*, A7: *A. gayana*, A8: *A. gilanica*, A9: *A. gracilis* A10: *A. haussknechtii*, A11: *A. haussknechtii*, A12: *A. hyaline*, A13: *A. hyaline*, A14: *A. leptophylla*, A15: *A. lorestanica*, A16: *A. mirheydari*, A17: *A. moghanica*, A18: *A. persica*, A19: *A. schizostephana*, A20: *A. schizostephana*, A21: *A. Susiana*, A22: *A. microcephala*, A23: *A. odontostephana*, C1: *C. austriaca*, C2: *C. wiedemanniana*.

Discussion

The species investigated in the present study cover most of Iranian *Anthemis* species. The pollen grains of the genus are homologous, generally in the type of aperture (tricolporate) and ornamentation (echinate). The most common pollen grain shape in *Anthemis* is spheroidal, or prolate (in *A.* sect. *odonthostephana* and related species). The pollen morphology of studied species is similar to previously introduced types, like *Anthemis* type (Stix 1960, Moore *et al*. 1991, Erdtman *et al*. 1969); *Achillea* type (Faegri *et al*. 1989), and *Anthemis arvensis* type (Punt & Hoen 2009).

The conducted analysis confirms prior published results (Oberprieler *et al*. 2007, Çeter *et al*. 2013, Özbek *et al*. 2016). Pollen shape, exine ornamentation, spine numbers, length and density have diagnostic value to separate most taxa of the genus *Anthemis*.

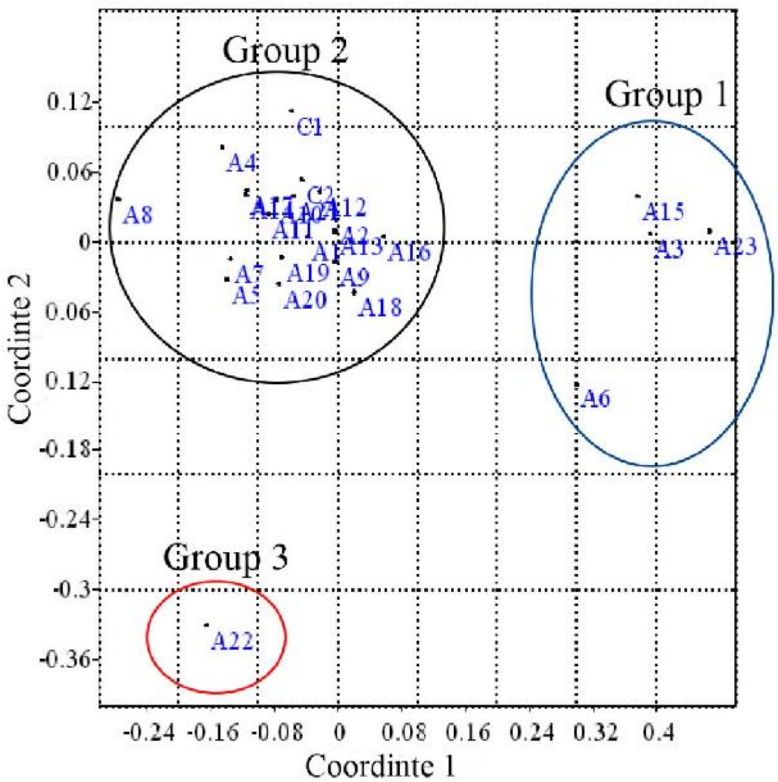

Fig. 4. Non-metric MDS analysis based on main pollen grain morphological characters. A1: *Anthemis atropatana*, A2: *A. austroiranica*, A3: *A. brachystephana*, A4: *A. bushehrica*, A5: *A. candidissima*, A6: *A. gayana*, A7: *A. gayana*, A8: *A. gilanica*, A9: *A. gracilis* A10: *A. haussknechtii*, A11: *A. haussknechtii*, A12: *A. hyaline*, A13: *A. hyaline*, A14: *A. leptophylla*, A15: *A. lorestanica*, A16: *A. mirheydari*, A17: *A. moghanica*, A18: *A. persica*, A19: *A. schizostephana*, A20: *A. schizostephana*, A21: *A. Susiana*, A22: *A. microcephala*, A23: *A. odontostephana*, C1: *C. austriaca*, C2: *C. wiedemanniana*.

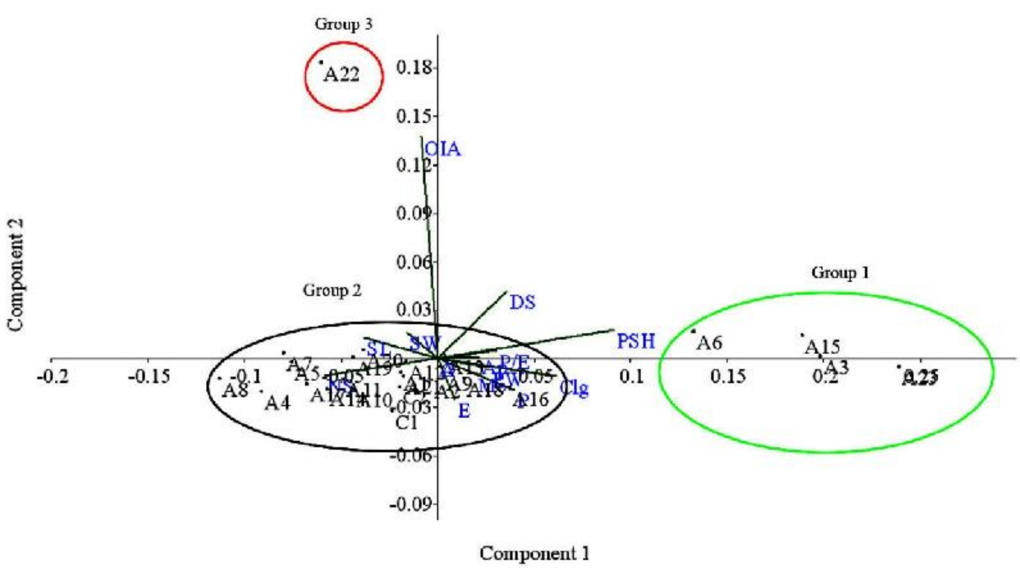

Fig. 5. PCA analysis based on main pollen morphological characters.

There is no doubt that, *Anthemis* and *Cota* are closely related genera. Altan & Akyalçin (2017) also stated that, morphological properties of pollen grains belonging to *Anthemis* and *Cota* have similarities. Our findings indicated that, pollen morphology of *Cota* species have more similarity with *A.* sect. *Anthemis*. In other word, pollen grains results do not support the molecular segregation (Presti *et al.* 2010) of *Cota* and *Anthemis*. Oberprieler (2001) in his polygenetic study showed that, *Anthemis* is also closely related to *Tripleurospermum.* By comparing the findings of this study and Çeter *et al*. (2013) results, this affinity is confirmed too.

Dividing of the studied species into three sections (Eig 1938, Yavin 1970, Iranshahr 1986, Oberprieler 1998) on the basis of floral structure is approved by palynological data. The three groups found in our analyses show the same differentiation.

According to Eig (1938), Yavin (1970), and Oberprieler (1998) *A.* sect. *Odontostephana* (incl. *A. odontostephana*) has many unique morphological characters. The shape of pollen grains in this section is prolate, while the most common shape of pollen in *Anthemis*, *Cota*, and *Tripleurospermum* is spheroidal (Çeter *et al*. 2013, Özbek *et al*. 2016). Further, morphological characters like spine number, spine length, apocolpium and mesocolpium are different from other sections. Therefore, pollen grains traits in contrast to molecular data (Presti *et al.* 2010) support *A.* sect. *Odontostephana* as an independent taxon. Flann (2009) considered *Anthemis gayana* as synonym of *A. odontostephana.* According to Olanj *et al*. (2017), pollen grain of *A. gayana* has spheroidal shape, P and E mean value are 28.64 and 30.65 µm, respectively. In this study, two types of pollen grains were observed in *A. gayana* specimens, oblate (P/E = 1.12) and spheroidal (P/E = 9.8) (Table 2), the oblate pollen type has large number. Palynological data confirm the affinity of *A. gayana* and *A. odontostephana* and does not support molecular findings.

The palynological features of most investigated species of *A.* sect. *Anthemis* are similar in size, shape, sculpture and colpus. These characters do not have diagnostic value to separate most species of this section. However, *A. brachystachys* and *A. lorestanica* that belong to *A.* sect. *Anthemis,* their pollen traits are similar to *A.* sect. *Odontostephana*. These results indicated that, *A.* sect. *Anthemis* does not form a homogenous group.

Anthemis sect. *Maruta* is morphologically characterized by subulate pales (Yavin 1970, Presti *et al.* 2010). According to morphological and molecular data, this section shows some similarities with *A.* sect. *Anthemis* (Yavin 1972, Grierson & Yavin 1975, Presti *et al.* 2010). In this section, *A. microcephala* was examined, the pollen characters of this species (incl. pollen size, ornamentation in spine, and spine density) is similar to *A. macrotis* (Oberprieler & Vogt 2006) and *A. cotula* (Altan & Akyalçin 2017). This similarity supported by phylogenetic tree (Clade C in Presti *et al.* 2010). Statistical analysis confirmed the independence of this section.

Conclusion

Although, the general features of *Anthemis* pollen grains showed high concordance, our palynological analyses highlighted that pollen grains morphology is good taxonomic marker and can be utilized for infrageneric classification of genus *Anthemis.* The division of the studied species into three section on the basis of floral structure was approved by palynological data and revealed that, *A.* sect. *Anthemis* does not form a homogenous group. This study, also supported the reunion of *Cota* with *Anthemis.* It is worth mentioned that, the result obtained from the analysis of palynological data were found to more compatible with the accepted conventional taxonomic classification of the genus *Anthemis* rather than the phylogenetic approaches using molecular data. Clearly, further research will be required to validate *Anthemis* species taxonomy.

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