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PALYNOLOGICAL SURVEY OF THE GENUS HYPERICUM (HYPERICACEAE) IN IRAN AND ITS TAXONOMIC IMPORTANCE

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In this research, pollen grains of ten species and two subspecies of the genus *Hypericum* in Iran belonging to four sections were studied using light and scanning electron microscopy. The pollen grains are monad, isopolar to subisopolar and heteropolar, prolate, subprolate, spheroidal and prolate- spheroidal in shape, small to medium in size. The outline of pollen grains varies from round to triangular, quadrangular and ovate from polar view and elliptical, tetrahedral, quadrangular, round and ovate from equatorial view; 3 and 4 syncolporate to 3, 4 and 6 zonocolporate. Based on exine sculpturing, pore shape, size and muri thickness, the examined species are divided in two main types including scrobiculate and micro reticulate and 2 subtypes including small pore / thick muri and large pore / thin muri. The pores are arranged from irregular to regular-irregular with regular intervals. The current result revealed taxonomically important palynological data of the genus *Hyperic*um. These traits can be used for infrageneric classification, especially at sectional and species levels.

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Key words: *Hypericum*; Hypericaceae; taxonomy; pollen morphology; Iran

مطا لعه گرده‌شناسی سرده Hypericum در ایران و اهمیت تاکسونومیک آن

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در این مطالعه ده گونه و دو زیرگونه از سرده Hypericum در ایران، متعلق به چهار بخشه توسط میکروسکپ نوری و الکترونی مورد بررسی قرار گرفت. دانه‌های گرده به صورن موناد، جور قطب تا تقریباً جور قطب و ناجور قطب، دارای اشکال استوانه‌ایی، تقریباً استوانه‌ایی، کروی و کروی–استوانه‌ای، اندازه کوچک تا متوسط می‌باشند. طرح کلی دانه‌های گرده از نمای قطبی گرد، سه گوش، چهار گوش، تخم مرغی و از نمای استوایی بیضوی، چهارگوش، مخروطی، گرد و تخم مرغی؛ منظم، منظم–نامنظم و نامنظم؛ دارای دریچه‌های ۳ الی ۴ شیاری–منفذی پیوسته و ۳، ۴ و ۶ شیاری–منفذی ناپیوسته می‌باشند. گونه‌های مورد مطالعه، براساس تزیینات اگزین، شکل و اندازه منفذ، ضخامت دیواره‌ها به دو گروه اصلی (زگیل‌دار و ریز مشبک) و دو زیر گروه (منافذ کوچک/دیواره‌ها ضخیم و منافذ بزرگ/دیواره‌ها نازک) تقسیم می‌شوند. منافذ دارای فواصل منظم، منظم–نامنظم

و نامنظم می‌باشند. نتایج تحقیق حاضر اهمیت تاکسونومیک صفات ریخت‌شناسی گرده را در سرده آشکار ساخت. این صفات می‌توانند برای رده بندی تحت سرده‌ای، به ویژه در سطوح بخشه و گونه مورد استفاده قرار گیرند.

INTRODUCTION

Hypericum L. as a species rich (with ca. 500 representatives) and the largest genus in the Hypericaceae family (Nürk 2011), comprising herbs and shrubs, distributed mainly in temperate zones of northern hemisphere (Eurasia and North America). However, some species of *Hypericum* occur in mountains and higher altitudes of equatorial regions and southern hemisphere (Robson, 1977; Nürk2011; Meseguer and Sanmartin, 2012). Iranian species of this genus grow mainly in north, northwest and center of Iran and form floristic elements of Hyrcanian mountainous areas, Irano-Turanian, Mediterranean and Zagros elements. They generally prefer steep slopes of rocky and calcareous cliffs and margin of mountainous forests (Robson, 1968; Azadi, 1999). Robson (1968) introduced 21 species in the area covered by Flora Iranica. Robson (1977) and Assadi (1984) reported *H. fursei* N. Robson and *H. dogonbadanicum* Assadi as two endemics of N and SW of Iran. In Flora of Iran, Azadi (1999) identified 19 species, 4 subspecies arranged in 5 sections (comprising *Campylosporus* (Spach) R. Keller, *Hypericum*, *Hirtella* Stef., *Taeniocarpum* Jaub. & Spach. and *Drosanthe* (Spach) Endl.), and two doubtful species including *H. heterophyllum* Vent. and *H. olivieri* (Spach) Boiss. In addition to considerable pharmaceutical importance (Ernst, 2003; Maggi, 2004; 2010; Bruni and Sacchetti, 2009; Ozturk & al., 2009; Nürk and Crockett, 2010; Crockett and Robson, 2011), the genus *Hypericum* has been subject of various investigations due to taxonomical complexities (Robson, 1981, 1985, 1996, 2001, 2006, 2010; Nürk and Blattner, 2010). Primarily, the basic studies on pollen of Hypericaceae were reported by Erdtman, 1952 and Aytug & al 1971. Then, different authors reported various pollen morphological data of the genus *Hypericum* (Khan, 1969; Thomas, 1970; Clarke, 1975, 1976, 1981; Barros, 1984). This was followed by palynological survey of the selected species of the genus (Martonfi & al., 2002; Ocak & al., 2012; Sentark, 2012; Avato, 2005; Matzka, 2001; Meseguer and Sanmartín, 2012) plus six Iranian species including *H. perforatum*, *H. tetrapterum*, *H. androsaemum*, *H. fursei*, *H. hirsutum* (Mahmoudi Otaghvari & al., 2015) and *H. dogonbadanicum (*Bayat & al., 2015). The main aims of this research are to provide a detailed account of pollen morphological features of 10 species and 2 subspecies of *Hypericum* belonging to four sections that has not been extensively studied yet. In addition, identification of pollen diagnostic characters for taxonomic treatment at inter- and intra-specific levels, are among other important objectives of this study.

MATERIALS AND METHODS

In the current survey, a total of 12 taxa (including 10 species and 2 subspecies) belonging to four sections (*Hypericum*, *Hirtella* Stef., *Taeniocarpum* Jaub. & Spach. and *Drosanthe* (Spach) Endl.) of Iranian species of *Hypericum* were undertaken for pollen morphological analysis, by light (LM) and scanning electron microscope (SEM). Pollen grains were collected from both mature flowers of freshly collected specimen (during 2015-2016) and herbarium materials. The voucher specimens are deposited in the herbaria as indicated in table 1. For LM analysis, pollen grains were acetolysed according to Harley (1992), using Olympus light microscope, and photographed by Dino-Eye camera model AM-423x. At least 30 grains were examined from each sample and several individuals of the same species (table 2). For SEM observation, pollen grains were mounted on the stubs with double-sided cellophane tape and then coated in a sputter coater with 25nm of gold-palladium at an accelerating voltage of 10–15KV, in Guilan University. The samples were photographed by Tescan Vega scanning electron microscope; model VEGA/TESCAN in Razi Metallurgical Research Center (RMRC), Tehran. The pollen terminology in general follows Erdtman (1952), Punt & al. (2007).

Table 1. Voucher specimens of the studied taxa.

Species	**Collecting data**
1. Sect. *Hypericum* 1.1 *H. triquetrifolium* Turra	Kurdestan, Marivan to Khav, 1350 m, Ghahremani & Mozaffarian, 18325 (TARI).
2. Section: *Hirtella* Stef. Subsect. *Platyadenum* N. Robson 2.1 *H. scabrum* L.	Tehran,Haraz road, 5-8 Km to Polur, 2200m, Amin & Bazargan, 75718 (TARI).
2.2 *H. asperulum* Jaub. & Spach	Azarbaiejan, From band Uromieh to Ziveh Margavar region, 1500m, Ghahreman & Aghustin, 13573 (TUH).
2.3 *H. hirtellum* Boiss.	Tehran, Polur, Golkaryeh, 2000m, 13566 (TUH).
Subsect. *Stenadenum* N. Robson 2.4 *H. elongatum* subsp. *micrcalycinum* (Boiss. & Heldr.) N. Robson	Azarbaiejan, 48 km from Tabriz to Marand, Givan village, Mishudagh Mountain, 1700m, Sabzi & Imani, 6918 (Tabriz Herbarium).
2. 5 *H. elongatum* subsp. *apiculatum* N. Robson	Azarbaiejan, Arasbaran protected area, 1300m, Ghahremani & Kasebi, 6918 (Tabriz Herbarium).
2. 6 *H. davisii* N. Robson	Azarbaiejan, Kaleybar to Ahar. 20Km to Ahar, Sambran pass, 1735m, Azadi & Nickchehre, 75666 (TARI).
2. 7 *H. helianthemoides* (Spach) Boiss.	Ardabil, Givy, 1400-1700m, Azadi, 75761 (TARI).
2. 8 *H. vermiculare* Boiss. & Hausskn. ex Boiss.	Tehran, Damavand to Firuzkuh, 27 Km to Firuzkuh, 2050-2150m, Azadi, 75722 (TARI).
3. Section: *Taeniocarpium* 3. 1 *H. linarioides* Bosse	Kurdestan, west of Sanandaj, 1705m, Maroofi, 1259 (Kurdistan Herbarium).
3.2 *H. armenum* Jaub. & Spach	Mazandaran, Youish, Baladeh, Kamarbon, 3200, Naqinezhad & Gholizadeh, 4366 (Babolsar Herbarium).
4. Section: *Drosanthe* (Spach) Endl. 4.1 *H. hyssopifolium* Vill.	Azarbaiejan, West of Tabriz, 1700, Mozaffrian, 25859 (TARI).

Table 2. Pollen morphological characters of studied species of the genus *Hypericum* of the current survey and former study of Mahmoudi Otaghvari & al., 2015. Abbreviations: Polar axis length (P), Equatorial axis width (E), ratio of Polar axis / Equatorial axis (P/E), Colpus length (Cl); Apocolpium index (AI): Distance between the apices of two ectocolpi (d) / equatorial diameter (D); Mesocolpium thickness (Meso), Outline from polar view (PO), Outline from equatorial view (EO), Triangular (T), Round (Ro), elliptical (EL),Oval (Ov), Tetrahedral (Th), Quadrangular (Q); Apertures number and types (ANT), Syn-colporate (Syn-colr), Zono-colporate (Z-colr); Polarity (Pol), Isopolar (Iso), Sub-isopolar (Sub-iso), Heteropolar (Het), Colpus margin (Cm), Rolled (Rol), Flat (FL); Operculum (O),Pollen size (Ps), Medium (Me), Small (Sm); Pollen shape (Psh), Prolate (Pr), Prolate-spheroidal (PS), Sub- prolate (SubP), Spheroidal (Sph); Irregular (Ir), Regular (Re), Regulare-Irregular (Re-Ir) pollen.

Species and subspecies	**P (**µm)	**E (**µm)	**P/E**	**Cl (**µm)	**d (**µm)	**D (**µm)	**AI** (d/D)	**Meso (**µm)
1. **Sect. *Hypericum*** 1.1 *H. triquetrifolium*	(25.1-26.5) 25.8 ±0.51	(11.9-14.5) 13.2 ±0.93	1.95	(24.6-25.1)24.9 ±0.21	–	(18.5-24.5) 21.5 ±2.54	–	(7.2-7.8) 7.5 ±0.17
2. Sect.: *Hirtella* Subsect. *Platyadenum* 2.1 *H. scabrum*	(26.5-28.6) 27.5 ±0.65	(20.5-22.5) 21.5 ±0.66	1.27	(20.7-21.3)21 ±0.21	(5.1-5.7) 5.4 ±0.25	(24.3-24.9) 24.6 ±0.23	0.22	(6.6-7.2) 6.9 ±0.23
2.2 H. asperulum	(23.1-23.7)23.4 0.23	(19.4-20.1) 19.7 ±0.28	1.18	(18.123.9)21 ±2.45	(3.9-4.5) 4.2 ±0.23	(21-21.6) 21.3 0.25	4.29	(11.8-12.4) 12.1 ±0.25
2.3 *H. hirtellum*	(25.1-25.3) 25.2 ±0.10	(18.8-19.7) 19.2 ±0.35	1.31	(16.5-17.1)16.8 ±0.23	(1.9-2.5) 2.2 ±0.23	(20.7-21.3) 21 ±0.24	9.56	(7.6-8.2) 7.9 ±0.25
Subsect. *Stenadenum* 2.4 *H. elongatum* subsp. *microcalycinum*	(24.8-28.7) 26.7 1.77±	(17.5-21.9) 19.7 ±1.89	1.35	(22.6-23.2)22.9 ±0.23	–	(20.1-23.9) 22 ±1.38	–	(8.6-9.2) 8.9 ±0.23
2.5 *H. elongatum* subsp. *apiculatum*	(24.3-26.4) 25.3 ±0.93	(19.7-23.1) 21.4 ±1.39	1.18	(17.1-17.6)17.3 ±0.19	–	(18.6-23) 20.8 ±1.84	–	(11.5-12.1) 11.8 ±0.24
2.6 *H. apricum*	(28.3-26.5) 27.4 ±0.60	(23.1-16.5) 19.8 ±2.6	1.38	(22.1-20.9)21.5 ±0.51	–	(17.8-13.3)15.6 ±1.77	–	(11.1-10.5)10.8 ±0.24
2.7 *H. davisii*	(25-25.2) 25.1 ±0.10	(17.2-18.9)18 ±0.7	1.39	(21.8-24.1)22.9 ±0.85	–	(25.9-26.5)26.2 ±0.23	–	(9.3-9.9)9.6 ±0.23
2.8 *H. helianthemoides*	(24.4-27.8) 26.1 ±1.31	(22.7-25.5) 24.1 1.12	1.08	(26.2-26.8)26.5 ±0.25	(3.2-3.8) 3/5 ±0.23	(23.9-24.5)24.2 ±0.25	0.14	(6.3-6.9)6.6 ±0.24
2.9 *H. vermiculare*	(27.1-27.5) 27.3 ±0.15	(19.3-19.9) 19.6 ±0.24	1.39	(18.1-18.6)18.3 0.19	(3.3-3.9) 3.6 0.23	(21.9-25.5)23.7 ±1.53	0.15	(9-9.7)9.4 ±0.27
3**. Sect. *Taeniocarpium*** 3.1 *H. linarioides*	(30.3-30.9) 30.6 ±0.23	(16-16.9)16.4 ±0.38	1.86	(26.8-27.4)27.1 ±0.24	(6.6-7.2) 6.9 ±0.23	(13.3-17)15.1 ±1.58	0.45	(9.7-10.3)10 ±0.24
3.2 *H. armenum*	(17.3-21.6) 19.4 ±1.63	(15.1-18.9)17 ±1.51	1.14	(15.9-16.5)16.2 ±0.23	(4.2-4.8) 4.5 ±0.23	(14-14.6)14.3 ±0.23	0.31	(10.9-11.5)11.2 ±0.24
4**. Sect. *Drosanthe*** 4.*1 H. hyssopifolium*	(23.3-26.9) 25.1 ±1.43	(20.1-22.1)22.1 ±0.94	1.13	(19.3-19.9)19.6 ±0.23	(3-3.6) 3.3 ±0.23	(19.1-19.7) 19.4 ±0.25	0.17	(14.6-15.2)14.9 ±0.23

Table 2. Continued.

Species and subspecies	PO	EO	ANT	OT	Pol	Cm	O	Ps	Psh
1. Sect. *Hypericum* *H. triquetrifolium*	Ro	El	3-Syn-colr	Re	Iso	Rol	+	Me	Pr
2. Sect.: *Hirtella* Subsect. *Platyadenum* *H. scabrum*	T	Th	6-z-colr	IR	Het	Rol	-	Me	Subp
H. asperulum	R	R-Ov	3-Z-colr	Re	Iso	Rol	+	S	Subp
Subsect. *Stenadenum* *H. elongatum* subsp. *apiculatum*	Ro-Q	Ro-Q	4- Syn-colr	Re-Ir	Sub-iso*	Rol	+	Me	SubP
H. elongatum subsp. *microcalycinum*	T	Ov-El	4- Syn-colr	Re-Ir	Sub-iso*	Rol	+	Me	Pr
H. hirtellum	Q	Th	4- z-colr- 4-syncolr	Ir	Het*	Rol	+	Me	Subp
H. apricum	T	Q	4-Syn-colr	Re-Ir	Sub-iso	Rol		Me	Pr
H. davisii	T	Th	4-Syn-colr	Ir	Het	Rol	+	Me	Pr
H. helianthemoides	T	Th	4-Syncolr	Ir	Het*	Rol	-	Me	Pr-sph
H. vermiculare	Q	Th	4-Syncolr	Ir	Het*	Rol	-	Me	Subp
Sect. *Taeniocarpium* *H. linarioides*	Ro	El	3- z-colr	Re	Iso	Rol	-	Me	Pr
H. armenum	Ro	Ov-Ro	3-z-colr	Re	Iso	Fl	+	S	Sph
Sect. *Drosanthe* *H. hyssopifolium*	T	Ro-Ov	3-z-colr	Re	Iso	Rol	+	Me	Pr-Sphr

RESULTS

The pollen morphological features are presented in tables 2–3 and figures 1-4. Our findings shows that pollen grains are monad, isopolar, subisopolar or heteropolar; prolate (P/E= 1.35-1.95), subprolate (P/E=1.18-1.31), spheroidal (P/E=1.14) and prolate-spheoroidal (P/E=1.08-1.13) in shape; small (19.4-23.4 µm) to medium (25.1-30.6 µm) in size (Erdtman 1952). The outline of pollen grains vary from round to ovate, triangular and quadrangular from polar view (fig. 1 A-F), elliptical to round (fig. 1 G-I) and tetrahedral from equatorial view (fig. 1 J-L). The outline of pollen are regular in *H. triquetrifolium, H. armenum, H. asperulum, H. hyssopifolium* and *H. linarioides*, regular -irregular in *H. elongatum* subsp. *apiculatum, H. elongatum* subsp. *microcalycinum, H. apricum* and irregular in *H. helianthemoides, H. vermiculare, H. scabrum, H. hirtellum* and *H. davisii*. The maximum (30.6 µm) and minimum polar axis (19.4 µm) were observed in *H. linarioides* and *H. armenum* respectively. The maximum equatorial axis (24.1 µm) was recorded in *H. helianthemoides*, while minimum equatorial axis (13.2 µm) belongs to *H. triquetrifolium*. Mesocolpium varied from 6.6 µm in *H. helianthemoides* to 14.9 µm in *H. hyssopifolium*. Minimum (16.2 µm) and maximum colpi lengths (27.1µm) were recorded in *H. linarioides* and *H. armenum* respectively. Apocolpium Index (AI) (External distance between two colpi/pollen grain diameter and d/D (Punt & al., 2007) differed from 14.3µm in *H. armenum* to 26.2 µm in *H. davisii*. Among the studied taxa, apertures number and types changed from 3- syncolporate in *H. triquetrifolium*; 3-zonocolporate in *H. asperulum, H. hyssopifolium, H. armenum* and *H. linarioides*; 4- zononcolpaorate in *H. hirtellum;* 6- zonocolporate in *H. scabrum;* 4-syncolporate in *H. hirtellum, H. elongatum* subsp. *microcalycinum, H. elongatum* subsp. *apiculatum, H. helianthemoides* and *H. vermiculare* and *H. davisii* all the examined species had rolled colpus margin, (except *H. armenum* and *H. hyssopifolium*, with relatively rolled margin) and psilate colpus membrane (fig. 2. A). In some studied species, operculum was present and obviously protruded e. g. *H. hyssopifolium* (fig. 2. A) and *H. armenum* (fig. 4. E and F). In addition, we observed equatorial bridge in *H. elongatum* subsp. *apiculatum* (fig. 3. K), *H. asperulum* and fastigium in *H. hirtellum* (fig. 4), in different species of the genus. Based on exine sculpturing, pore shape and muri thickness the examined species are divided in two main types and 2 subtypes (presented in table 3, figs. 2-4). Type I: This type includes pollen grains with scrobiculate exine sculpturing and occurred in *H. hyssopifolium* (fig. 2. C) and *H. asperulum* (fig. 2. F). In these species pores are small (0.17-0.18 µm) and round in shape. Type II: This type comprises, micro reticulate exine sculpturing and recognized in 13 species (table 2). This type is divided in to two subtypes. Type II sub type A: includes pollen grains with small pore (0.16-0.40 µm) and thick muri (0.30-0. 95µm) and recorded in *H. linarioides* (fig. 2. I), *H. davisii* (fig. 2. L), *H. scabrum* (fig. 3. C), *H. triquatrifolium* (fig. 3. F) and *hirtellum* (fig. 4. I). Type II sub type B: this subtype consists of pollen grains with large pore (lumen) (0.24-0.75 µm) and thin muri (0.20-0.35 µm), it was identified in *H. helianthemoides, H. elongatum* subsp. *microcalycinum* (fig. 3. I), *H. elongatum* subsp. *apiculatum* (fig. 3. L), *H. apricum* (fig. 4. C), *H. armenum* (fig. 4. F), *H. vermiculare* (fig. 4. L).

Our findings showed that the pores are spaced irregularly in *H. apricum, H. vermiculare, H. hyssopifolium* and *H. asperulum*, regular-irregular in *H. linarioides, H. davisii*, H. *scabrum, H.triquetrifolium, H. elongatum* subsp. *apiculatum, H. elongatum* subsp. *microcalcicum, H. armenum* and regular in *H. helianthemoides* and *H. hirtellum*.

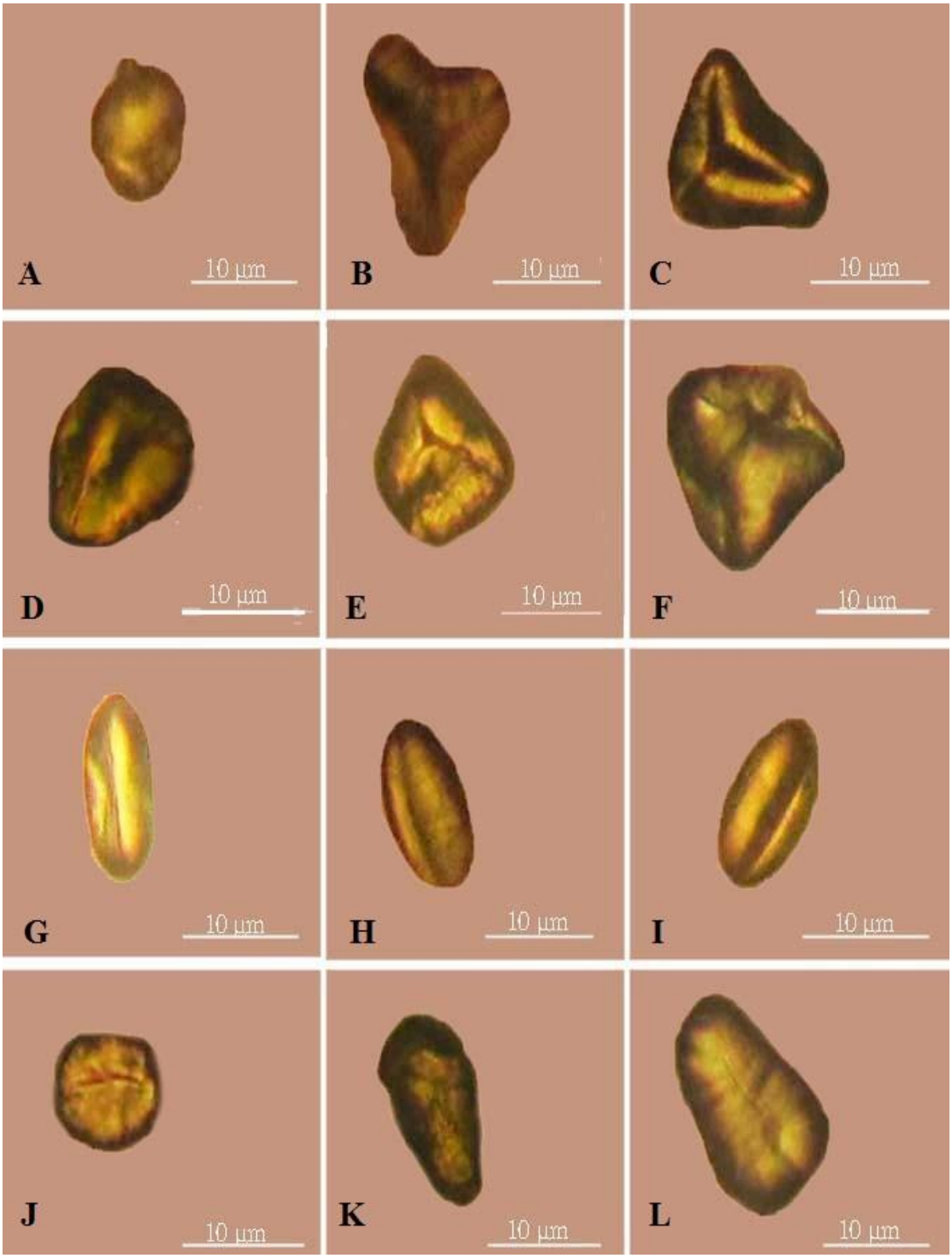

Fig. 1. The LM micrographs of pollen grains in :A, *H. armenum*; B, *H. helianthemoidae*; C, *H. scabrum*; D and L, *H. elomgatum* subsp *apiculatum*; E, *H. hirtellum*; F and K, *H. elongatum* subsp. *microcalycinum*; G, *H. asperulum;* H, *H. triquetrifolium*; I, *H. linarioides;* J, *H. hyssopifolium.*

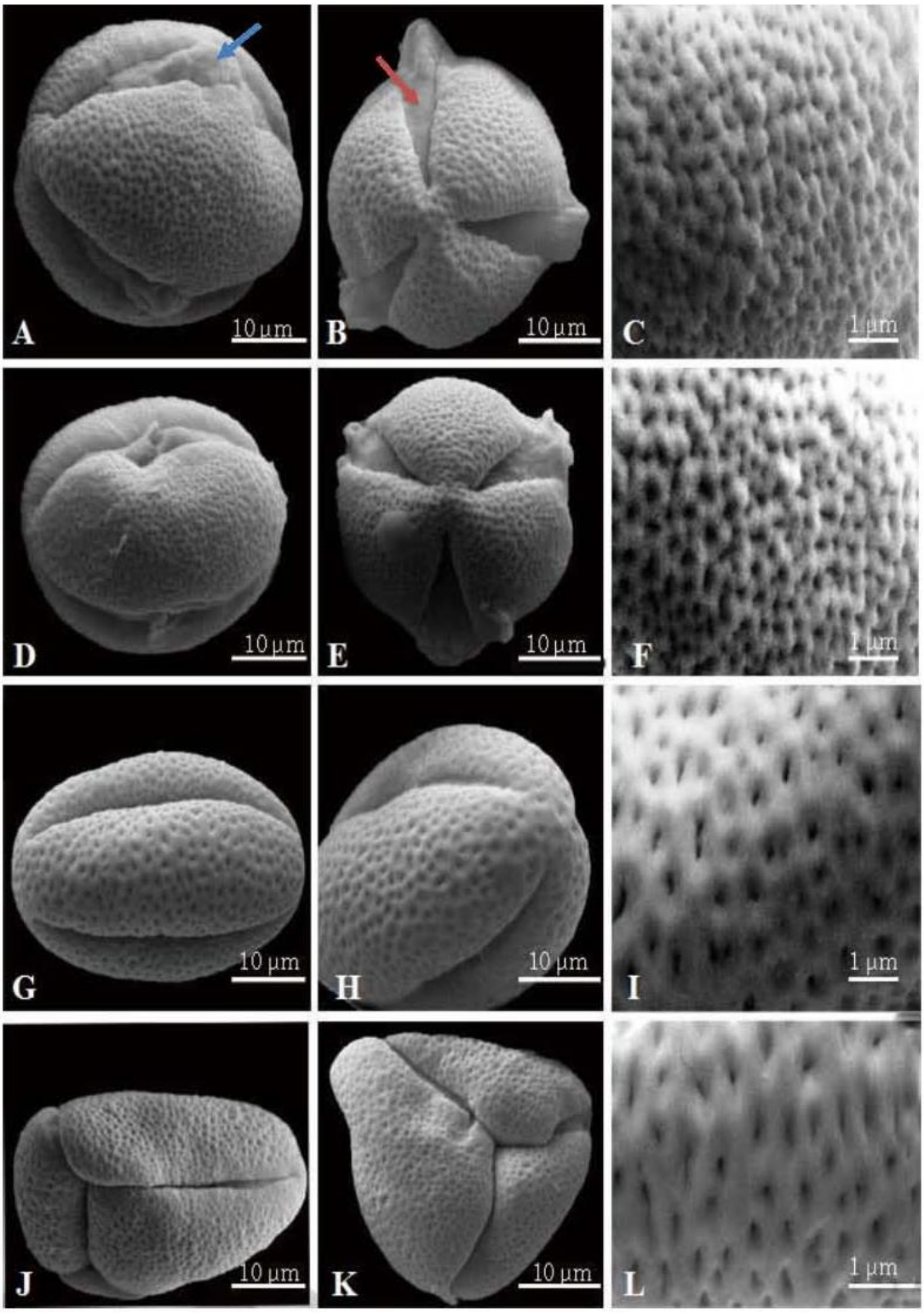

Fig. 2. The SEM micrographs of pollen grains in *Hypericum.* A-C, *H. hysopifolium*; D-F, *H. asperulum*; G-I, *H. linarioides*; J-L, *H. davisii*. Red arrow indicates smooth colpus membrane and the blue one shows operculum.

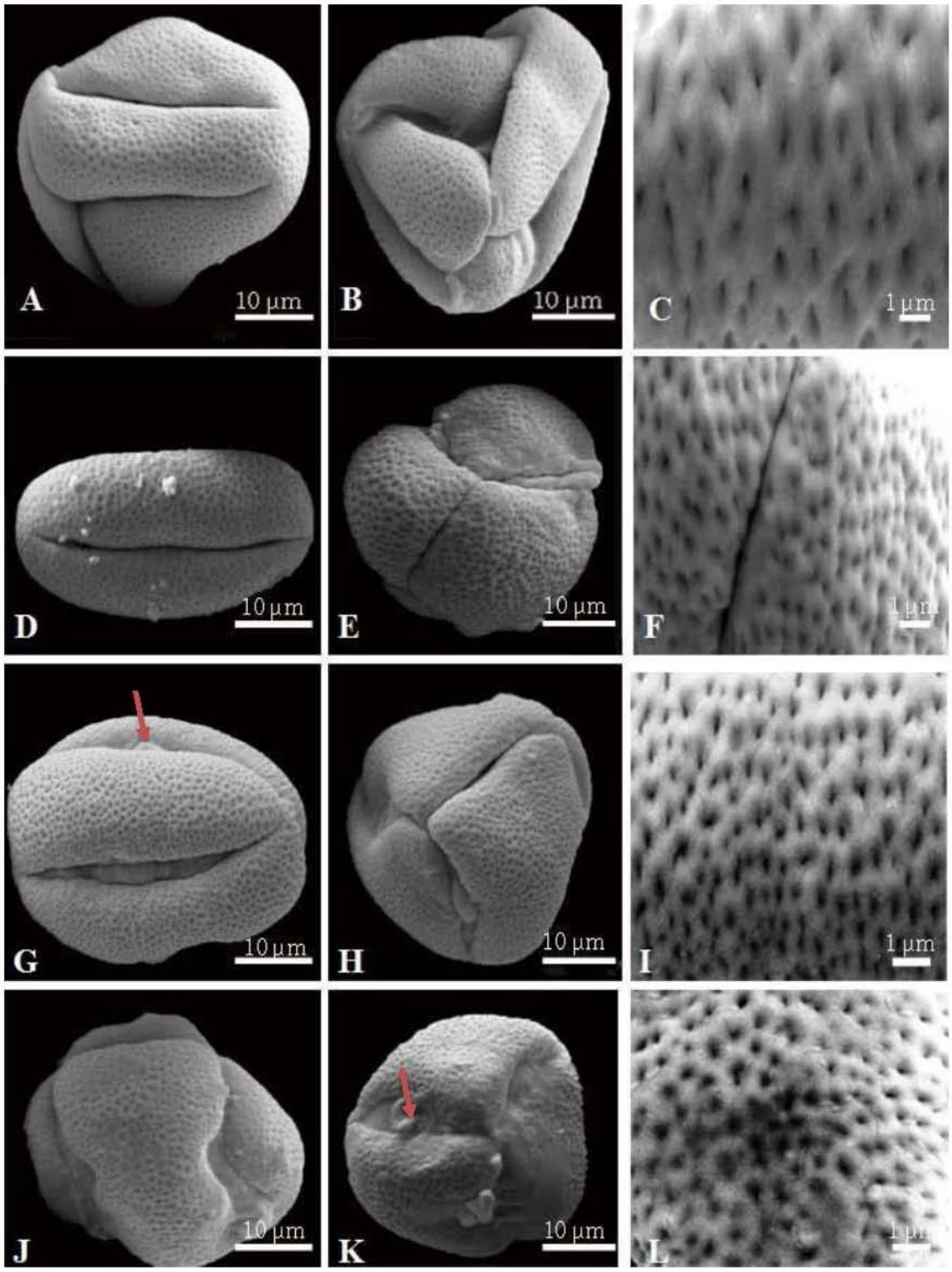

Fig. 3. The SEM micrographs of pollen grains in *Hypericum.* A-C, *H. scabrum*; D-F, *H. triquetrifolium*; G-I, *H. elongatum* subsp. *microcalycinum*; J-L *H. elongatum* subsp. *apiculatum*. Arrow indicates equatorial bridge.

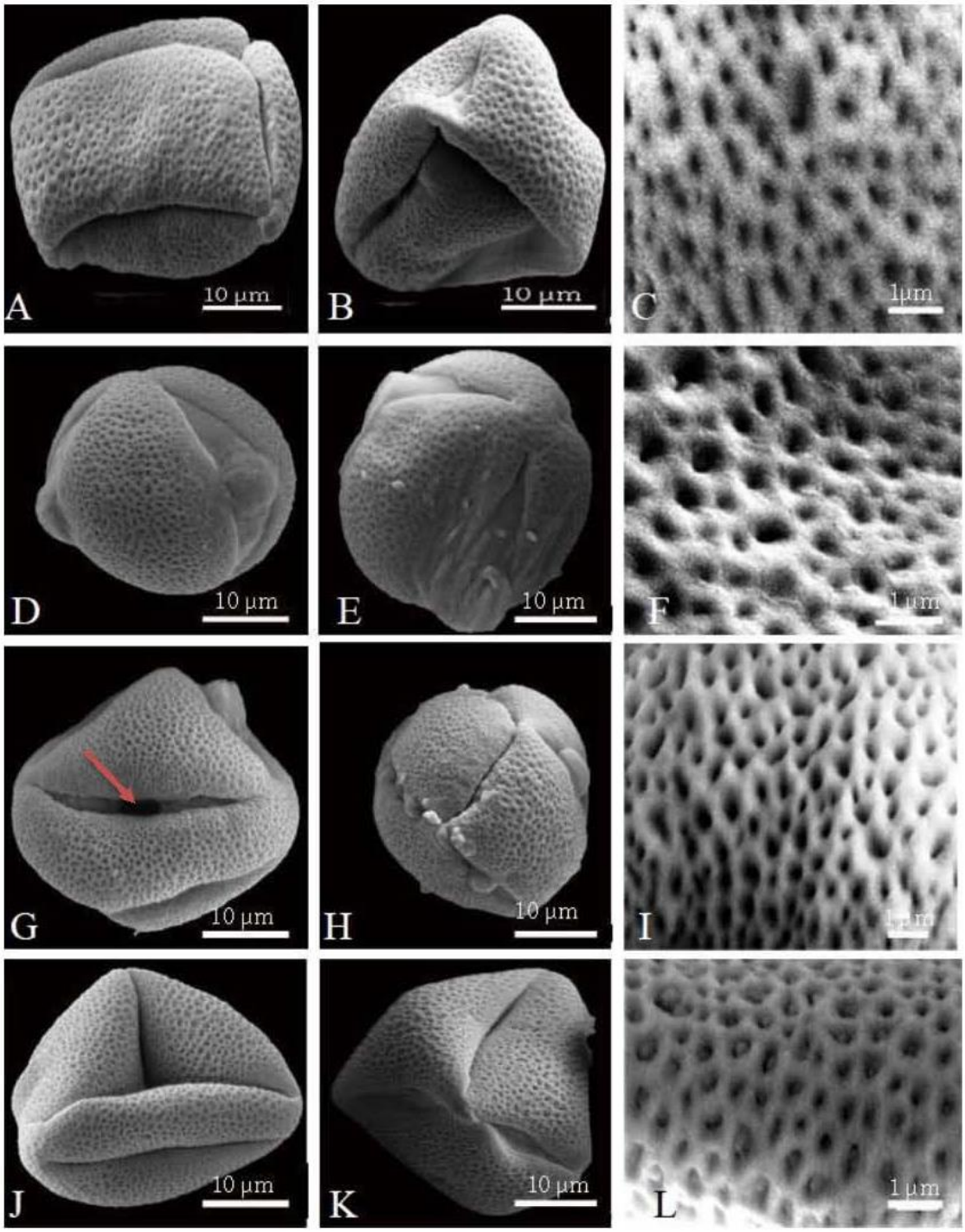

Fig. 4. The SEM micrographs of pollen grains in *Hypericum.* A-C, *H. apricum*; D-F, *H. armenum*; G-I, *H. hirtellum*; J-L, *H. vermiculare.* Arrow indicates fastigium.

Table 3. Grouping based on exine sculpturing, pore shape and muri thickness; Abbreviations: Muri thickness (Mt); Pore (Pd); Pore shape (PS): Round (Ro), Elliptical (EL), Linear (L), Polygonal (Pol); Pore arrangements (PA); Regular (R), Irregular (Ir).* indicates, species from Mahmoudi Otaghvari & al., (2015).

Section/ Sub section/Species and subspecies	**Mt (µm)**	**Pd (µm)**	**PS**	**PA**	**Sculpturing**
Type I 1. *H. hyssopifolium* (Sect. *Drosanthe*)	(0.20-0.40) 0.3	(0.14-0.2) 0.17	Ro	Ir	**Scrobiculate**
2. *H. asperulum* (Sect. *Hirtella*, Sub sect. *Platyadenum*)	(0.35-0.40) 0.35	(0.16-0.2) 0.18	Ro	Ir	
Type II **Sub type A**					
3. *H. linarioides* (Sect. *Taeniocarpium*)	(0.50-0.95) 0.72	(0.24-0.27) 0.20	Ro-L	R-Ir	
4. *H. scabrum* (Sect. *Hirtella*, Sub sect. *Platyadenum*)	(0.50-0.7) 0.60	(0.20-0.24) 0.22	Ro-L	R-Ir	
5.**H. tetrapterum* (Sect, *Hypericum*)	>0.6	(0.210-0.269) 0.23	-	-	
6. *H. triquetrifolium* (Sect. *Hypericum*)	(0.50-0.68) 0.59	(0.16-0.25) 0.20	Ro	R-Ir	Small pore and thick muri
7. *H. davisii* (Sect. *Hirtella* Subsect. *Stenadenum*)	(0.50-0.68) 0.59	(0.29-0.39) 0.34	Ro	R-Ir	
8. **H. fursei* (Sect. *Taeniocarpium*)	-	(0.211-0.314) 0.26	-	Ir	
9. *H. hirtellum* (Sect. *Hirtella*, Sub sect. *Platyadenum*)	(0.30-0.52) 0.40	(0.20-0.40) 0.30	Ro	R	
10 **H. androsaemum* (Sect. *Androsaemum*)	-	(0.184-0.221) 0.20	-	Ir	**Micro-reticulate**
Sub type B					
11. **H. hirsutum* (Sect. *Taeniocarpium*)	>0.6	(0.574-0.673) 0.62	-	Ir	
12. *H. armenum* (Sect. *Taeniocarpium*)	(0.30-0.4)0.35	(0.24-0.68) 0.58	Ro-L	R-Ir	
13. *H. vermiculare* (Sect. *Hirtella* Sub sect. *Stenadenum*)	(0.20-0.30) 0.30	(0.50-0.60) 0.55	Ro	Ir	
14. *H. elongatum* subsp. *microcalycinum* (Sect. *Hirtella* , Sub sect. *Stenadenum*)	(0.30-0.40) 0.35	(0.49-0.68) 0.58	Ro-L	R-Ir	Large pore and thin muri
15. *H. elongatum* subsp. *apiculatum* (Sect.: *Hirtella*, Sub sect. *Stenadenum*)	(0.30-0.40) 0.35	(0.30-0.68) 0.49	PoL-R	R-Ir	
16. **H. perforatum* (Sect. *Hypericum*)	>0.6	(0.443-0.562) 0.48	-	-	
17. *H. helianthemoides* (Sect. *Hirtella*, Sub sect. *Stenadenum*)	(0.25-0.40) 0.32	(0.40-0.45) 0.42	Pol-R	R	
18. *H. apricum* (Sect. *Hirtella*, Sub sect. *Stenadenum)*	(0.25-0.30) 0.25	(0.30-0. 75) 0.52	Ro	Ir	

DISCUSSION

Palynological analysis of selected species of the genus *Hypericum* revealed important pollen morphological characters, especially pollen outline, numbers and types of apertures, colpus length; presence and absence of operculum; exine sculpturing type, pore shape, size and arrangements. The current pollen morphological data are in agreement with that of previous authors (Mahmoudi Otaghvari & al, 2015 and Bayat & al., 2015). Based on our findings, the pollen grains were regular (in two species and one subspecies), regular –irregular (in two species and one subspecies) and irregular (in 5 species) in outline. Irregular pollen has been already reported by previous workers (Martonfi & al., 2002; Mesegure and Sanmartin, 2012) in other species e.g. *H. fursei*, *H. desetangsii*, *H. montanum*. Clarke (1981) explained production of 50 to 100% of irregular pollen in different section of the genus (e.g. in Sect. *Hirtella* Stef). Size of pollen grain varied from small to medium. However, medium pollen type was dominant among the examined species (in 10 representatives). Some researchers believed that the pollen size in the genus *Hypericum* is affected by hybridization and ploidy level of the species and lack taxonomic importance (Asker & Jerling, 1992; Horandl, 2004; Matzk & al., 2003). In the examined species, apertures number and types changed from 3-syncolporate (in 2 species), 3-zonocolporate (in 4 species), 6-zonocolporate (in one species), and 4-syncolporate (in 6 species and 2 subspecies). Among the studied species *H. hirtellum* had both 4-zono and syn-colpotare pollen grains. Previous researches (Ocak & al., 2013; Martonfi & al., 2002) also reported variation in number and types of apertures (from 2 to 4 syncolporate, 3 to 4- zonocolporate, 6, 8 and 12-pericolpate) in different species of *Hypericum*. These traits have been considered as diagnostic evidences and good tool for separating species and subspecies (Walker and Doyle 1975; Moore & al 1991). Rolled colpus margin with smooth external edge are another pollen characteristic feature in *Hypericum* (Ocak & al., 2013; Martonfi & al., 2002). This has been observed in all the studied taxa (except *H. armenum*). SEM observation also revealed other important palynological evidences of the genus such as operculum (which is coherent exine structure covering an aperture) (punt & al 2007), equatorial bridge (exine connection between the margins of a colpus in the equatorial region) (Pascoe 2007; punt & al 2007), and fastigium (cavity in colporate grains appearing as separation part of the exine from the domed sexing in the region of endoaparature; (Reitsma 1966; Martonfi & al., 2002). These traits are good tools for separating species of this genus (Hebed & al., 1988; Hebed and Chinepa 1990). Based on exine sculpturing, muri thickness, pore size and shape the examined species are divided in two main types. Scrobiculate exine sculpturing comprises shallow muri forming reticulate pattern surrounding small lumen of less than 1 µm considered as pores (Martonfi & al., 2002; Punt & al). The average distance between pores are greater than their diameters (Vezey & al. 1992) in *H. hyssopifolium* and *H. asperulum*. Microreticulate exine sculpturing is the most abundant and recorded in 11 examined species. This type of exine ornamentation was formerly reported in *H. bithynicum*, *H. confertum*, *H. olympicum*, *H. orientale* and *H. adenotrichum* (Ocak, & al. 2013), *H. tetrapterum*, *H. perforatum*, *H. androsaeum* (Mahmoudi Otaghvari & al., 2015), and *H. dogonbadanicum* (Sect. *Campylosporus*) *(*Bayat & al., 2015). However, Ocak, & al. (2013) reported other exine sculpturing in different species of the genus e.g. granulated in *H. fursei*, perforate-microreticulate in *H. venustum*, *H. heterophyllum*, *H. calycinum*, *H. avicularifolium*, *H. montbretii*. Our results show that the exine sculpture type classes are not able to delimit species of the same sections, but they can serve as a useful tool for species identification.

Palynological study of selected species of the genus *Hypericum*, render informative data and can be used for classification purpose, especially at sectional, species and subspecies levels. In Iran, the genus *Hypericum* includes 19 species and 4 subspecies arranged in 5 sections (Robson, 1968 and Azadi, 1999). Among them, Sect. *Hirtella* Stef, is the largest section (Robson 1986), consists of perennial herbaceous plants having black glands on margin of sepals and petals; clawed petals; stamens in three bundle and elongated gland on the capsule surface. According to our results, these species possess identical pollen morphological characters (polarity, pollen shape and size, type and number of apertures; exine sculpture; pore shape and arrangement). Sect. *Hirtella* Stef includes two subsections: Subsect. *Platyadenum* N. Robson and Subsect. *Stenadenum* N. Robson). Plants with marginal obconical glands, glandular sepal apex, preferring rocky and stony-gypsy steep slopes of NW, W, E and C of Iran, characterize the first subsection. The palynological data of three studied species (*H. scabrum*, *H. hirtellum* and *H. asperulum)* of this subsection, including pollen shape; aperture types, exin sculpture (type I and subtype A) also supports a close relationship among these species. *Hypericum scabrum* resembles *H. hirtellum* in having micro reticulate exine sculpture, small pore and thick muri, zono-colporate aparature and irregular folded pollen of medium in size. On the other hand, the exine sculpturing of pollen grains in *H. asperulum* is scrobiculate, with thin muri and smaller pore diameter,zono-colporate, regular pollen of small in size. The second subsection, possess

pollen grains of medium in size; exine sculpturing of type II. sub type B (except for *H. davisii*) and syn-colporate pollen grains with or without operculum. Robson (1968) and Azadi (1999) placed *H. Helianthemoides*, *H. vermiculare* , *H. apricum* , *H. davisii* and *H. elongatum* in this subsection, because of their round - elliptical gland at calyx margin and glandular tip of calyx. The two studied subspecies: *H. elongatum* subsp. *microcalcycum* and *H. elongatum* subsp. *apiculatum*, grow on rocky slopes and forests of N and NW Iran (Robson 1968; Azadi1999), share several pollen morphological characters e.g. pollen of medium size; regular and regular-irregular, subisopolar; micro-reticulate sculpture with large perfora and thin muri (type II sub type B) arranged in regular-irregular intervals. However, they differed by their pollen and perforation shapes. *Hypericum vermiculare* and *H. helianthemoides* are placed close to each other because of their capsule and inflorescence shape (Robson 1968). Both species show irregular (folded), heteropolar pollen; micro-reticulate sculpture with large perfora with thin muri (type II sub type B). However, perora shape and arrangement can be helpful to distinguish the two species. *Hypericum apricum* also resembles to *H. vermiculare* by microreticulate sculpture, (type II sub type B), perfora and arrangement. Nevertheless, it is characterized by irregularly spaced perfora and regular-irregular pollen. *Hypericum davisii* is identified by exine sculpturing of type II subtype A, thick muri, small, round regular-irregularly spaced perfora. This species is recognized by its round-ovate buds, oblong-elliptical sepals, narrow cylindrical inflorescence (Azadi 1999).

Section *Taeniocarpium* Jaub. & Spach., consists of four species (*H. armneum*, *H. linarioides*, *H. hirsutum* and *H. fursei*), characterized by their erect or ascending stem; reddish petals, without claw*;* and linear glands on external surface. The result supports the former palynalogical data *(*Mahmoudi Otaghvari & al. 2015), and exhibit pollen morphological affinities among different species of the section. This includes medium size (in *H. armneum*, *H. linarioides*, and *H. hirsutum*), zonocolporate, regular (in *H. armenum* and *H. linarioides*), regular - irregular (in *H. fursei* and *H. hirsutum*), rolled colpus margin (*H. linarioides, H. hirsutum* and *H. fursei*), micro-reticulate exine sculpture of type II, subtype A (in *H. fursei* and *H. linarioides*) and subtype B (in *H. armenum* and *H. hirsutum*) pollen grains. The current result is in controversy with current classification (Robson 1968; Azadi1999), in which *H. linarioides*, *H. fursei* and *H. armenum* (for their glabrous stem, leaves and calyxes); *H. fursei* and *H. armenum* (for their dense glands at upper half of the calyx, cylindrical inflorescence) regarded as closely related species. While *H. hirsutum* is separated from them by its hairy stem, leaves and calyx.

Hypericum hyssopifolium is the only representative of Sect. *Drosanthe* (Spach) Endl., characterized by oblong-lanceolate obtuse sepals with marginal sessile glands; obovate petals covered by colored and black marginal glands (Robson, 1968; Azadi ,1999). Based on the present analysis, among the studied species, *H. hyssopifolium* and *H. asperulum (Hirtella* Stef, Subsect. *Platyadenum*) have scrobiculate exine sculpturing. This does not support the relationship between the two species.

Sect. *Hypericum* comprises three herbaceous species (*H. tetrapterum* and *H. perforatum* and *H. triquetrifolium*) which have common seed and petal characters morphological features (Robson, 1968; Azadi, 1999). Among them, *H. triquetrifolium* and *H. tetrapterum (*Mahmoudi Otaghvari & al., 2015), resemble to each other by their exine sculpturing pattern (type II sub type A). However, based on Robson, (1968) and Azadi (1999), *H. triquetrifolium* approaches to *H. perforatum* for its stem morphology (having two longitudinal line). Our finding showed that, pollen morphological characters of Sect. *Hypericum* do not support morphological studies and current classification (Robson, 1968; Azadi, 1999).

Sect. *Campylosporus* is identified by tree, shrubs, with dark glands, permanent petals, 5 bundle stamens, 5 more or less fused styles. It includes *H. dogonbadanicum* growing in W and SW Iran, in Zagros Mountain, at 1000-1500 m a.b.s (Azadi, 1999). Despite to morphological differences, it exhibits similar palynological evidences (Bayat & al. 2015) especially exine sculpturing pattern (type II) with other representatives of the genus especially those having micro reticulate sculpturing.

In summary, pollen morphology is very useful in delimitation of *Hypericum*. These traits are taxonomically informative and can be used for separation of different taxonomic ranks (sections, species and subspecies levels). However, the importance of pollen morphological characters and the species relationship should be discussed based on molecular phylogenetic study of Iranian species of the genus which is in urgent need.

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