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Pollen morphological traits analysis in the genus *Hymenocrater* Fisch. & C. A. Mey., (Lamiaceae)

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Abstract

Hymenocrater Fisch. & C. A. Mey., a perennial genus of the Lamiaceae family, is mainly distributed in Iran. These aromatic plants produce several biologically important secondary metabolites. Although several investigations have been conducted on the genus, no inclusive palynological study is available for these species. In this evaluation, we studied pollen grain morphological traits in 10 *Hymenocrater* species, of which five are endemic to Iran. The pollen grains were acetolyzed according to the Erdtman method, and up to 25 pollen grains were examined per species using scanning electron microscopy and light microscopy. Our findings revealed that all species had the 6-zonocolpate monad pollen grains, except for a species that generate octocolpate pollen grains. Pollen size varied from large (in most species) to small. Pollen grains' shape differed as prolate, subprolate or spheroidal. The equatorial outlines were detected as truncate, obtuse convex or rarely circular. The outlines in ambitus were obtuse convex or circular. Moreover, most species exhibited a bireticulate exine surface sculpturing pattern and rarely reticulate. According to UPGMA clustering, these species were clustered into four groups; PCA biplot revealed that some species were characterized by the diagnostic palynological characteristics. However, the species clustering pattern did not agree with those which were detected by phylogenetic, anatomy and micromorphological data in the previous studies. Our findings indicated that, among the evaluated characteristics, the pollen grains shape and their ambitus were more variable than others and had a limited taxonomic value for the genus. However, other evaluated characteristics lack a taxonomic importance in species delamination or clustering due to their infraspecific variabilities or stability among the species.

Keywords Aromatic plant · Gol-e-Arvaneh · Nepetoideae · Palynology · Taxonomic value

1 Introduction

Hymenocrater Fisch. & C. A. Mey. is a genus of Nepetinae Coss. & Germ. (Nepetoideae-Lamiaceae) and comprises 11 species that are mainly distributed in Iran (Pojarkova 1954; Rechinger 1982). Jamzad (2012) listed nine species for Iran, five of which are endemic to the country. In Persian, the genus is named Gol-e-Arvaneh (Morteza-Semnani et al. 2016; Tabaripour et al. 2021).

Hymenocrater species are shrubs or perennial aromatic herbs with numerous erect, glabrous or pubescent branches. The leaves are ovate or broad ovate, dentate and acute. These species have numerous, sessile or short-pedicled flowers with a long and indistinctly 2-lipped corolla in different shaped inflorescences (Rechinger 1982; Jamzad 2012).

According to Morteza-Semnani et al. (2016), species of the genus synthesize several important secondary metabolites such as phenolic acids, terpenoids, essential oil and flavonoids, which exhibit different biological properties, including antioxidant, antidiabetic, antimicrobial, antiparasitic and anticancer.

There are several investigations on the leaf and stem anatomy (Jafari and Jafarzadeh 2008), chemical composition of essential oil (Morteza-Semnani et al. 2012; Sabet Teimouri et al. 2012; Shahriari et al. 2013), flavonoid compounds (Gohari et al. 2009; Al-Anee et al. 2015), leaf indumentum

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and nutlet micromorphology (Serpooshan et al. 2014), morphology and genetic diversity (Tabaripour et al. 2021) of the *Hymenocrater* species. In addition, Jafari and Jafarzadeh (2008) evaluated the pollen grains morphological characteristics in four species of the genus, including *H. platystegius*, *H. calycinus*, *H. bituminosus* and *H. elegans*, and indicated their taxonomical importance, while no comprehensive study is available on the palynology of all *Hymenocrater* species in Iran and the world.

Due to morphological similarity and taxonomic complexity in the genus *Hymenocrater* (Tabaripour et al. 2021), in this evaluation, we studied pollen grains morphology in 10 *Hymenocrater* species. This investigation was aimed at: (1) describing pollen morphological characteristics in some species for the first time; (2) comparing palynological characteristics of the studied species, and (3) examining the taxonomical importance of the pollen grain morphological traits at the infrageneric level.

2 Material and methods

Plant material – We investigated the pollen grains characteristics in 10 species of the genus *Hymenocrater*. We identified the plant samples according to the morphological descriptions and keys is available in Flora of Iran (Jamzad 2012) and Flora Iranica (Rechinger 1982). The herbarium samples of these species were housed at Shahid Beheshti University (HSBU), Iranian Research Institute of Plant Protection (IRAN), and Wien (W) (Table 1).

Light microscopy (LM) – The pollen grains were prepared for light microscopy (LM) study based on the acetolysis method (Erdtman 1960). We measured 25 acetolyzed pollen grains per each species using an Olympus light microscope and a digital camera. Measurements included equatorial and polar axes length, length and width of ecotoaperture (colpus), apocolpium and mesocolpium width. Image tools (ver. 2) was used for these measurements.

Scanning electron microscopy (SEM) – We directly transferred pollen grains to some stubs were covered with a double-sided transparent tape. Then, the stubs were coated with a gold layer using a JFC–1600 Auto Fine Coater and photographed with an SU 3500 scanning electron microscope at 15 kV. The investigated palynological traits included: equatorial (E) and polar (p) axes length and their ratio, equatorial and ambitus shape, ecotoaperture length and width, endoaperture (porus) diameter, exine sculpturing type and dimensions. We used Erdtman (1960) and Moore et al. (1991) terminologies for descriptive terms. We determined the shape of pollen based on the polar/equatorial axes length ratio (P/E ratio). Additionally, we termed the pollen grains as spheroidal, if $P/E = 0.88–1.14$, the grains are termed prolate and oblate, if $P/E >$ ca 1.2, and $P/E <$ ca. 0.8, respectively, based on Punt et al. (2007).

Statistical analyses – We calculate the mean and standard deviation for the quantitative pollen grains traits, subsequently data were standardized and employed for unweighted pair group method with arithmetic mean (UPGMA) and PCA biplot analyses (Podani 2000). The Euclidean distance was used for as similarity index in clustering analyses. We used Past version 2 software for statistical analyses (Hammer et al. 2001).

3 Results

General description of pollen grain morphology – All the examined species have the 6-zonocolpate monad pollen grains, except for *H. oxyodontus* which has the octocolpate pollen grains**.** A description of the pollen grains morphology of these species is presented using some SEM micrographs (Fig. 1) and light images (Fig. 2). The quantitative pollen

Table 1 Locality address and voucher number of the evaluated species (asterisks indicate the endemic species)

Species	Localities	Voucher no
H. altimuranus Rech.f	Afghanistan	W1983-0007198
H. bituminosus Fisch. & C.A. Mey	Iran, Qazvin province, 5 km to Bahram Abad	HSBU2014446
*H. calycinus** (Boiss.) Benth	Iran, Mazandaran province, Sari, Malkhast village, margin of Badab-e Surt	HSBU2014443
*H. elegans** Bunge	Iran, Mazandaran province, Baladeh road to Nur, after Iva village	HSBU2014440
H. incanus Bunge	Iran, Markazi province, Arak, Gavar	HSBU2014445
H. longiflorus Benth	Iran, Kurdistan province, Marivan, Bandul village	HSBU2014438
*H. oxyodontus** Rech. f	Iran, Semnan province, Shahroud, Koh-e Peyghambaran	1529(IRAN)
*H. platystegius** Rech. f	Iran, Razavi Khorasan province, Kashmar	HSBU2014447
H. sessilifolius Benth	Afghanistan	W1978-0017299
*H. yazdianus** Rech. f	Iran, Yazd province, Shir Koh	HSBU2014444

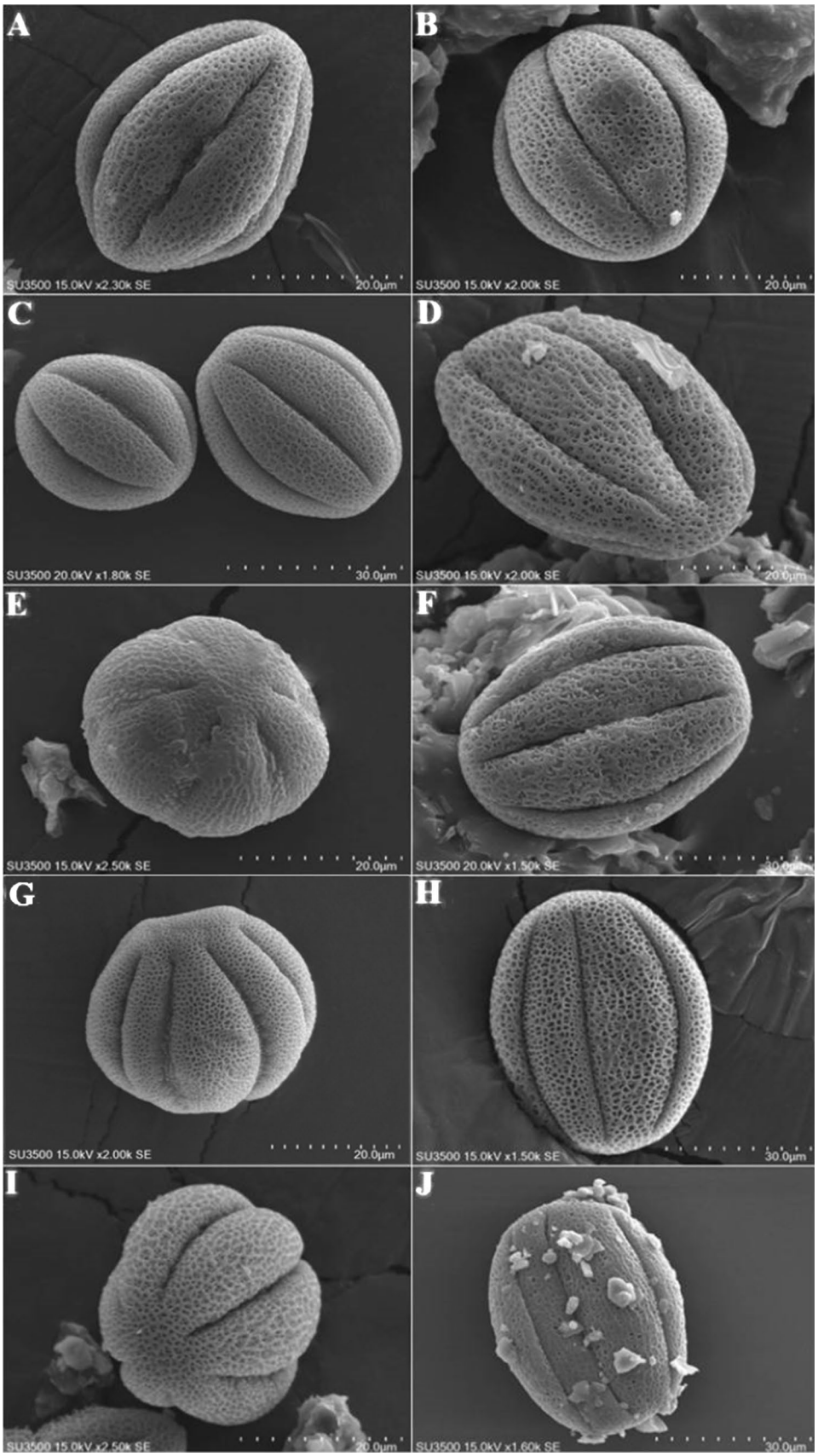

Fig. 1 SEM micrographs of the pollen grains in *Hymenocrater* species (Lamiaceae): equatorial view (**A**–**D**, **F**–**H**, **J**) and ambitus (**E**, **I**). **A** *H. altimuranus,* **B** *H. bituminosus,* **C** *H. calycinus,* **D** *H. elegans,* **E** *H. incanus,* **F** *H. longiflorus,* **G** *H. oxyodontus,* **H** *H. platystegius,* **I** *H. sessilifolius,* **J** *H. yazdianus*

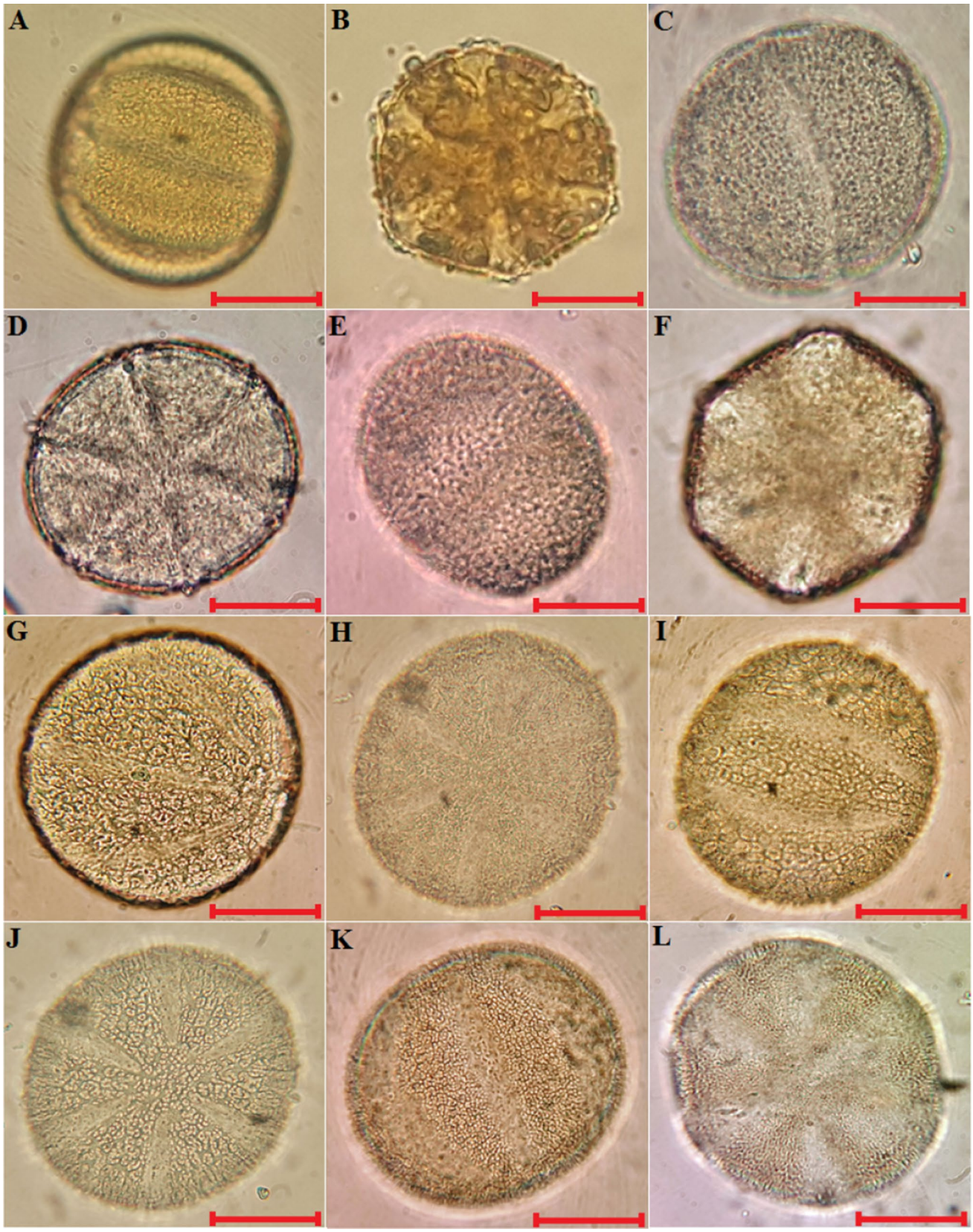

Fig. 2 Light image of the pollen grains in *Hymenocrater* species (Lamiaceae): **A** *H. altimuranus* (equatorial view), **B** *H. sessilifolius* (ambitus), **C**, **D** *H. bituminosus* (equatorial and ambitus views), **E**, **F** *H. calycinus* (equatorial and ambitus views), **G**, **H** *H. elegans* (equatorial and ambitus views), **I**, **J** *H. longiflorus* (equatorial and ambitus views), **K** *H. platystegius* (equatorial view), **L** *H. yazdianus* (ambitus).The scale bar is 25 µm

grain characteristics are listed in Table 2, and qualitative features are summarized in Table 3.

Based on Erdtman's (1960) pollen size classification, a majority of species have the large and rarely small pollen grains as in *H. calycinus*.

According to the P/E ratio, various shapes of the pollen grains were detected among the studied species, prolate (*H. altimuranus*, *H. longiflorus*, *H. platystegius* and *H. yazdianus*), subprolate (*H. calycinus*, *H. sessilifolius* and *H. elegans*) and spheroidal (*H. bituminosus*, *H. incanus* and *H. oxyodontus*). In most of the evaluated species, the equatorial outline view was truncate, whereas in some others it was obtuse convex (*H. sessilifolius*, *H. oxyodontus* and *H. incanus*) or rarely circular (*H. bituminosus*).

Two shapes of pollen ambitus were found among the species: It was obtuse convex in most species (*H. yazdanius*, *H. altimuranus*, *H. oxyodontus*, *H. longiflorus*, *H. elegans* and *H. calycinus*), and in the rest of the species, it was circular. Bireticulate is the more frequent type of exine sculpturing, while in some species (*H. altimuranus*, *H. yazdanius* and *H. oxyodontus*) it is determined as reticulate (Fig. 3).

PCA analysis revealed that the first three components comprise 82% of the total variation (Table 4). Moreover, according to PCA loading (Fig. 4) some traits such as polar and equatorial axes length, P/E ratio, endoaperture diameter, ecotoaperture length, pollen shape and exine ornamentation were positively correlated. However, among them some characteristics (such as polar axis length, endoaperture diameter and ecotoaperture length) strongly affected the principal components ($P \geq 0.8$). Meanwhile, some others (apocolpium index, exine ornamentation and equatorial view shape) had a negative correlation with the principal components.

The largest (60.67 µm ± 1.47) and shortest (23.74 µm ± 3.40) polar axis length were recorded in *H. longiflorus* and *H. calycinus*, respectively. These conditions hold true for the equatorial axis length and also mesocolpium width. *H. longiflorus* had the largest ecotoaperture length (53.36 µm ± 1.68), while its shortest one (20.74 µm ± 0.97) was detected in *H. calycinus*. The largest (0.76) value of the apocolpium index was registered in *H. incanus* and the smallest one (0.41) in *H. altimuranus*.

Table 2 Quantitative palynological characteristics of studied *Hymenocrater* species (Lamiaceae) (all values are in µm)

Species	Polar axis length (*P*)	Equatorial axis length (*E*)	*P*/*E* ratio	Width of Mesocolpium	Ecotoaperture length	Endoaperture diameter	AI
H. altimuranus	41.11 ± 2.31	29.45 ± 0.61	1.39	11.00 ± 1.65	37.87 ± 1.34	0.71 ± 0.15	0.41
H. bituminosus	38.35 ± 1.78	33.59 ± 2.45	1.14	14.26 ± 0.68	33.97 ± 1.53	0.79 ± 0.22	0.52
H. calycinus	23.74 ± 3.40	18.88 ± 1.97	1.32	6.38 ± 0.78	20.74 ± 0.97	0.54 ± 0.09	0.46
H. elegans	51.09 ± 2.58	38.44 ± 3.71	1.33	14.94 ± 1.32	43.77 ± 3.85	0.96 ± 0.33	0.55
H. incanus	28.21 ± 0.55	26.28 ± 0.54	1.07	10.90 ± 1.54	24.27 ± 0.51	0.37 ± 0.07	0.76
H. longiflorus	60.67 ± 1.47	43.54 ± 1.55	1.39	15.05 ± 3.72	53.36 ± 1.68	1.27 ± 0.39	0.56
H. oxyodontus	39.77 ± 4.47	38.91 ± 0.02	1.02	11.94 ± 4.30	33.07 ± 2.01	0.61 ± 0.19	0.28
H. platystegius	49.18 ± 3.65	36.25 ± 7.95	1.36	12.96 ± 2.60	43.45 ± 3.72	1.20 ± 0.31	0.46
H. sessilifolius	37.72 ± 4.04	29.73 ± 4.00	1.27	9.60 ± 1.56	36.19 ± 4.56	0.52 ± 0.26	0.68
H. yazdanius	33.75 ± 0.54	25.00 ± 0.52	1.35	12.75 ± 0.74	30.77 ± 1.02	1.04 ± 0.36	0.55

Table 3 Qualitative pollen grains traits of the studied species

Species	Pollen shape	Exine ornamentation	Equatorial view shape	Ambitus shape
H. altimuranus	Prolate	Reticulate	Truncate	Obtuse convex
H. bituminosus	spheroidal	Bireticulate	Circular	Circular
H. calycinus	Subprolate	Bireticulate	Truncate	Obtuse convex
H. elegans	Subprolate	Bireticulate	Truncate	Obtuse convex
H. incanus	spheroidal	Bireticulate	Obtuse convex	Circular
H. longiflorus	Prolate	Bireticulate	Truncate	Obtuse convex
H. platystegius	Prolate	Bireticulate	Truncate	Circular
H. oxyodontus	Spheroidal	Reticulate	Obtuse convex	obtuse convex
H. sessilifolius	Subprolate	Bireticulate	Obtuse convex	Circular
H. yazdanius	Prolate	Reticulate	Truncate	Obtuse convex

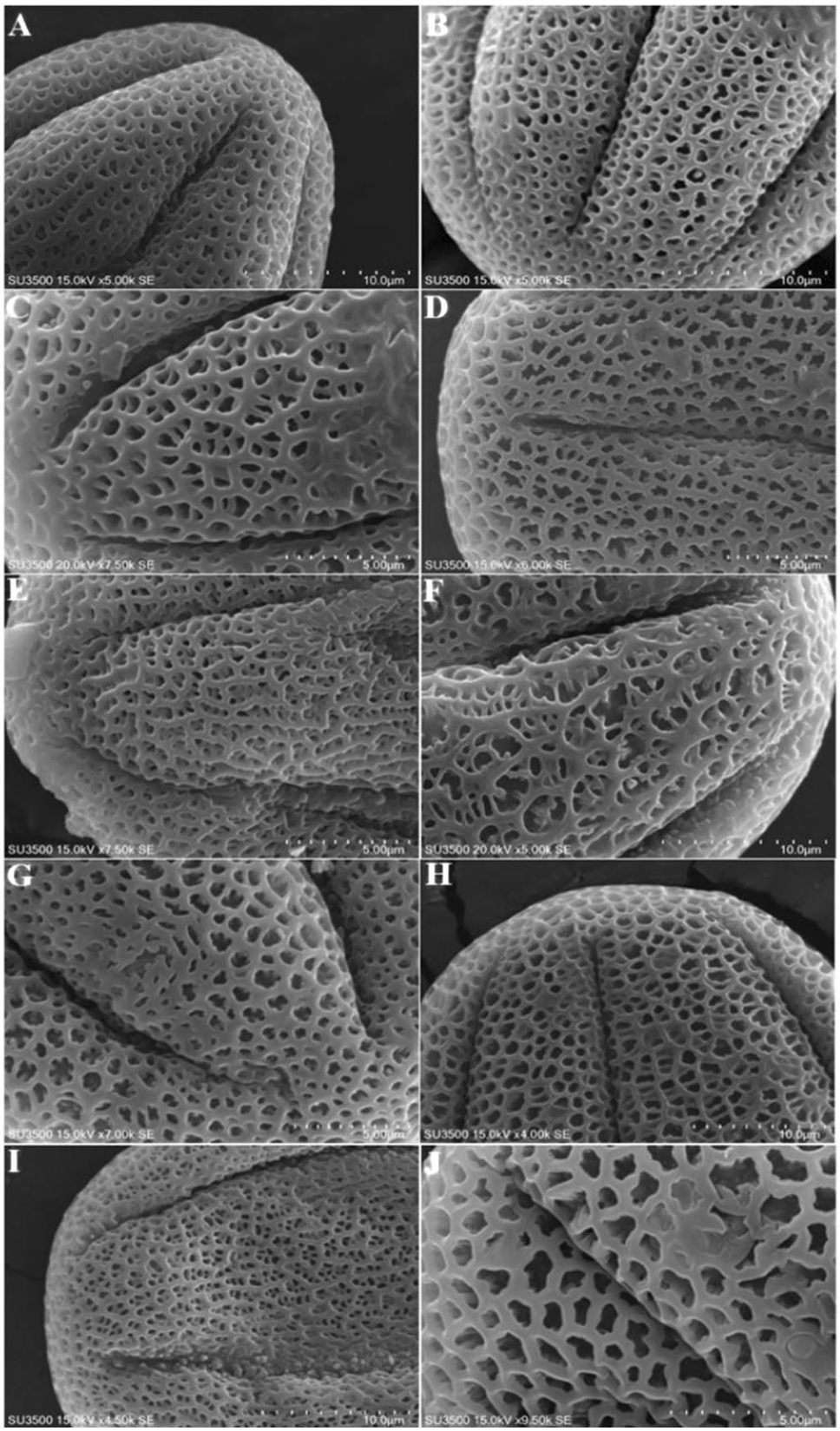

Fig. 3 SEM micrographs of exine ornamentation in *Hymenocrater* species (Lamiaceae). **A** *H. altimuranus,* **B** *H. bituminosus,* **C** *H. calycinus,* **D** *H. elegans,* **E** *H. incanus,* **F** *H. longiflorus,* **G** *H. oxyodontus,* **H** *H. platystegius,* **I** *H. sessilifolius,* **J** *H. yazdianus*

Table 4 Eigenvalue and percentage of variation of the principle components

PC	Eigenvalue	% Variance
1	0.048912	43.779
2	0.0272868	24.423
3	0.0175317	15.692
4	0.00803929	7.1956
5	0.00609069	5.4515
6	0.00337007	3.0164
7	0.000403724	0.36136
8	5.85873E-05	0.052439
9	3.16745E-05	0.028351

Species clustering – Based on the UPGMA tree (Fig. 5), the studied species were clustered into four groups: (I) *H. oxyodontus*, (II) *H. yazdanius*, (III) *H. elegans*, *H. platystegius*, *H. altimuranus* and *H. longiflorus* and (IV) *H. bituminosus*, *H. incanus*, *H. calycinus* and *H. sessilifolius*. In addition, PCA biplot of these species and pollen grains characteristics revealed that some species were characterized by a special feature(s), which was useful in their identification (Fig. 6). For example, *H. oxyodontus* and *H. yazdianus* were characterized by the exine ornamentation pattern and sculpturing type. Moreover, the highest value of the mesocolpium width, equatorial and polar axes length were calculated

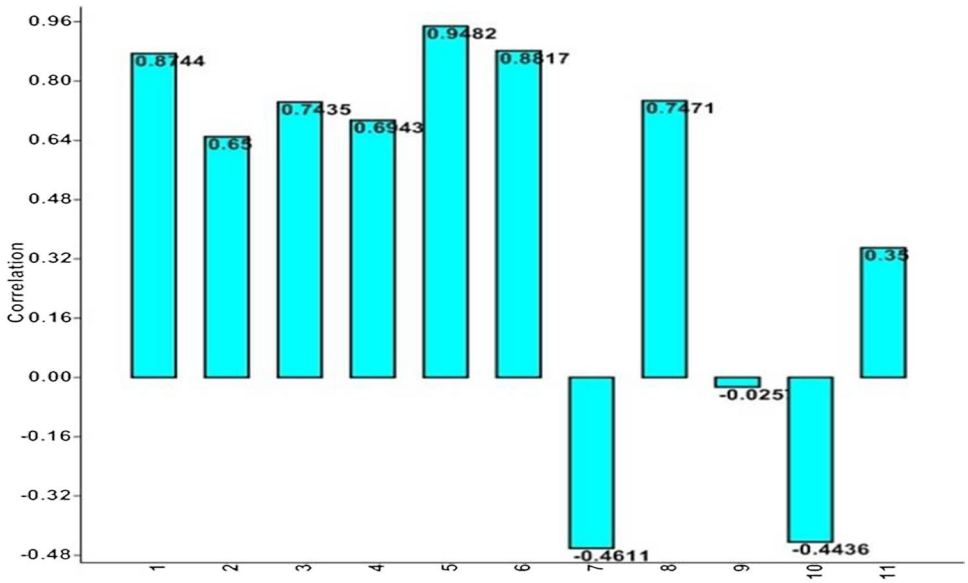

Fig. 4 PCA loading 1 of pollen morphological characteristics of the evaluated species. 1: polar axis length, 2: equatorial axis length, 3: polar/equatorial axes length, 4: mesocolpium diameter, 5: Pori diameter, 6: colpi length, 7 apocolpium index, 8: pollen shape, 9: exine ornamentation, 10: equatorial view shape, 11 ambitus

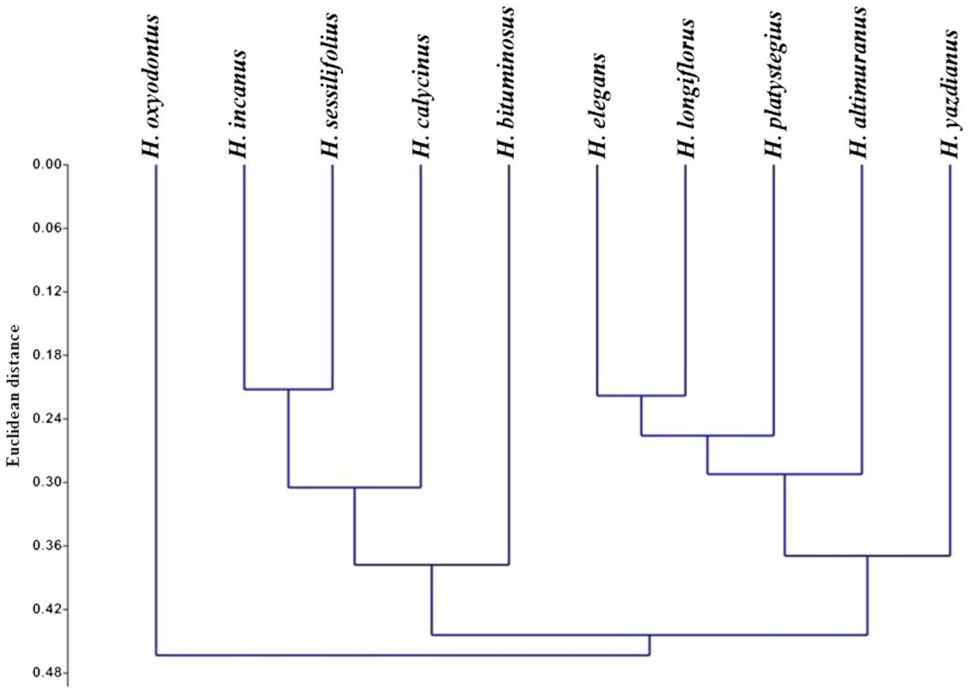

Fig. 5 UPGMA clustering of the evaluated *Hymenocrater* species (Lamiaceae) according to pollen grain characteristics

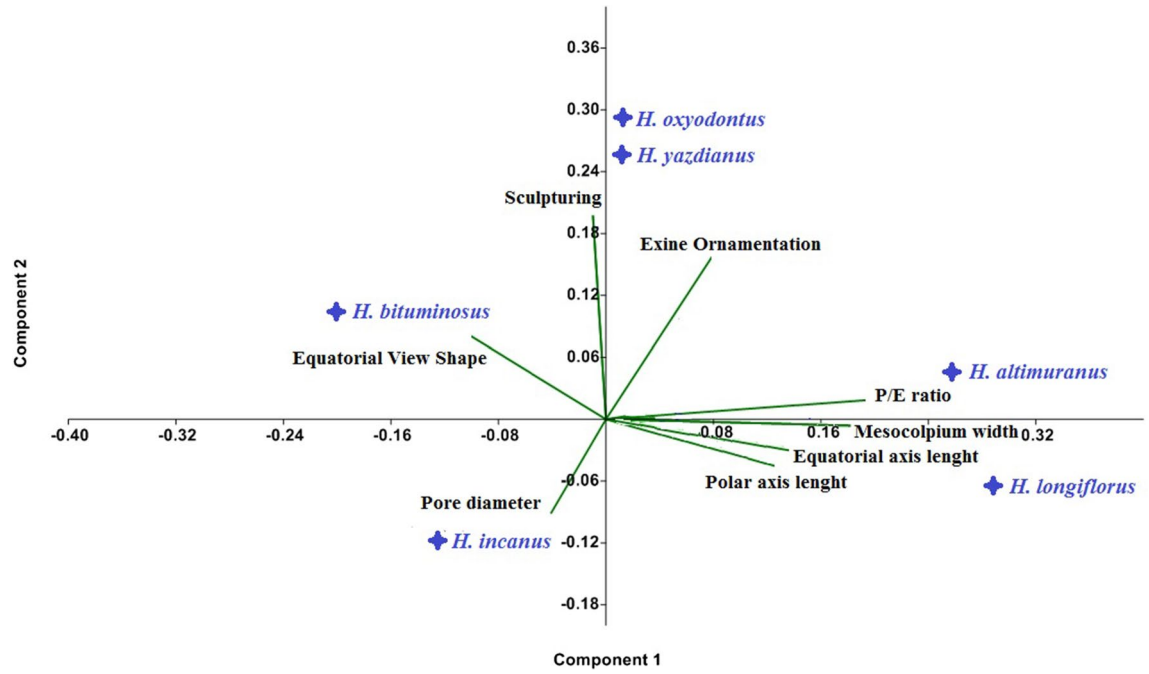

Fig. 6 PCA biplot of the palynological characteristics and *Hymenocrater* species (Lamiaceae)

for *H. longiflorus.* We observed the highest *P*/*E* ratio in *H. altimuranus*.

4 Discussion

Pollen grains morphology – Almost all species had the large pollen grains, but we observed the small pollen grains in *H. calycinus*. Similar variations were reported in different genera of the subfamily Nepetoideae, such as *Nepeta* L. (Talebi et al. 2020b), *Glechoma* L. and *Marmoritis* Benth. (Jang and Hong 2010), *Dracocephalum* L. (Naderifar et al. 2015), *Cedronella* Moench, *Drephanocaryum* Pojark., *Lophanthus* Adans. (Moon et al. 2008) and also *Lallemantia* Fisch. & C.A. Mey. (Koohdar et al. 2018).

PCA analysis revealed that most of the examined quantitative characteristics varied significantly among the species. However, the quantitative traits of pollen grains lack taxonomic value because pollen grains size variation among diverse populations of *Hymenocrater* species was detected in the previous study. For example, our measurement of the polar and equatorial axes length in *H. bituminosus* and *H. longiflorus* greatly differed from those have been reported for the mentioned species by Moon et al. (2008). In addition, the similar variations were reported for other Lamiaceae genera, for instance: *Mentha* L. (Celenk et al. 2008), *Nepeta* (Talebi et al. 2020b) and *Lycopus* L. (Moon and Hong 2003).

The length of the polar and equatorial axes and their variations are very important, because the overall shape of the pollen grains is derived from the P/E ratio. Erdtman (1952) indicated that the pollen grains shape is determined according to the *P*/*E* ratio. The polar axis length is strongly correlated with the equatorial axis length; meanwhile, pollen grains' shape can differ significantly among various populations of each species, excluding the effect of preparation techniques. Such difference was recorded in diverse families such as Lamiaceae and Fabaceae (Talebi et al. 2020a, b).

Most of the evaluated species had the hexacolpate pollen grains. However, the octocolpate pollen grains were detected in *H. oxyodontus. Hymenocrater* belongs to the subfamily Nepetoideae, which its taxa are characterized by the hexacolpate and tri-nucleate pollen grains (Talebi et al. 2020b). Meanwhile, a previous investigation (Moon et al. 2008) indicated a few species of the subfamily Nepetoideae have the octocolpate pollen grains such as *Glechoma hederacea* L., *H. bituminosus*, *H. longiflorus*, *Meehania urticifolia* (Miq.) Makino and *Nepeta viscida* Boiss. These results revealed that some species generate pollen grains with a different number of aperture, which is called aperture heteromorphism (Till-Bottraud et al. 1995).

We found that the exine sculpturing pattern rarely varied among the studied species and had no taxonomic value. In this regard, most of the evaluated species had the bireticulate exine sculpturing pattern; therefore, we could not identify most of species using this feature.

Our findings agreed with a previous investigation on the pollen grains morphological characteristics in the genus, which detected the exine ornamentation as bireticulate in *H. bituminosus*, *H. calycinus*, *H. elegans* and *H. platystegius* (Jafari and Jafarzadeh 2008). Although we detected the exine sculpturing pattern in *H. bituminosus* and *H. longiflorus* as bireticulate, Moon et al. (2008) reported it as microreticulate in these species. Additionally, the most common sexine ornamentation type in the tribe Nepeteae is bireticulate, which is consisted of angular primary lumina with a fine secondary reticulum. These findings indicate the infraspecific variation in the exine sculpturing pattern. A similar result was reported in diverse *Nepeta* species (Talebi et al. 2020b).

A similar pattern was found for the ambitus, for which only two forms were detected among these species: circular and obtuse convex. However, the obtuse convex shape was more frequent among the species. A similar pattern was observed in other Lamiaceae genera such as *Nepeta* (Talebi et al. 2020b) and *Callicarpa* L. (Ma et al. 2016).

Meanwhile, the equatorial shape was a relative variable characteristic, and three forms of it were recorded. Its more frequent shape was truncate, and other forms have been rarely observed. This finding demonstrated that the equatorial shape had a diagnostic value in the genus.

We found the similar reports from other Lamiaceae genera, such as *Nepeta* (Talebi et al. 2020b) and *Dracocephalum* (Naderifar et al. 2015), and from other families, such as Rubiaceae (*Crucianella* L. Parsapanah and Beygom Faghir 2021) and Leguminosae (*Onobrychis* Mill. Talebi et al. 2020a).

Another relative variable qualitative trait was the pollen shape, of which three forms were detected among the studied species. All the detected forms had a nearly equal frequency. Meanwhile, our findings did not completely agree with the previous palynological evaluation in the genus (Jafari and Jafarzadeh 2008). They reported the pollen grains shape as prolate spheroidal in *H. elegans* and *H. platystegius*, while we observed them as subprolate and prolate in *H. elegans* and *H. platystegius*, respectively.

These results indicate that the pollen grain shape can differ intra-specifically due to preparation method, phenotypic plasticity and polyploidy. A similar finding was reported in some other genera of the subfamily Nepetoideae, such as *Nepeta* (Talebi et al. 2020b) and *Dracocephalum* species (Naderifar et al. 2015). However, Celenk et al. (2008) suggested that the difference in pollen grains size among various populations of each taxon can be related to various preparation methods.

Species clustering – The species clustering pattern, based on the pollen characteristics, did not agree with those were obtained in the previous anatomical and micromorphological study of the genus (Serpooshan et al. 2014), in which *H. bituminosus*, *H. calycinus*, *H. elegans*, *H. oxyodontus* and *H. platystegius* were clustered close together as a group. In the current study, the mentioned species were divided into two groups and placed far from each other. In addition, *H. oxyodontus* and *H. elegans* were grouped based on Serpooshan et al. (2014) findings, whereas we observed these species being placed separately. Additionally, Serpooshan et al. (2018) evaluated the phylogenetic relationship among *Hymenocrater* and allies genera such as, *Lophanthus*, *Marmoritis* and *Nepeta*, using nrITS, plastid *trnL* intron and *trnL-F* intergenic spacer DNA sequences. Their findings indicate that none of these genera is monophyletic. However, the pattern of *Hymenocrater* species clustering was not similar with our obtained results.

Recently, Tabaripour et al. (2021) have employed morphological and cp-DNA sequences to delimit *Hymenocrater* species. The clustering pattern of species widely varied from those obtained by our study. However, the clustering pattern of species based on micromorphological characteristics (Serpooshan et al. 2014) is more similar to our study than those obtained by molecular and morphological characteristics (Tabaripour et al. 2021).

In conclusion, pollen grains in most *Hymenocrater* species were hexacolpate, monad and radially symmetrical. Their sizes differed from large to rarely small. PCA analysis revealed significant variation for some quantitative traits. Among the qualitative traits, exine sculpturing pattern and ambitus shape scarcely differed and had no taxonomic value. Meanwhile, some others, such as general pollen shape and equatorial outline view, were relatively more variable and had a taxonomic value.

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Authors contribution All the authors contributed to the study's conception and design. RT performed material preparation and data collection. MSH performed the statistical analyses. The first draft of the manuscript was written by SMT and revised by RT and MSH. All authors read and approved the final manuscript.

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Availability of data and materials Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent for publication We hereby declare that we participated in this research and the paper's development. We have read its final version and give consent for the publication.

Ethical approval Not applicable.

Informed consent All authors agreed to the publication of the present work.

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