Micromorphology of leaf trichomes in *Onosma* (*Boraginaceae*) and their systematic relevance in Iran

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Abstract. This study provides the first detailed description of leaves and nectar trichome characteristics in *Onosma*. The indumentum characteristics of 32 *Onosma* taxa collected from Iran are given in it, as well as the results obtained from LM and SEM investigations. Three groups have been recognized within the genus, relatively in agreement with the infrageneric taxa of *Onosma* that Boissier had originally described. The obtained results were confirmed by many molecular, palynological and morphological studies. Furthermore, a diagnostic key has been provided according to which the indumentum, accompanied by other morphological evidences, shows distinct boundaries between sections, subsections and species in *Onosma*. Although there are taxa which cannot be determined solely using trichome morphology, these evidences can be a very useful tool in distinguishing between many of the currently recognized *Onosma* taxa.

Key words: *Boraginaceae*, Iran, *Onosma*, morphology, systematics, trichome

Introduction

Onosma L. is a genus of the tribe *Lithospermeae* Dumort., belonging to the large family of *Boraginaceae* which comprises *ca.* 150 species (Weigend & al. 2009; Cecchi & Selvi 2009; Kolarčik & al. 2010) distributed mainly in West and Central Asia and in the Mediterranean area, and growing in dry, sunny, rocky, sandy, and steppe habitats (Javorka 1906; Meusel & al. 1978).

Stems and leaves of the *Boraginaceae* are covered with hairs which may be glandular or eglandular (Metcalf & Chalk 1950). Density of these hairs varies with different habitats. Due to the presence of dense trichomes, the *Boraginaceae* family is occasionally referred to as the "shagy coat family" (Simpson 2006). The family *Boraginaceae* is characterized by a great diversity of setae forms. Many authors (Al-Nowaihi & al. 1987; Selvi & Bigazzi 2001; Diane & al. 2003; Taia 2006;Ventrella & Marinho 2008; Perveen 2009) have proved that the setae characters support isolation of many genera and species of *Boraginaceae*. Trichomes are widely distributed on the reproductive and vegetative parts of *Boraginaceae*. Most descriptions have been published in the context of the general studies of *Boraginales*, and many of them were published in the last decades (e.g. Metcalf & Chalk 1950; Johnston 1952, 1953a,b, 1954a,b; Solereder 1908). Published are also some new studies on the trichome morphology of *Boraginaceae* (Al-Nowaihi & al. 1987, Selvi & Bigazzi 2001, Diane & al. 2003, Taia 2006, Ventrella & Marinho 2008, Perveen 2009). Non-glandular trichomes associated with cystolith bodies in the basal portion and with partially calcified walls, called cystolith-hairs, are widely distributed in *Boraginaceae* and are responsible for the wrinkled leaf surface (Solereder 1908).

The taxonomic value of indumentums and their involvement in systematics and phylogenetics are well known in *Boraginaceae* and in the closest to it families (Metcalf & Chalk 1950, El-Gazzar & Watson 1968, Ahmad 1974, Elias & Newcombe 1979, Mathew & Shan 1983, Abu-Asab & Cantino 1987, Cantino 1990, Rahn 1992, Werker 2000, Gairola & al. 2009).

Trichome morphology has been widely used as a taxon delimitating character in *Onosma*. Boissier (1879) was the first to refer to the trichomes role in the taxonomy of *Onosma*. Subsequently, Shishkin (1953), Post (1966), Riedl (1967), Polunin (1969), Davis (1978), and Zohary (1978) had pointed out the trichome importance. Furthermore, Akcin & al. (2010) acted on Boissier ideas to differentiate taxonomically the species. Peruzzi & Passalacqua (2008) showed population variability of trichomes in the *Onosma echioides* (L.) L. complex.

The indumentum of *Onosma* leaves consists of three separate components: setae (seldom named hairs) arising from broad, often slightly raised, or pancake-shaped, multicellular tubercles; setules, occasionally shortened to tiny spinules or produced as hairs (pilies), stellately arranged around the base of the seta; and tiny hairs forming a pubescent, puberulous or tomentose surface between the setae. The setae occasonally form scabrous, strigose or sericeous indumentum (Davis 1978).

Boissier was the first to treat comprehensively this genus (1879) in *Flora Orientalis*. *Onosma* was classified there on the basis of indumentums into three sections, namely: *Podonosma*, *Protonsoma* and *Onosma*. Recent section was further divided into three subsections: *Haplotricha* (Boiss.) Gürke, *Heterotricha* (Boiss.) Gürke and *Asterotricha* (Boiss.) Gürke. While the *Asterotricha* group is characterized by stellate setae, *Haplotricha* lacks them, and *Heterotricha* possesses an intermediate indumentum type (Kolarčik & al. 2010). Furthermore, Khatamsaz (2001) followed Boissier's classification in the *Flora of Iran*. Anyway, the above-mentioned section was divided into two subsections, *Haplotricha* and *Asterotricha* (all taxa of *Heterotricha* and *Asterotricha* are given according to Boissier's classification), by Riedl (1967) and Davis (1978).

Owing to the importance of trichome evidence in the taxonomy of *Onosma*, some taxa were denominated on the basis of trichome features. For example, *Onosma sericeum* Willd. (sericeous setae or silky trichomes), *Onosma asperrima* Bornm. (aspersus setae or slightly rough trichomes), *Onosma bulbotricha* DC. (bulbous setae), *Onosma chrysochaeta* Bornm. (yellow setae, or golden trichomes), and *Onosma chlorotricha* Boiss. & Noë (green setae) (see Plate 1).

Taxonomic treatments within the genus *Onosma* are highly controversial (Teppner 1996) and many similar species were described on the basis of minor morphological differences (Kolarik & al. 2010). On the other hand, any perfect and detailed reference to some valuable morphological evidence in *Onosma* is lacking. Furthermore, the taxonomic range of sections and subsections is rather ambiguous. Therefore, it is important to understand the mentioned ranges, the existing variation and the major characteristics of the trichomes, when identifying the *Onosma* species. Thus an attempt was made in this study at identifying the unique characters of the trichomes of each taxon, so as to expand the knowledge on trichome diversity and clarify the ambiguities in the systematics of this genus.

Material and methods

In our research, trichome morphology of 32 species of the genus *Onosma* from Iran was studied on the basis of SEM and LM microscopy techniques (Plates 1-7). Voucher information can be found in Table 1. Materials used for this study were taken from wild populations and from herbarium samples in HSBU, TARI, WU and W (herbarium abbreviations according to Thiers 2008). The trichomes were investigated on the cauline leaves and nectar ring. The indumentums of the upper leaf surface of all studied species were examined by light and scanning electron microscope. For light microscopy (LM), dried leaves were photographed by Dinolite Microscope AM-413T. Furthermore, dried leaves were mounted on stubs using double-sided adhesive tape. Samples were coated with 12.5–15 nm of gold. Subsequently, the coated leaves were examined and photographed with Cam Scan MV 2300 Electron Microscope. The general trichome terminology follows Riedl (1967), Davis (1978) and Harris & Harris (2001).

The trichomes were measured by Image Tools ver. 3 (Fig. 2). These measurements included seven quantitative and three qualitative characteristics, namely: setae type, setae orientation, setae color, setae length, setae density, setae ornamentation diameter, presence of tubercle pili (small hairs at the base of tubercles), length and density of tubercle pili, tubercle diameter, pili density, and presence of nectar trichomes (Table 2). Some 20–30 setae were assessed for quantitative characteristics. Setae density was categorized in a relative comparison between the studied taxa.

Table 1. The examined species of genus *Onosma*.

Taxa	Locality	Collector	
Onosma rostellata Lehm.	Kurdistan, Bayangan, 1450 m	Mehrabian	HSBU-2010244
Onosma orientale L.	Khuzistan, Masjed Soleyman, Andika, 600 m,	Mozaffarian	TARI-63017
Onosma albo-rosea Fisch.	Kermanshah, Kerend-e Gharb, 45 km west of Kerend, Rijab,900 m,	Lashkar & Hatami	TARI-167
Onaosma armena DC.	Azarbaijan, Maku to Khoy, 2400 m	Assadi& Mozaffarian	TARI-30353
Onosma bilabiata Boiss.	Azarbaijan, southern slopes of Mt Sahand, 2300–2600 m	Assadi& Mozaffarian	TARI-30764
Onosma dasytricha Boiss.	Gohkiloye va Boyer Ahmad: 11 km from Dogonbadan to Ghoram (VPI), 1400 m	Assadi& Abohamzeh	TARI-38584)
Onosma hebebulba DC.	Azarbaijan,45km Kerend, Dalahoo, 2000 m	Assadi	TARI-60835
Onosma iranshahrii Ghahreman & Attar.	Kurdistan, Marivan to Paveh, Gardan-e Tat, between Dezli and Hanigarmaleh,	Mozaffaraian	TARI-75701
Onosma kurdica Teppner	Kurdistan in Mt Hamzeh Arab SE Bijar, 2600 m	Lamond,Termeh & Rechinger	WU-3231
Onosma rasychaena Boiss.	Zanjan, Mahneshan, Angoran Protected Area, Mt Belgheis, 2700 m	Mehrabian	HSBU-2010281
Onosma elwendica Wettst.	Tehran,Lashkarak, 1900 m	Mehrabian	HSBU-2010247
Onosma Gaubae Bornm.	Tehran, Damavand, Chenar to Tar, 2500 m	Mozaffarian	TARI-37319
Onosma nervosa H.Riedl.	Esfahan, Ferydonshahr, Mt Venizan, 2500 m	Mozaffarian	TARI-77244
Onosma stenosiphon Boiss.	Kerman, Mt Chopar	Kanani	HSBU-2010237
Onosma longiloba Bunge	Semnan, 20 km Mohamad Abad from Firuzkuh,	Pahlevani	HSBU-2012-100
Onosma asperrima Bornm.	Fars, Nurabad: Doshman-Ziari Region, Ab Zalu village, Kuhe Tasak, 1900–2500 m	–	TARI-45772
Onosma dichroantha Boiss.	Golestan, Golestan National Park,1500 m	Heidari, Ghorbani & Habibi	HSBU-2007300
Onosma microcarpa DC.	Markazi, Arak, Gavar, 2000 m	Mehrabian	HSBU-2010244
Onosma macrophylla Bornm.	Kermanshah, Malavi to Eslam Abad, 1200 m	Mozaffarian	TARI-64384
Onosma pachypoda Boiss.	Azarbaijan, Yam,Misho Dagh Mt., 1800 m	Mehrabian	HSBU-2010602
Onosma platyphylla H.Riedl.	Hamedan, Nahavand, on the road to Nurabad, above Gamasiab, Kuh-e Garin, 2500 m	Assadi	TARI-75138
Onosma bodeana Bornm.	Tehran, Sohanak, 2200 m	Mehrabian	HSBU-2010256
Onosma bulbotricha DC.	Zanjan to Mahneshan, 2200 m	Mehrabian	HSBU-2010238
Onosma cornuta H.Riedl.	Kurdistan, Bijar to Takab, 1600 m,	Mehrabian	
Onosma kilouyensis Boiss. & Hausskn.	Khuzestan, Dehdez, Kuh Sefid, 2700 m	Mozaffarian	TARI-74528
Onosma kotschyi Boiss.	Fars, south of Estahbanat, 2200 m	Mozaffarian	TARI-46999
Onosma olivieri Boiss.	Kermanshah, Iranshahr & Zargani, 1500 m		IRAN-2901
Onosma sabalanica Ponert	Aerdabil, Meshkin Shahr, Mt Sabalan, 2900 m	Mehrabian	HSBU-2010249
Onosma sericea Willd.	Kurdistan, Sanandaj, Abidar, 1730 m	Mehrabian	HSBU-2010273
Onosma straussii H.Riedl.	Markazi, Arak, Gavar, 2178 m	Mehrabian	HSBU-2010-232
O. subsericea Freyn	Azarbaijan, Khalkhal to Givi, 1800 m,	Wendelbo & Assadi	TARI-27837

Table 2. Measurements of the trichomes (Plate 1-7): A – setae type (I, II, III)., B – setae status (adpressed – Ad, patents – Pa)., C – setae length., D – setae color., E – setae density (medium – Mi, dense – D), F – tubercle pili length, G – tubercle pili density, H – tubercle diameter, I – small pili density, J – setae ornamentation diameter, K – nectar pili.

Species	Setae type (A)	Setae staus (B)	Setae length (mm) (C)	Setae color (D)	Setae density (E)	Tubercle pili length (mm) (F)	Tubercle pili density (G)	Tubercle diameter (mm) (H)	Small pili density (I)	Setae ornamentation dimeter (µm) (J)	Nectar pili (K)
Onosma rostellata Lehm.	I	Pa	1.37 1.01–2.36	white	M	–	–	0.27 0.10–0.45	M	11.96 9.32–15.44	–
Onosma orientale L.	I	Pa	0.64 0.50–0.72	white	M	–	–	0.09 0.06–0.15	M	4.45 3.42–7.77	–
Onosma longiloba Bunge	I	Pa	1.59 0.98–1.90	white	M	–	–	0.14 0.10–.020	D	12.67 8.81–13.97	+
Onosma platyphylla H. Riedl.	I	Pa	1.26 0.74–2.13	white	M	–	–	0.53 0.35–0.76	–	9.31 5.61–13.15	–

Table 2. Continuation.

Species	Setae type (A)	Setae staus (B)	Setae length (mm) (C)	Setae color (D)	Setae density (E)	Tubercle pili length (mm) (F)	Tubercle pili density (G)	Tubercle diameter (mm) (H)	Small pili density (I)	Setae ornamentation dimeter (μm) (J)	Nectar pili (K)
Onosma bulbotricha DC.	I	Pa	1.32 0.87–1.84	white	M	–	–	0.37 0.26–0.48	M	8.99 7.05–11.69	+
Onosma dichroantha Boiss.	I	Pa	1.09 0.74–1.51	white	M	–	–	0.28 0.23–0.35	M	7.04 5.62–9.25	–
Onosma microcarpa DC.	I	Pa	1.15 0.74–2.21	White–yellow	M	–	–	0.30 0.17–0.47	M	8.15 5.47–10.28	–
Onosma sabalanica Ponert	I	Pa	0.95 0.58–1.53	White–yellow	D	–	–	0.07 0.03–0.13	M	5.06 3.41–6.44	–
Onosma kotschyi Boiss.	I	Pa	1.11 0.68–1.42	white	M	–	–	0.17 0.13–0.22	M	9.85 5.50–13.14	–
Onosma asperrima Bornm.	I	Pa	0.97 0.52–1.35	white	M	–	–	0.44 0.32–0.60	–	20.60 11.25–24.87	–
Onosma cornuta H.Riedl.	I	Pa	1.51 0.76–1.89	grey	D	–	–	1.22 0.83–1.55	M	13.62 7.37–18.69	+
Onosma sericea Willd.	I	Ad	0.77 0.54–1.06	grey	M	–	–	0.08 0.06–0.11	M	5.36 3.71–6.43	–
Onosma subsericea Freyn	I	Ad	1.21 0.85–1.51	grey	M	–	–	0.17 0.11–0.27	D	8.05 5.69–10.48	–
Onosma bodeana Bornm.	I	Ad	0.77 0.43–1.07	grey	M	–	–	0.93 0.74–1.21	–	absent	–
Onosma pachypoda Boiss.	I	Ad	0.90 0.72–1.08	white	M	–	–	0.10 0.06–0.12	M	13.26 7.94–16.36	–
Onosma kilouyensis Boiss.	I	Pa	1.59 1.16–2.10	white	M	–	–	0.37 0.21–0.55	L	11.34 6.49–16.15	–
Onosma gaubae Bornm.	I	Pa	1.04 0.67–1.48	white	M	–	–	0.19 0.11–0.24	D	12.24 8.90–18.37	–
Onosma macrophyllum Bornm.	II	Ad	1.16 0.80–1.54	white	M	0.25 0.17–037	M	0.25 0.17–0.37	D	6.85 4.33–9.81	–
Onosma elwendica Wettst.	II	Ad	1.18 0.82–1.89	white–yellow	D	60.76 44.68–87.34	M	0.50 0.39–0.65	D	10.20 7.41–13.96	–
Onosma olivieri Boiss.	II	Ad	1.07 0.73–1.46	yellow	M	47.14 35.79–54.61	L	0.26 0.17–0.37	M	6.19 3.95–9.15	+
Onosma nervosa H.Riedl.	II	Ad	0.87 1.06–1.38	white–yellow	M	0.04 0.03–0.05	D	0.37 0.26–0.51	M	12.31 10.02–16.07	–
Onosma stenosiphon Boiss.	II	Ad	1.05 0.70–1.73	white	M	104.18 61.57–167.67	M	0.42 0.35–0.58	D	9.61 8.52–11.64	+
Onosma pabotii H.Riedl.	II	Ad	0.92 0.66–1.11	white	M	83.78 62.68–100.47	L	0.11 0.08–0.14	M	10.44 7.62–12.64	–
Onosma straussii H.Riedl.	II	Ad	1.34 1.28–1.80	white	M	66.92 40.62–138.11	D	0.50 0.18–0.72	D	8.09 4.36–11.65	+
Onosma dasytricha Boiss.	III	Ad	1.30 1.09–1.67	grey	D	0.10 0.08–0.14	M	0.10 0.07–0.15	L	16.47 9.90–20.04	–
Onosma albo–rosea Fisch.	III	Pa	1.36 0.87–1.71	grey	D	175.07 118.22–243.42	D	0.18 0.16–.023	L	19.38 10.97–29.88	–
Onaosma armena DC.	III	Pa	1.13 1.00–1.27	white	D	277.16 209.96–401.50	M	0.21 0.19–.026	–	7.52 7.30–9.95	–
Onosma rasychaena Boiss.	III	Pa	1.51 1.32–2.05	white	M	227.72 144.36–292.76	D	0.24 0.17–0.28	L	14.13 8.06–20.49	–
Onosma kurdica Teppner	III	Pa	1.10 0.91–1.33	white	D	207.39 170.24–233.50	M	160.72 152.95–186.31	M	absent	–
Onosma bilabiata Boiss.	III	Pa	1.17 0.66–1.67	white	M	177.44 154.40–210.61	M	0.18 0.13–0.22	M	8.81 6.64–10.40	–
Onosma iranshahri Ghahreman & Attar.	III	Pa	0.99 0.68–1.12	white	M	200.68 149.12–265.70	D	1.14 0.09–0.22	L	6.94 3.83–1051	+
Onosma hebebulba DC.	III	Pa	1.30 0.77–2.62	white	M	136.38 95.84–207.35	M	0.19 0.14–.024	M	9.32 7.29–13.12	–

Results

The quantitative and qualitative characters of trichomes show considerable variation among the species. In most taxa, both leaf surfaces are more or less densely covered with different types of unicellular trichomes forming an indumentum of variable texture and density. Trichomes on the leaves include setae (long trichomes with tubercles) and small pili that occur between the setae. Furthermore, there are scattered or dense pili (rays) on the tubercale base of some taxa. In the nectar ring, there are short and white pili only. Setae vary between adprresed to patent. (Plate 1, Fig. 13). On the basis of this evidence, three types of trichomes were identified in the studied taxa:

Type I. Setae with glabrous tubercles – Plates 1-4 (Figs 1-48)

Trichomes consist of galbrous tubercules at the base. Dense or sparse papilae cover the setae surface. This type occurs in sect. *Protonosma*, sect. *Podonosma* and sect. *Onosma* subscect. *Haplotricha*. Sparse setae of low density (*O. orientale*) to dense setae (*O. microcarpa and O. bubotricha*) are found in the mentioned type. Setae length ranges between 0.64 mm (*O. rostellata*) and 1.59 mm (*O. kiliuyensis*). Pili have dominantly covered all studied species, with the exception of *O. asperrima* and *O. platyphylla*. The tubercule diameter is 0.08 mm (*O. sericea*) to 1.22 mm (*O. cornuta*) and the setae ornamentation diameter ranges between 4.45 μm (*O. orientale*) and 13.26 μm (*O. cornuta*), with the exception of the ornamentations in *O. bodeana*. The setae are of medium strong (*O. subsericea, O. kilouyensis*) to strong density (*O. sabalanica* and *O. cornuta*). Pili density ranges between medium (*O. bulbotricha* and *O. dichroantha*) and strong (*O. longiloba* and *O. subsericea*), with the exception of *O. platyphylla, O. bodeana and O. platyphylla*. Setae color varies between white (*O. stenosiphon, O. dichroantha*) and white-yellow (*O. microcarpa, O. sabalanica*) to grey (*O. sericea, O. bodeana* and *O. subsericea*). Setae status is adprresed (*O. sericeum, bodeana, O. pachypodum*) to patent (*O. longiloba, O. microcarpa*).

Type II. Setae with sparse pilies at base – Plate 5 (Figs 49-60)

Seate consist of glabrous tubercles at the base and dense or sparse papilae on their surface. The mentioned type occurs in sect. *Onosma* subscect. *Heterotricha*. Type II dominantly represents sparse, white (*O. elwendica, O. pabotii*) or yellow and dense setae (*O. nervosa, O. olivieri*). Setae length ranges between 0.87 mm (*O. nervosa*) and 1.34 mm (*O. dasytricha*). Tubercle diameter ranges between 0.11 (*O. paboti*) and 0.50 (*O. elwendicum* and *O. straussii*). Setae ornamentations diameter is 6.85 μm (*O. macrophylla*) to 12.31 μm (*O. nervosa*). Small hair density varies between strong (*O. Gaubae* and *O. elwendica*) to medium (*O. olivieri* and *O. paboti*). Tubercle pilies range between 0.04 mm (*O. nervosa*) and 47.14 mm (*O. olivieri*), and low (*O. macrophylla, O. olivieri*) to medium density (*O. elwendica, O. stenosiphon*). Setae color varies between white (*O. macrophylla, O. stenosiphon*) and white-yellow (*O. nervosa, O. olivieri, O. straussii*). Setae status is only adprresed.

Type III. Setae with radial or asteroidal pilies – Plate 6-7 (Figs 61-83)

Setae consist of tubercles with semi-dense or dense tubercle pilies at the base. The mentioned type differs from Type II by the longer and denser tubercle pilies at the base of setae. It is observed in sect. *Onosma* subscect. *Asterotricha*. Type III represents sparse (*O. armena, O. rascheyana*), adprressed and silky setae (*O. dasytricha*). Radial setae are dense (*O. albo-rosea, O. rascheyana*) to medium dense (*O. hebebulba, O. bilabiata*). Setae length ranges between 0.99 mm (*O. iranshahri*) to 1.36 mm (*O. albo-rosea*). Pilies predominate in all members of the mentioned type. Setae color is white (*O. hebebulba, O. bilabiata*) to grey (*O. albo-rosea, O. dasytricha*) in that type. Setae ornamentation diameter ranges between 6.94 μm (*O. iranshahri*) and 19.38 μm (*O. albo-rosea*). Nectar hairs are seen only in *O. iranshahri*. Pili density is low (*O. rasychaena, O. dasytricha*) to medium (*O. hebebulba, O. bilabiata*). The dominant species have patent status and only *O. dasytrichum* had adpressed status of the setae.

The three-trichome type found in the examined species is described in detail below and summarized in Table 2. We have provided a diagnostic key derived from the indumentum data study and from the most valuable characters in the basic references (Boissier 1879; Popov 1953; Riedl 1967; Davis 1978) (e.g. Tab. 3-4).

Plate 1. (Figs 1-12) SEM of studied species.

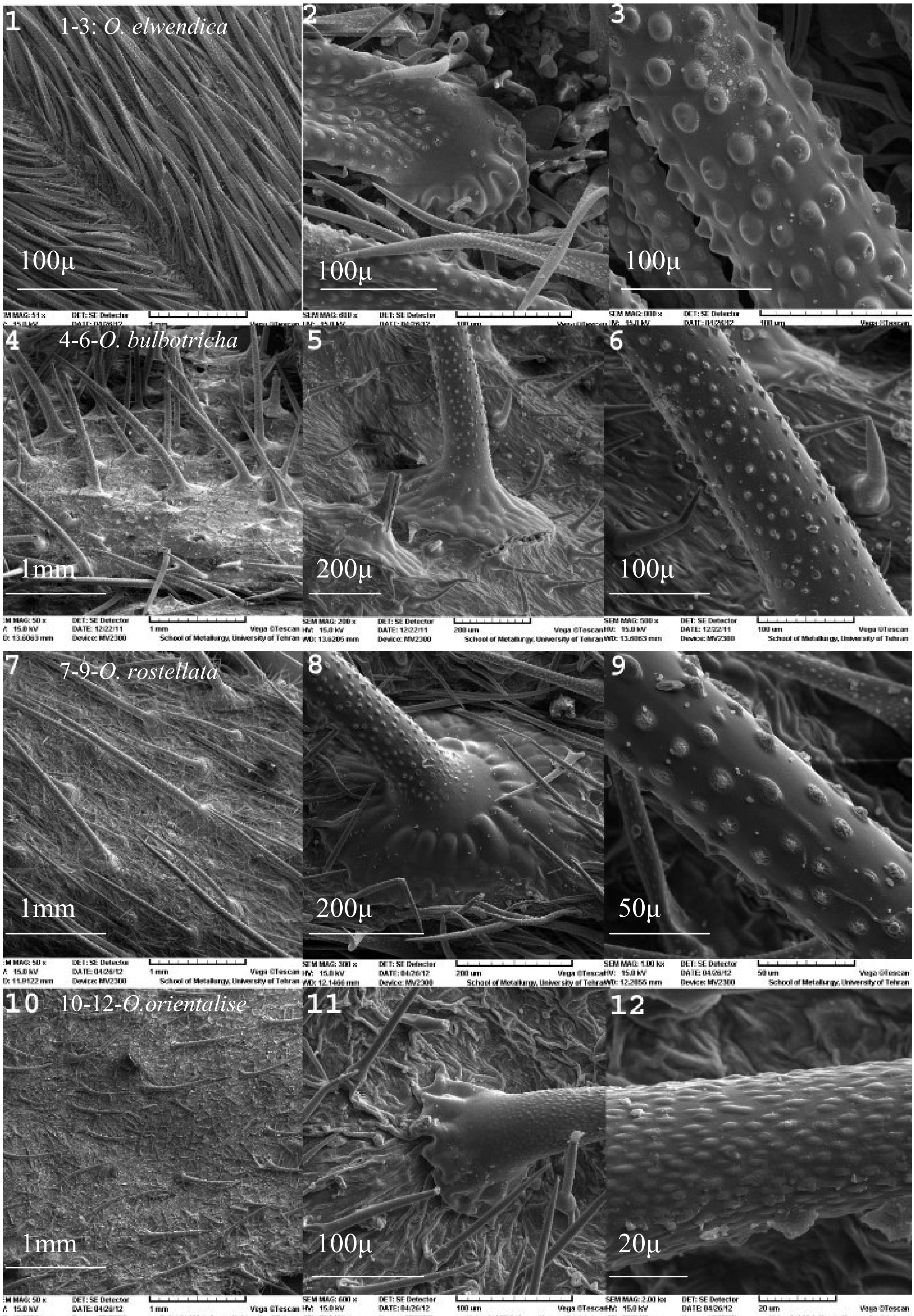

Plate 2. (Figs 13-24) SEM of studied species.

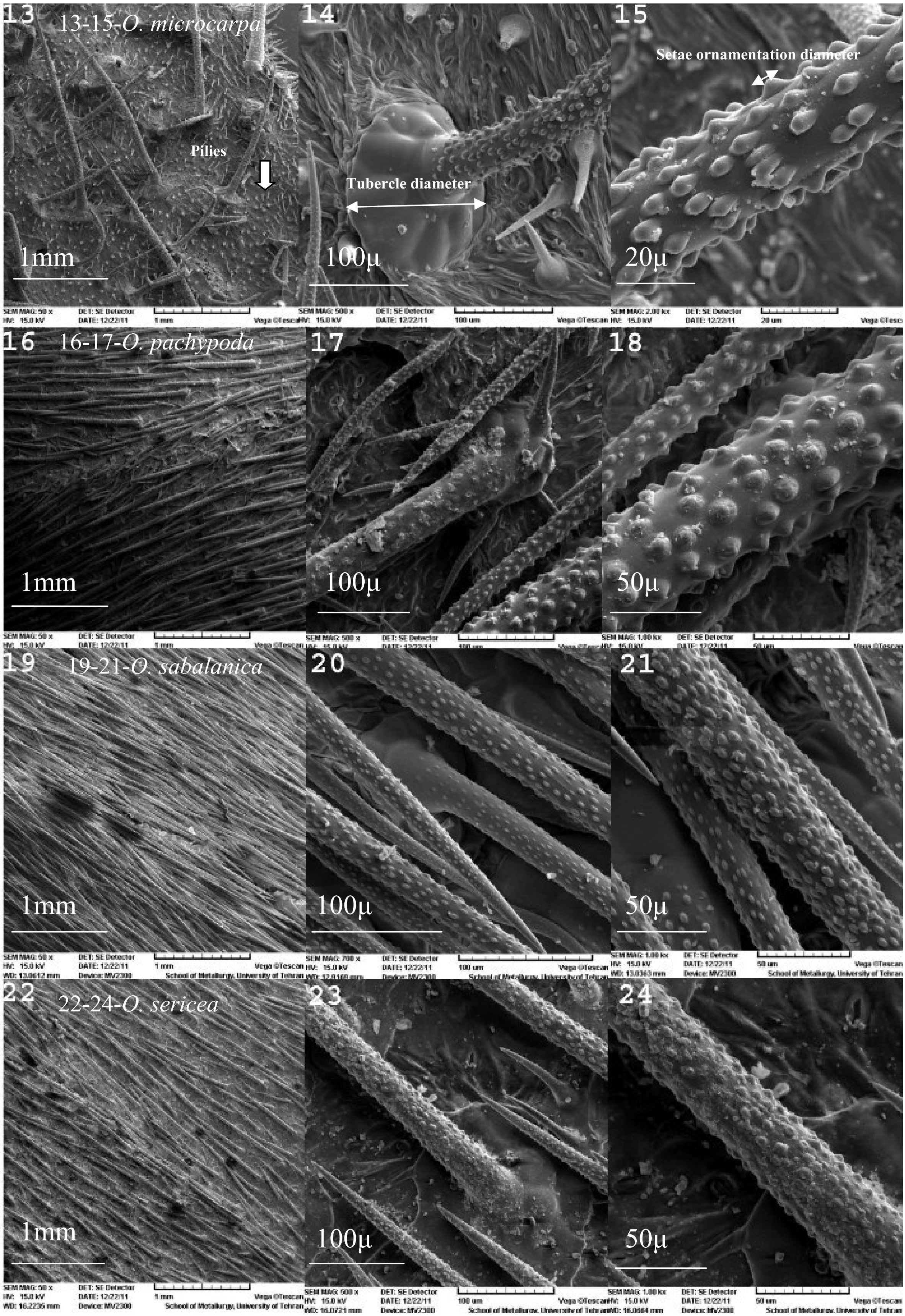

Plate 3. (Figs 25-36) SEM of studied species.

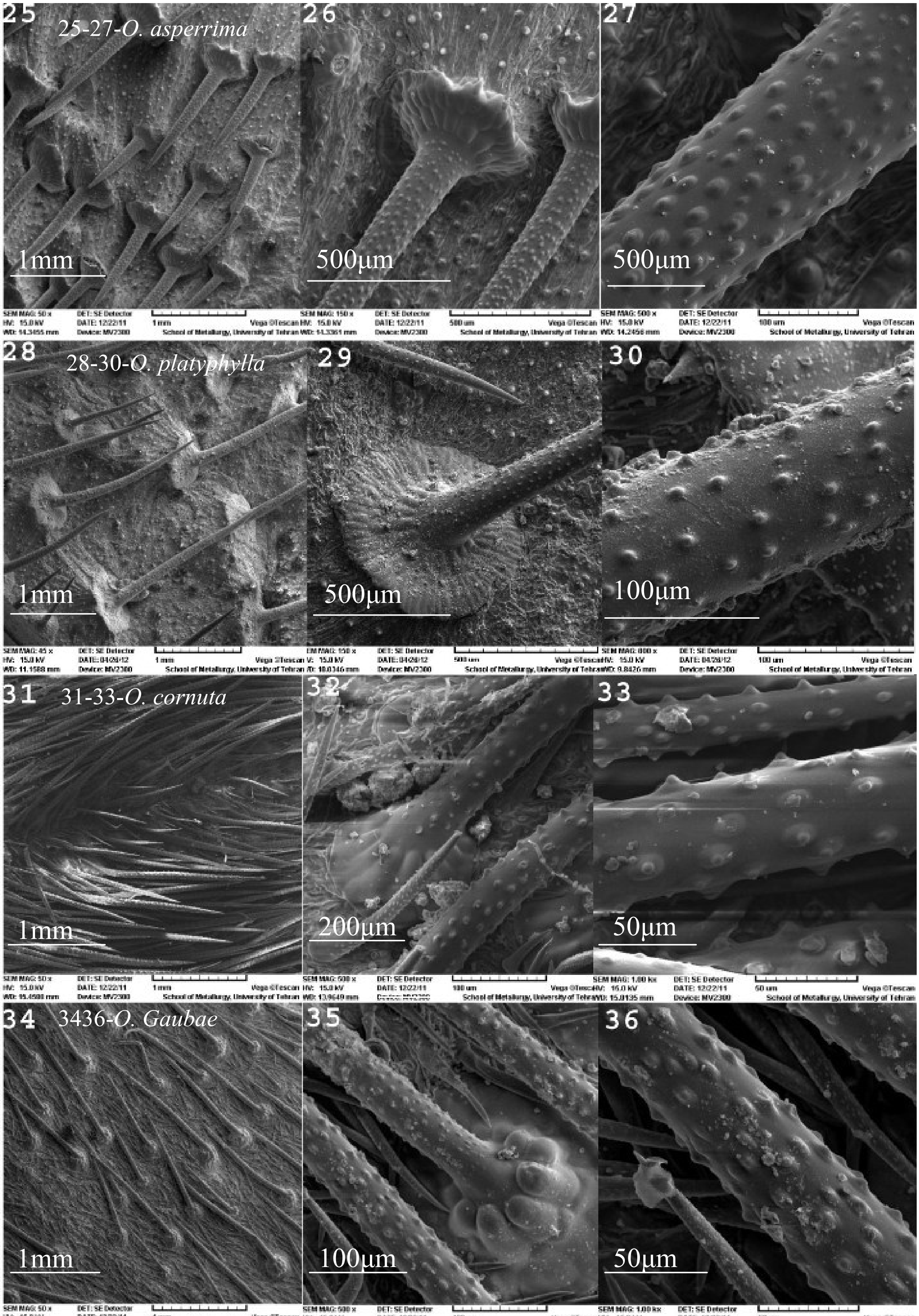

Plate 4. (Figs 37-48) SEM of studied species.

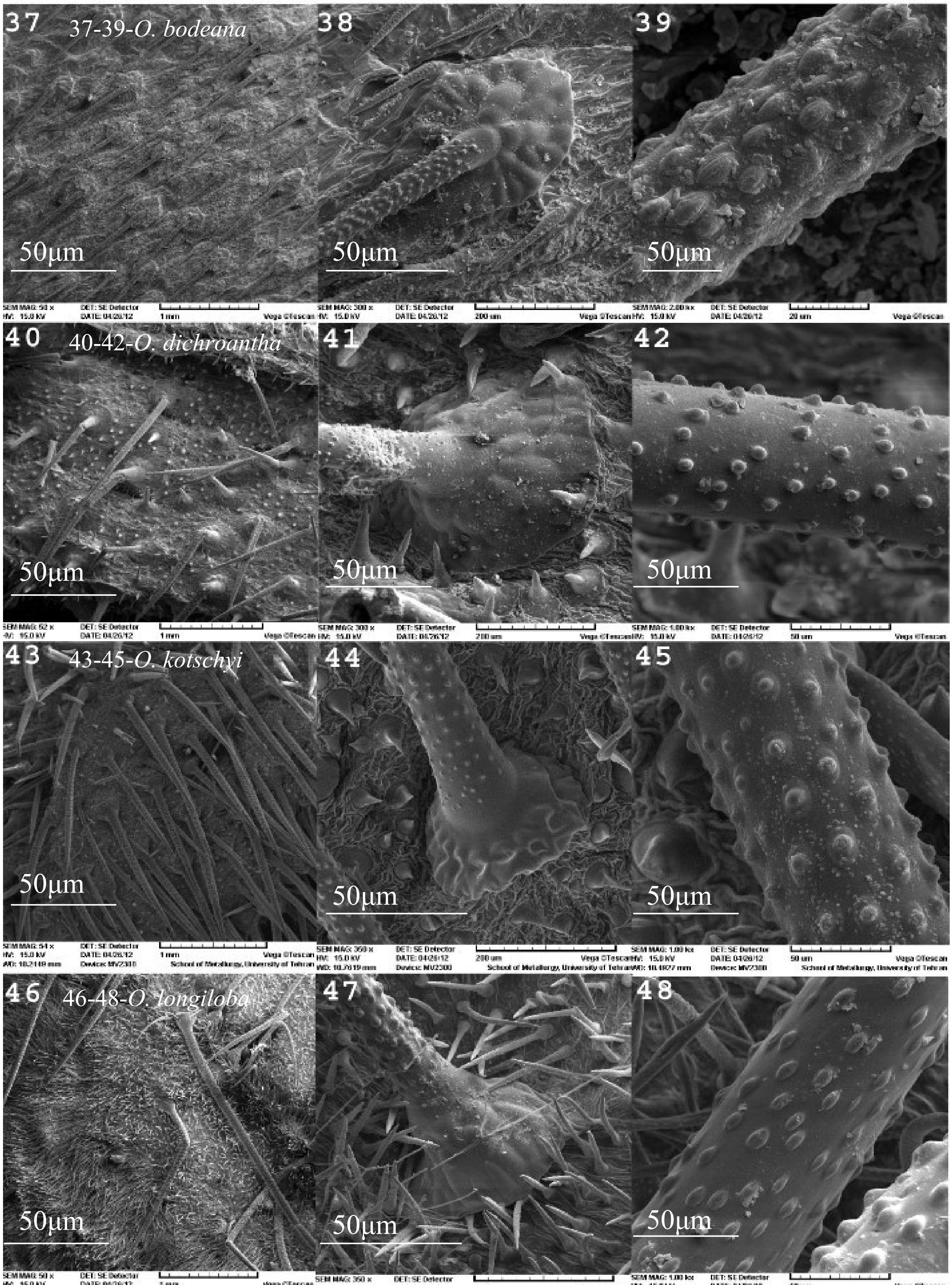

Plate 5. (Figs 49-60) SEM of studied species.

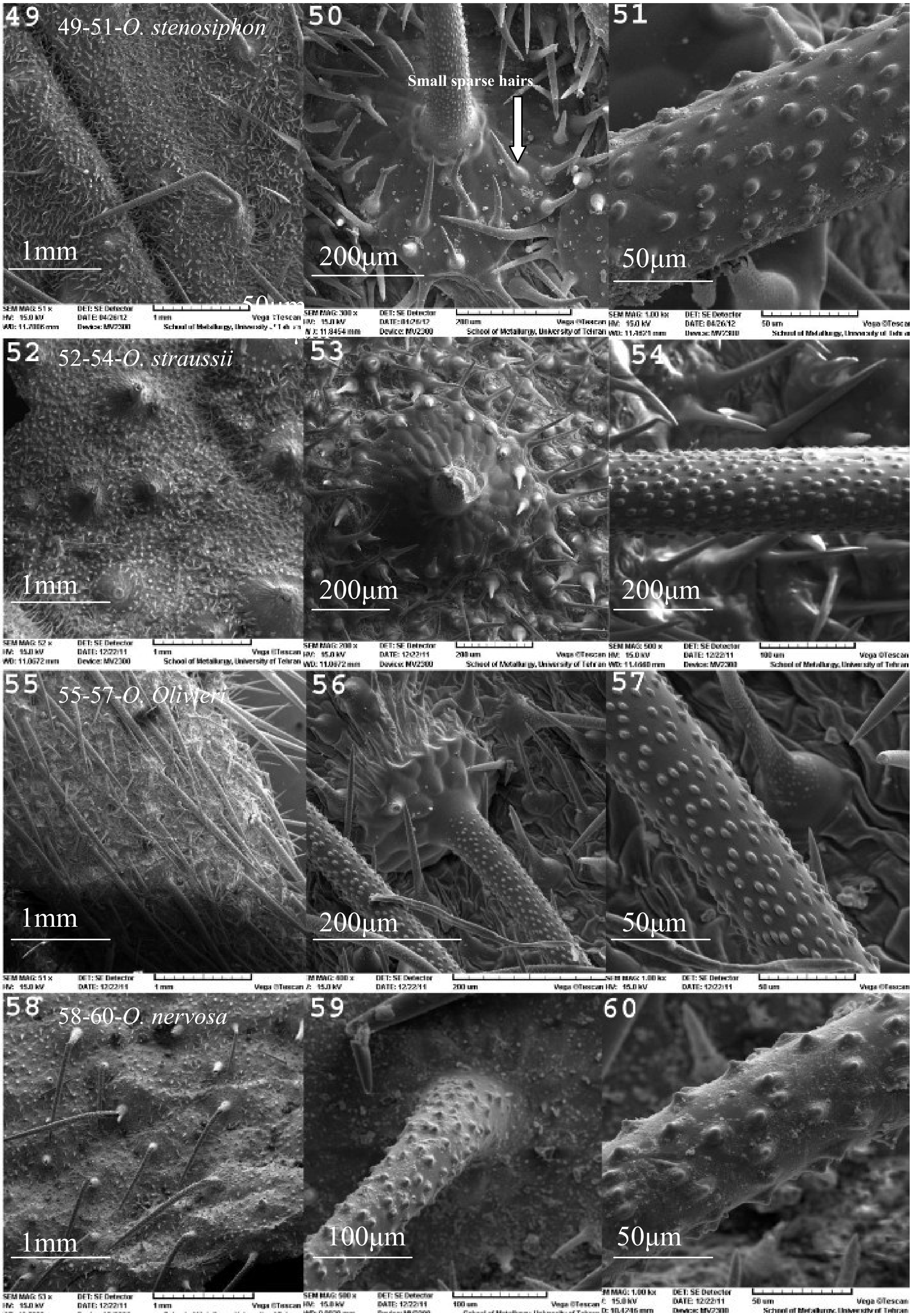

Plate 6. (Figs 61-72) SEM of studied species.

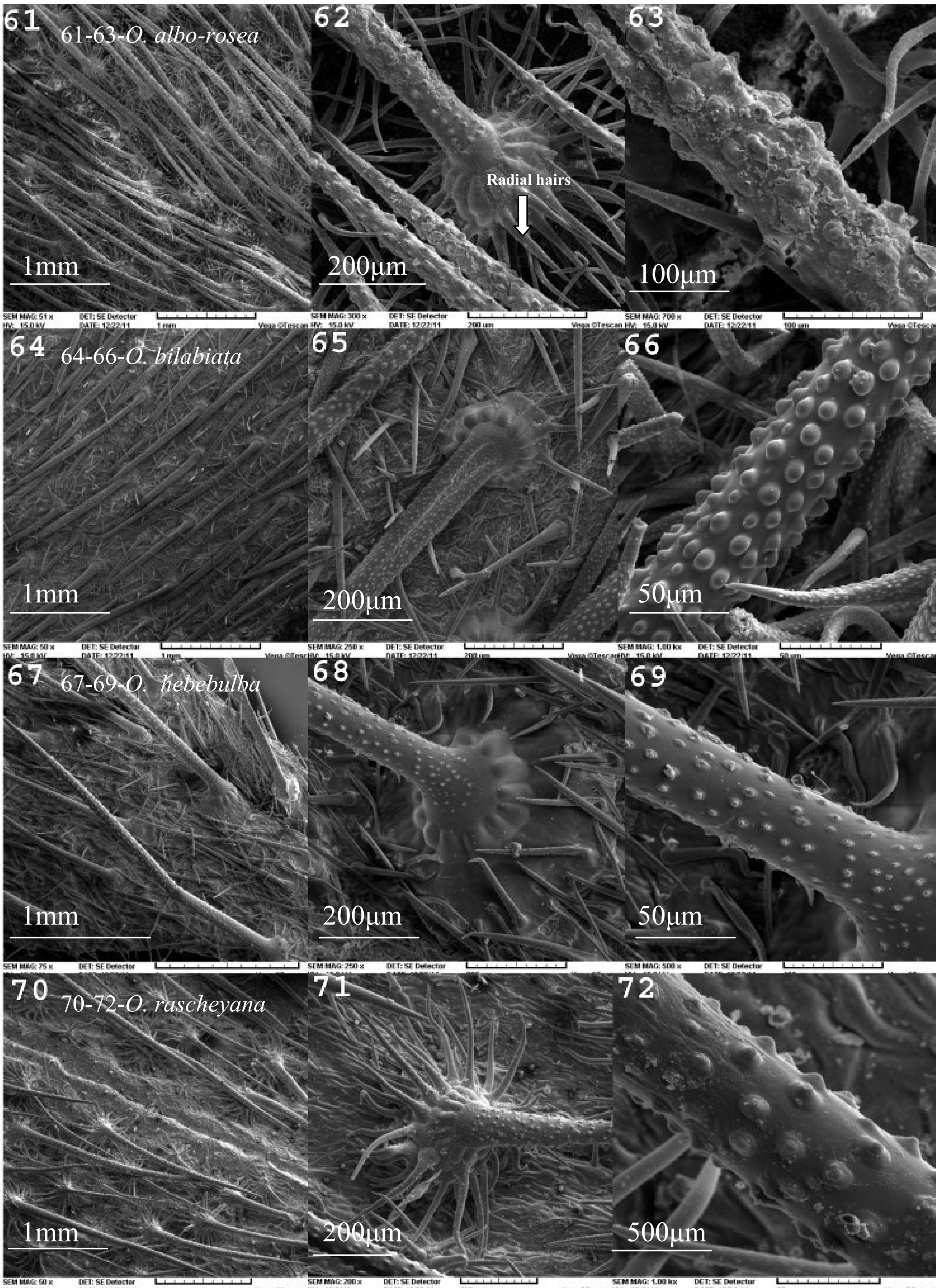

Plate 7. (Figs 73-83) SEM of studied species.

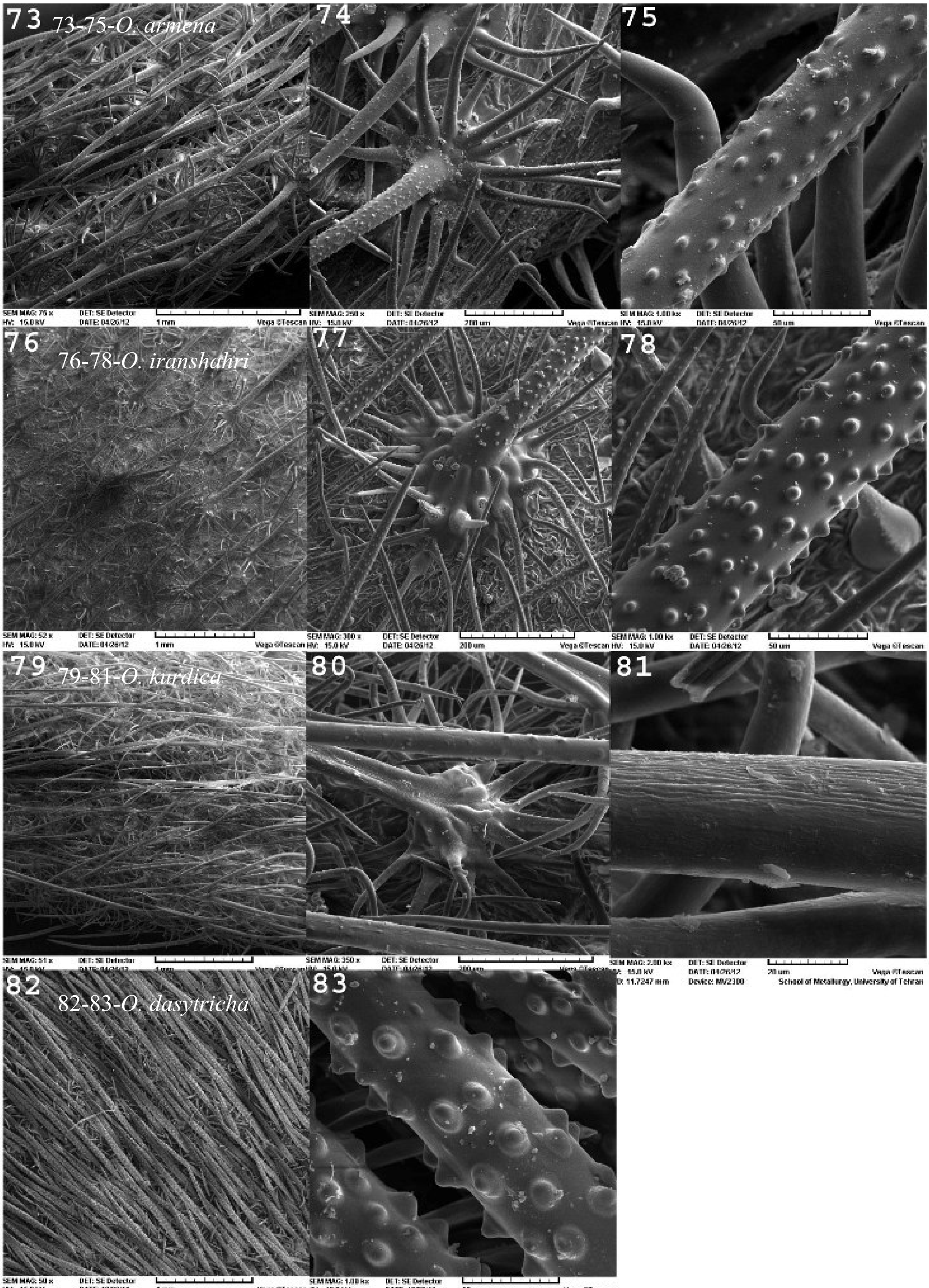

Table 3. Key to studied sections according to trichome features.

1	Large setae with simple base (absence of short trichomes at base)	2
–	Large setae with sparse and asteroidal pilies at base	4
2	Setae hooked	*sect. Podonosma*
–	Setae unhooked	3
3	Anthers connected along the side	*sect. Protonosma*
–	Anthers connected at base or free	sect. *Onosma* Subsect. *Haplotricha*
4	Large setae with small sparse pilies	sect. *Onosma* Subsect. *Heterotricha*
–	Large setae with asteroidal pilies	sect. *Onosma* Subsect. *Asteroricha*

Table 4. Key to studied species according to the trichome features.

1	Setae glabrous at base (absence of short pilies at base)	2
–	Large setae with sparse or asteroidal pilies at base	22
2	Setae hooked and loose (*Sect. Podonosma*)	*O. orientale*
–	Setae unhooked	3
3	Anthers connected along the side	*O. rostellata*
–	Anthers connected at base or free	(subsect. *Haplotricha*)4
4	Setae adppressed and grey	12
–	Setae patent and white or whitish–yellow	5
5	Nectar ring with small pilies	6
–	Glabrous nectar ring	7
6	Dense pilies, tubercles small. Corolla violet; corolla lobe up to 5 mm	*O. longiloba*
–	Scattered pilies, tubercles large. Corolla pale–yellow; corolla lobe up to 2 mm	*O. bulbotricha*
7	Setae whitish–yellow	8
–	Setae white	9
8	Setae dense. Leaves lanceolate–linear. Calyx 15–17 mm long	*O. sabalanica*
–	Setae sparse. Leaves spathulate. Calyx 8–12 mm long	*O. microcarpa*
9	Small hairs on leaf surface present	10
–	Small hairs on leaf surface absent	11
10	Corolla campanulate–tubular, 25–40 mm.	*O. dichroantha*
–	Corolla tubular, 8–11 mm.	*O. kotscyi*
11	Tubercles large. Anthers exserted	*O. platyphylla*
–	Tubercles small. Anthers inserted	*O. asperrima*
12	Pilies absent. Corolla up to 30 mm long	*O. bodeana*
–	Pilies present	13
13	Setae patent. Nectar ring with pilies, levaes obovate–lanceolate	*O. cornuta*
–	Setae adpressed	14
14	Very dense pilies. Calyx linear. Leaves linear	*O. subsericea*
–	Pilies of medium density. Calyx wide. Leaves lanceolate	*O. sericea*
15	Tubercles with sparse pilies	16
–	Tubercles with asteroidal pilies	22
16	Nectar ring with pilies. Setae yellow	*O. olivieri*
–	Nectar ring glabrous	17
17	Setae yellow. Inflorescence paniculate	*O. nervosa*
–	Setae white	18
18	Setae of medium density. Corolla yellowish–blue	*O. elwendica*
–	Setae dense. Corolla yellow to red	19
19	Dense tubercle pilies. Corolla purple to pink. Inflorescens with apical cyme	*O. straussii*
–	Tubercle pilies of low density	20
20	Radial hairs of low density. Leaves spathulate. Corolla clavate– campanulate	*O. paboti*
–	Tubercle pilies of medium density	21
21	Annulus pilose. Inflorescence capitate. Corolla violet, 7–9 mm	*O. stenosiphon*
–	Annulus glabrous. Corolla yellow. Inflorescence laxe paniculate. Corolla yellow, 17–19 mm	*O. macrophylla*

Table 4. Continuation.

22	Setae grey	23
–	Setae white	24
23	Setae adpressed. Leaves obovate–lanceolate	*O. dasytricha*
–	Setae sparse. Leaves lanceloate–oblong	*O. albo– rosea*
24	Setae dense	25
–	Setae of medium density	26
25	Pilies absent. Corolla 16–20 mm. Leaves spathulate	*O. armena*
–	Pilies of medium density. Corolla 20–25 mm long. Leaves linear to lanceolate	*O. kurdica*
26	Pilies of low density. Corolla yellow to red. Leaves spathulate	*O. rascheyana*
–	Dense to medium dense tubercle pilies	27
27	Dense tubercle pilies. Annulus pilose. Corolla 25–30 mm long	*O. iranshahri*
–	Tubercle pilies of medium density	28
28	Corolla 20 mm. Calyx elongated into fruit, up to 27 mm long	*O. bilabiata*
–	Corolla up to 15 mm. Calyx not elongated.	*O. hebebulba*

Discussion

Our study shows the morphology and distribution of trichomes, which are of taxonomical significance at species and sectional level. We categorized these characters on the basis of discrimination values. The first group has diagnostic value between the sections and subsections. Setae types and tubercle pili properties fall into this group. The second type of seate may serve as differentiation value for a broad range of species. Setae status and pili density fall into this group. The third group includes some minor or unique characters which can be used for diagnosis of smaller taxonomic groups, or of some species. Setae color, setae density and nectar hair fall into this group. The last group shows some overlaps among the studied species, according to the setae length, tubercle diameter and setae ornamentation diameter.

Presence or absence of nectar pili was found useful in distinguishing between some species, as for instance, *O. cornuta* from *O. sericea* and *O. Olivieri* from *O. elwendica.* Setae can be used as a subsidiary character for delimitation of some species. For instance, on the basis of the trichome color *O. olivieri* differs from its closest species (*O. elwendica*), as well as in setae density. For example, *O. kurdica* and *O. rascheyana* (as close species), could be distinguished from each other on the basis of trichome density, because *O. kurdica* has dense trichomes, while the other species has medium dense trichomes.

Sect. *Podonosma* has short setae (setae length ≤0.64mm), while they are longer in other sections (setae length ≥ 0.92mm). Furthermore, some restricted and close species, such as *O. kurdica* (1.10 mm) and *O. rascheyana* (1.51 mm), *O. kotschyi* (0.97 mm) and *O. platyphylla* (1.26 mm) could be differentiated on the basis of setae length.

On the other hand, tubercle diameter and setae ornamentation diameter are hardly reliable for differentiation, because they have large overlaps in the studied species, with the exception of *O. bodeana* (sect. *Haplotricha*) and *O. kurdica* (sect. *Asterotricha*) which have no setae ornamentations. Pilies are distributed on the surface of all *Onosma* species and range in density from strong to low, with the exception of *O. asperrima*, *O. platyphylla* and *O. bodeana* belonging to subsect. *Haplotricha,* and *O. armena* from *subsect. Asterotricha.* As a weak delimitation character, the above-mentioned character can be used along with other characters for diagnosis of *O. sericea* from *O. subsericea*.

Setae curvation (hooks) is regarded as a unique character in *Onosma orientale* (sect. *Podonosma*) and can be used as a valuable character at section level. The tubercle pilies density on the margins or on the tubercles is valuable in delimiting some close taxa, which face certain complexities in determination: *O. iranshahri* (dense) and *O. bilabiata* (medium – dense), *O. rascheyana* (medium – dense setae) and *O. kurdica* (dense setae).

Setae orientation is from adprresed to patent and can be used for species differentation in some similar species. For example, in *O. dasytricha* (adprressed trichomes) and *O. albo-rosea* (patent trichomes), which have similar morphology, setae orientation can help delimit them from each other.

On the basis of ISSR molecular markers, Mehrabian et al (2011) have shown high taxonomic isolation between *O. rostellata* and the other *Onosma* taxa. Analysis of the pollen characters in some Iranian *Onosma* (Mehrabian & al. 2012) has shown high diffrentiation between *O. rostellata* and the other *Onosma* species. However, trichome studies (recent study) did not show obvious diffrentiation between them.

Davis (1978) pointed out the affinity of some species to transgress the boundary between sect. *Onosma* subsect. *Haplotricha* and *Asterotricha,* defined solely by the indumentum type, and did not accept the presence of an intermediate subsection (*Heterotricha*). Riedl (1967) did not accept the existence of subsect. *Heterotricha* and divided sect. *Onosma* in two: subsect. *Haplotricha* and subsect. *Asterotricha*. The analysis of representatives of the *Heterotricha* group, however, showed a bimodal chromosome (Teppner 1971) that probably originated from hybridization between the taxa of the *Haplotricha* and *Asterotricha* groups (Teppner 1971, 1972; Vouillamoz 2001, Mártonfi & al. 2008; Kolarčik & al. 2010). That division into three infrageneric groups seems to be supported also by other karyological data (Kolarčik & al. 2010).

On the basis of ISSR molecular markers and morphological evidences in *Onosma* species, Mehrabian & al. (2011) have shown some relatively distinct boundaries between *Haplotricha, Heterotricha* and *Asterotricha*. Kolarcik & al. (2010) confirmed differentiation of the above-mentioned subsections. Furthermore, on the basis of pollen morphology Binzet & al. (2010) have supported the presence of these subsections. Khatamasaz (2001) also confirmed this classification. Moreover, the mentioned sections have been currently treated as informal groups (Peruzzi & Passalacqua 2008). In our study, judging mainly by the indumentum type, three groups have been proved within the genus that coincide with the infrageneric taxa of *Onosma* that Boissier (1879) had originally described as sections.

On the basis of our results, we propose the use of trichome type for delimitation of sections and subsections. This study indicates that it is possible to distinguish three subsections within section *Onosma,* namely *Haplotricha, Heterotricha* and *Asterotricha.* It was found that there are smaller and sparse pilies on the tubercles of subsect. *Heterotricha,* there are longer pilies on the margins in subsect. *Asterotricha*, and absence of such pilies in subsect. *Haplotricha.*

Thus our research, as the first detailed study based on the SEM method, has exemplified all trichome evidences which is very important in the taxonomy of *Onosma*. Besides this, we have provided a diagnostic key, according to which the trichomes accompanied by other morphological evidences show distinct boundaries between sections, subsections and species in *Onosma*. Although there are taxa which cannot be determined solely by trichome morphology, this evidences can be a very useful tool in distinguishing between many of the currently recognized *Onosma* taxa. Moreover, our study covers a high percentage of the mentioned taxa and can be used for taxonomic differentiation and taxa determination by means of interpretation of the importance and effectiveness of indumentum evidences and possibility coincidence with other taxonomic characters.

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