

TRICHOME MICRO-MORPHOLOGY IN MARRUBIUM L. (LAMIACEAE) IN IRAN AND THE ROLE OF ENVIRONMENTAL FACTORS ON THEIR VARIATION

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The genus *Marrubium* L. belongs to Lamiaceae family and Lamioideae subfamily. It is present in Iran with 11 species from which only one is endemic (*M. procerum* Bunge). Most *Marrubium* species are distributed in steppes, arid and semiarid areas of Iran. In this research the trichomes of leaf and calyx surface were examined using Scanning Electron Microscope. Trichome micro-morphological characters in studied species showed that the main hair type is stellate with unequal rays, one of the rays 3-15 times longer than the others. The general trichome shapes are similar but there are variations in stellate trichomes based on their long branches length, the short branches length and the number of short branches. The effect of environmental condition i.e. arid areas and altitude are important factors for morphological variations and density of trichomes.

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Key Words: Trichome; micromorphology; environmental factors; *Marrubium*; Lamiaceae; Iran

مطالعات ریز ریخت شناسی کرک در جنس *Marrubium* L. تیره *Lamiaceae* در ایران و نقش عوامل محیطی بر گوناگونی آنها

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جنس *Marrubium* L. متعلق به تیره نعنائیان و زیر تیره Lamioideae است. این جنس در ایران با ۱۱ گونه حضور دارد که در بین آنها تنها گونه *Marrubium procerum* Bunge انحصاری ایران است. گونه‌های این جنس اکثراً در مناطق خشک ایران می‌رویند. در این تحقیق کرک‌های سطح برگ و کاسه گل با استفاده از میکروسکوپ الکترونی مورد بررسی قرار گرفتند. نوع غالب کرک ستاره‌ای است که عموماً با یک شاخه مرکزی بلندتر از سایر شاخه‌ها متمایز است. تعداد شاخه‌های کوتاه، طرز قرار گرفتن آنها و طول شاخه‌های بلند و کوتاه در گونه‌های مختلف متفاوت است. نتایج نشان می‌دهد تراکم کرک، طول شاخه بلند و تعداد شاخه‌های کوتاه در هر کرک با شرایط محیطی رویشگاه گونه‌ها مرتبط است.

INTRODUCTION

Marrubium L. belongs to Lamiaceae subfamily Lamioideae. In the Plant List data base (<http://www.theplantlist.org>) there are 120 species names for *Marrubium*, of which 49 are accepted species. The genus is distributed in temperate regions of Europe, north Africa and Asia (Boudjelal & al. 2012). The genus has 11 species in Iran of which only one is endemic (*M. procerum* Bunge), (Seybold, S. 1978; Rechinger, K.H. 1982; Jamzad, Z. 2012). Most species are distributed in the steppes, arid and semi-arid

areas of Iran. The genus is characterized with having stamens which are included in the corolla tube, calyx throat bearded and corolla tube with a ring of hair inside. The genus is also characterized by stellate trichomes which cover all plant parts.

Trichomes are one of the most important and useful characters for identification of the genera and species in Lamiaceae (Akgül & al. 2008; Azizian & Moore 1982; Jamzad 1999; Marin & al. 2008; Osman 2012; Serpooshan & al. 2014; Seyedi & Salmaki 2015). They show a range of variation within the family. Simple

glandular and non-glandular as well as peltate are the most common types of trichomes, but stellate and branched hairs are also characteristic of some genera. Stellate and branched hairs are rather common in subfamily Lamioideae and occur in many genera i. e. *Marrubium*, *Ballota*, *Stachys*, *Phlomis*, *Eremostachys*, *Phlomidioschema* and *Lagochilus*. These types of trichomes have also been observed in other subfamilies as Nepetoideae (i.e. some species groups in *Nepeta*; *Lavandula*; *Zhumeria*) & Pogostemonoideae (Bhatti, & Ingrouille 1995; Jamzad 1999; Upson & Andrew 2004)

The presence of stellate hair in Lamioideae, Nepetoideae & Pogostemonoideae shows that it is not a diagnostic character (trait) at subfamily level, not even at generic level but at sub-generic and species levels. In some genera there are both kinds of hairs; species with stellate and simple trichomes i. e. *Stachys* and *Nepeta*, (Lamioideae and Nepetoideae respectively) in which this character is diagnostic at subgeneric level. In Lamiaceae, some genera are characterized by having stellate trichomes in all their species, i.e. in *Marrubium* & *Lavandula*.

In this research, *Marrubium* was studied to examine the potential value of trichomes in the taxonomy of the genus. Meanwhile, referring to the wide distribution of some species within the genus, i.e. *M. astracanicum* and *M. vulgare*, morphological variation in trichomes and taxonomic complexities are observed, so the role of environmental factors in morphology of trichome were also evaluated.

We carried out a comprehensive study of the trichomes in almost all the native Iranian *Marrubium* species. Trichomes were examined using Scanning Electron Microscope (SEM). Here we report the result of this research which is part of a PhD thesis project undertaken by M. Ahvazi.

MATERIALS AND METHODS

Forty two accessions belonging to 10 species were examined. In most cases, more than 3 accessions of each species were examined except for *M. duabense* and *M. cordatum* for which we had only one accession. All specimens used in this study were from the TARI herbarium. The list of studied specimens and their voucher numbers are given in table 1. Macro-morphological characters were studied using an OLYMPUS stereo-microscope. For micro-morphological studies middle part of the calyx and leaves were fixed on stubs using double-sided adhesive tape. The samples were coated with gold, and observed with a VEGA \\\ TESCAN Scanning Electron Microscope (SEM). Characteristics of indumentum

including trichome types and sizes in calyx and leaf in each studied specimen are given in tables 2-4. The terminology used is based on Metcalfe and Chalk (1950), Roe (1971), Cantino (1990), Hardin (1976).

RESULTS

Two types of trichomes were observed in the studied taxa:

Type I. Simple trichomes which include glandular and non-glandular trichomes.

The results of the microscopic observations showed that most plant parts are covered with glandular and non-glandular trichomes. The glandular trichomes are of two main types, peltate and capitate or stipitate. Peltate trichomes consist of a very short stalk cell and a large head. Stipitate trichomes are with a basal cell, long stalk and a uni-cellular head. Capitate or stipitate trichomes are distributed both on the leaves and calyx in the studied species but with more density on the calyx, i.e. in *M. astracanicum*, *M. anisodon*, and *M. propinquum* (figs. 1 E, 1L, 1 U and 1 V respectively).

Type II. Stellate trichomes: the stellate trichomes are with unequal rays, one of the rays much longer than the others (mono-radial hairs). We observed three types of stellate trichomes regarding to their short branches, they are mono-seriate, bi-seriate and multi-seriate. The stellate trichomes have high variation in their short and long branches length. Long branches may be 1 to 5-cellular and short branches are usually 1, rarely 2-cellular. The number and the length of short branches may vary in different species (i.e. in *M. astracanicum*, fig. 1 A to K and *M. cuneatum*, fig 1 R). The characteristics of trichomes of calyx and leaf are given below.

Calyx

Calyces of forty two accessions belonging to ten species were studied. We observed glandular (peltate and stipitate) and non-glandular (stellate) trichomes in all species. Rate of density for all species is given in table 2. The number of short branches vary from 5 (*M. propinquum*) to 23 (*M. parviflorum*). The highest number of cells in long branches is 5 and belongs to *M. cordatum* (fig. 1 M, m) which has the longest branch (2899 μ m) and *M. parviflorum* (fig. 1 T, t) has the smallest size of long branches with two cells (321 μ m). Multi-seriate short branches were observed in *M. astracanicum* (fig. 1 F, f), *M. crassidens* (fig.1 P, p), *M. cuneatum* (fig.1 R, r) and *M. parviflorum* (fig. 1 T, t). Mono-seriate short branches were observed in *M. anisodon* (fig. 1 L, l) and *M. propinquum* (fig. 1V, v).

Table 1. Materials studied in this work. All collecting numbers belong to TARI Herbarium.

Species	Locality
<i>Marrubium anisodon</i> C.Koch.	Mazandaran: 50km of Chalus, 1300m, 17.7.1984, Assadi & Masoumi 51460.
<i>M. anisodon</i>	Mazandaran: Chalus, valley Vali-Aba village 2826.
<i>M. anisodon</i>	Baluchestan: E slope of Kuhe- Taftan from sangan 5325.
<i>M. anisodon</i>	Tehran: road from Nowbaran to Hamadan, between Dokhan and Tajarak 76208.
<i>M. astracanicum</i> Jacq.	Ardabil: Meshkinshahr, 2000m, 5.7.1995, Azimi 195.
<i>M. astracanicum</i>	Semnan: Shahrud, Kuh-e Ghatry, 2600m, 8.4.1973, Wendelbo & Foroughi 8968.
<i>M. astracanicum</i>	Tehran: near Polour, 2350m, 18.4.1942, Babakhanlou & Amin 12948.
<i>M. astracanicum</i>	Semnan: Shahrud, Kuh-e Abr, 2600m, 23.6.1974, Wendelbo & Foroughi 12950.
<i>M. astracanicum</i>	Tehran: to Abali, Cheshmeh-e Aala, 1350m, Dini & Arazm 12967.
<i>M. astracanicum</i>	Isfahan: N side of Kuh-e Dena, 3500m, 16.7.1983, Assadi & Abouhamzeh 46176.
<i>M. astracanicum</i>	Mazandaran: Between Damghan & Sari, 1600m, 17.5.1978, Wendelbo & Assadi 29562.
<i>M. astracanicum</i>	Ghazvin: Alamut, above the Evan village, 2200m, 10.7.1984, Assadi & Massoumi 51051.
<i>M. astracanicum</i>	Kermanshah: Islamabad to Ilam, 1800m, 22.6.1987, Assadi 60927.
<i>M. astracanicum</i>	Kurdistan: Marivan, Dizli, 2350m, 12.6.2000, Massomi & Nikchehreh 80198.
<i>M. astracanicum</i>	Lorestan: Nurabad to Nahavand, Islamabad, 2100m, 3.6.2005, Assadi & Mehregan 88994.
<i>M. cordatum</i> Nab.	Fars: North side of kuke-Dena, 6.8.1978, Assadi & Mozaffarian 31446.
<i>M. crassidens</i> Boiss.	Isfahan: Ardestan to Taleghan, 1700m, 15.5.1975, Wendelbo & Foroughi 11508.
<i>M. crassidens</i>	Isfahan: Shahreza Hoonjan, 2600m, 30.5.2000, Feyzi & Shams 12637.
<i>M. crassidens</i>	Kerman: Kuh-e Khabar, 2500m, 9.6.1973, Assadi & Miller 25213.
<i>M. crassidens</i>	Lorestan: Oshtorankuh, Tihun village, 2000m, 12.7.1981, Assadi & Mozaffarian 37120.
<i>M. cuneatum</i> Russell	Kermanshah, Islamabad, kerende gharb, 1600m, 8.7.2003, Shams 14000.
<i>M. cuneatum</i>	Hamadan: 25 km. S. Nahavand to Boroujerd road of Noorabad. Sarab. E. Gamasiab Rahdar Khaneh 289.
<i>M. cuneatum</i>	Kermanshah: 35 km. N. of Kermanshah to Kamyaran right side of the road, Parro mountains 306.
<i>M. cuneatum</i>	Hamadan: 5 km road of Malayer to Arak. Left side, Kuh-Sardeh heights 3294.
<i>M. cuneatum</i>	Tehran: N.W. of Tehran, Souleghan 12946(TARI)
<i>M. duabense</i> Murata	Khorasan: between Golestan forest & Bojnurd, 1800m, 17.7.2003 85520.
<i>M. parviflorum</i> Fisch. & C.A.Mey.	Semnan: 12km of Shahmirzad, 2400m, 26.7.1982, Assadi & Mozaffarian 40363.
<i>M. parviflorum</i>	Guilan: Deylaman towards Asiabar, around Bala-eishkouh village Moradi, 608
<i>M. parviflorum</i>	E. Azarbayjan: Marand, Kiamaki-Dagh after Miab 72795.
<i>M. parviflorum</i>	Tehran: Firuzkuh to Semnan, Torud village 80623.
<i>M. persicum</i> C.A.Mey.	Azarbayejan: 40km from Namin to Germe, 1900m, 21.6.1980, Mozaffarian 34623.
<i>M. persicum</i>	E. Azarbayejan: Meshkin-Shahr, Lahrud 7285.
<i>M. persicum</i>	Ardebil: Dashte Moghan, Bikdasht 9898.
<i>M. propinquum</i> Fisch. & C.A.Mey.	Azarbayejan; toward Aslanduz, 400, 29.5.1993, Ghahremani, 2452.
<i>M. propinquum</i>	Ardabil: Sadafarin (collector??) 9848.
<i>M. propinquum</i>	W. Azarbayejan: 5 km. from Ardabil to Astara 61638.
<i>M. vulgare</i> L.	Kohkilouyeh-va Boirahmad: 5km of Yasuj, 1750m, 18.5.1987, Bakhtiari & Iranzadeh 20.
<i>M. vulgare</i>	Yazd: Heart, 2200m, 18.3.1995, Baghestani & Jafarnejad 444.
<i>M. vulgare</i>	Khorasan: 15km of Ahmadabad, 1200m, 27.8.1977, Bhadresra 5129.
<i>M. vulgare</i>	Isfahan: Dran to Feridonshahr, 2500m, 3.6.2002, Nowrozi & Shams 13252.
<i>M. vulgare</i>	Sistan va Balochestan: Khash, at Taft mountain, 2000m, 26.4.1977, Assadi 22864.
<i>M. vulgare</i>	Fars: Takhte Jamshid, 12.5.2005, Jamzad 90359.

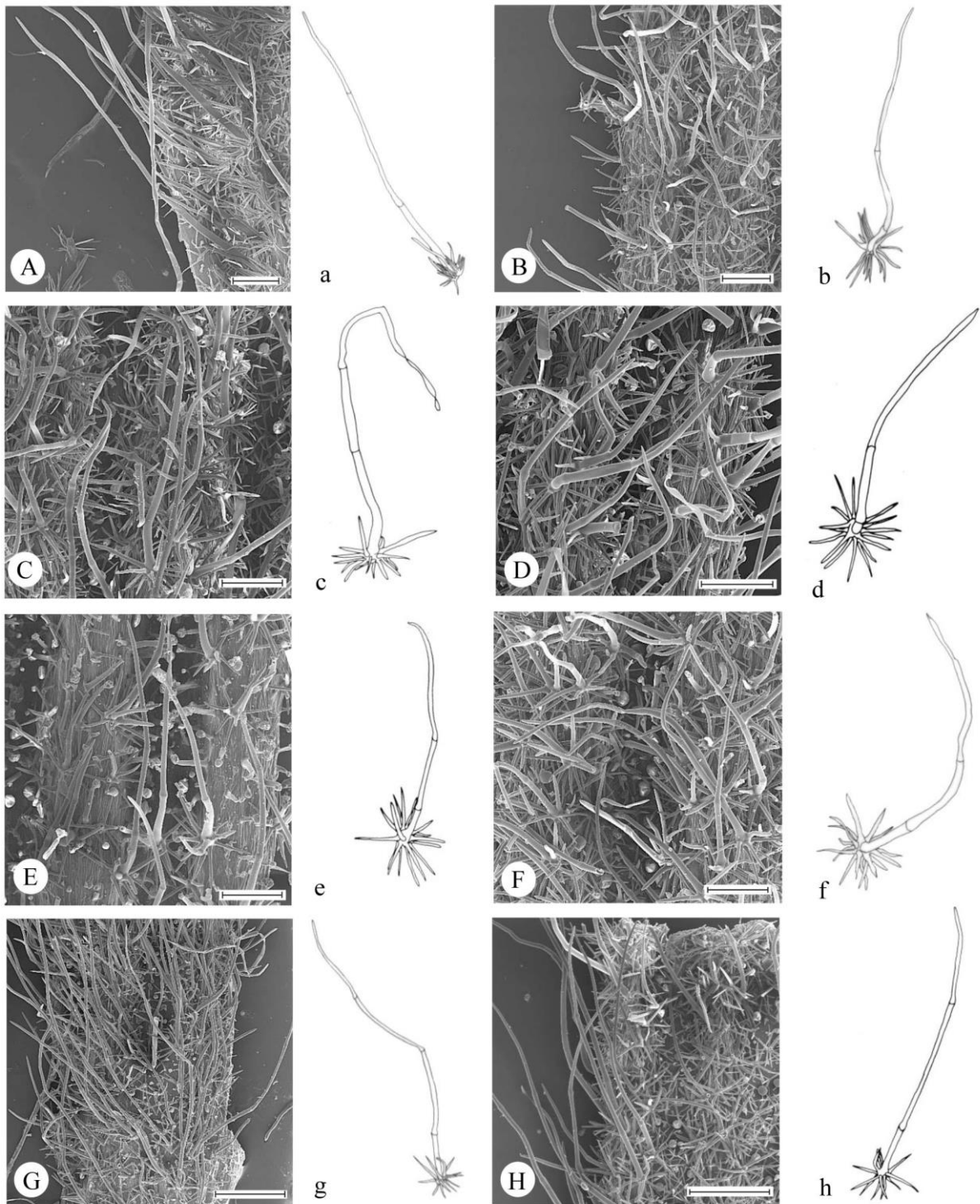


Fig. 1: Scanning electron micrographs of calyx indumentum in different species of *Marrubium*. A, a; B, b; C, c; D, d; E, e; F, f; G, g; H, h. different specimens of *M. astracanicum*. Scale bar = 200 μ m in A, B, C, D, E, F, H and 500 μ m in G.

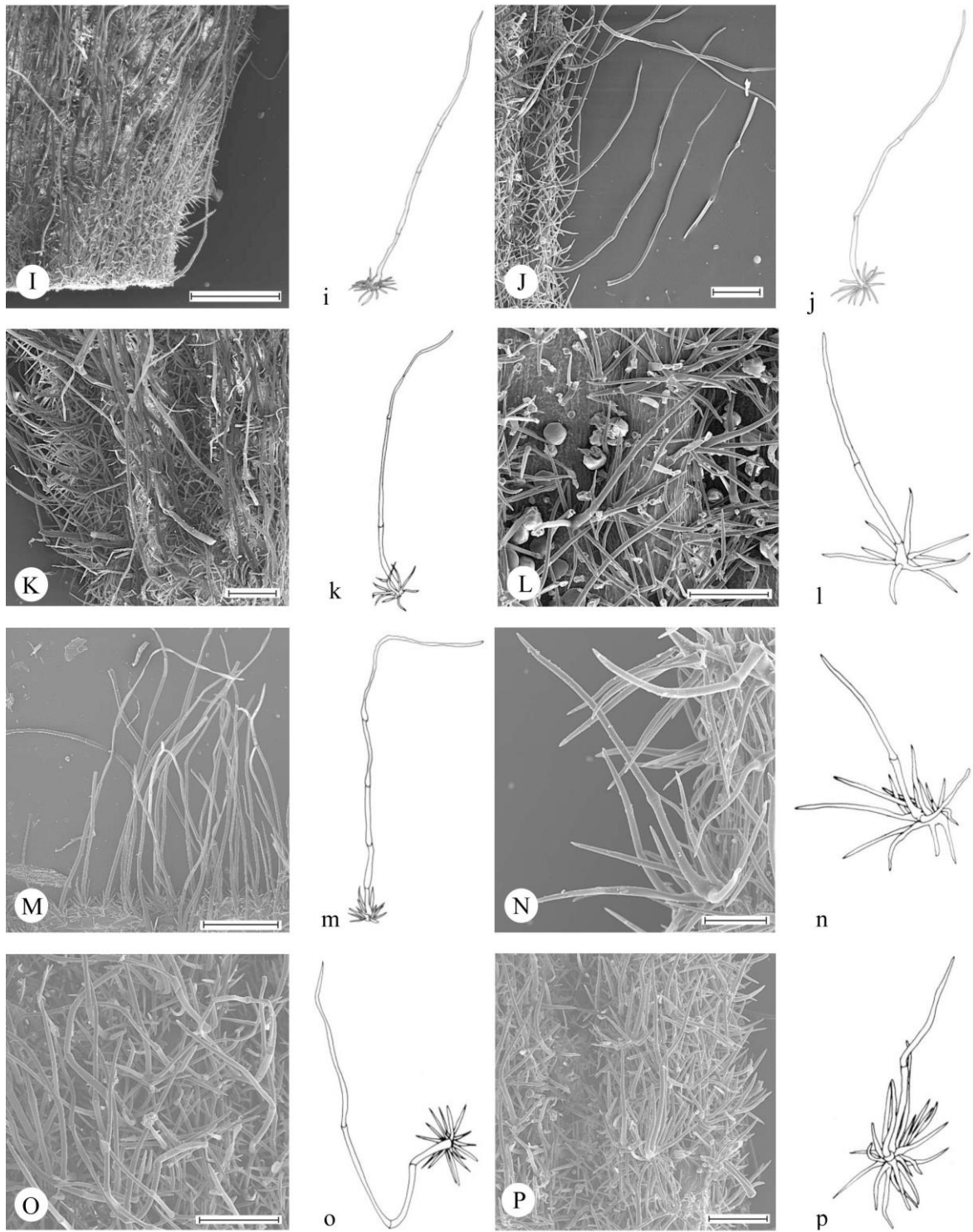


Fig.1. Continued. *M. astracanicum*: I, i; J, j, K, k; *M. anisodon*: L, l; *M. cordatum*: M, m; *M. crassidens*: N, n, O, o, P, p. I, M: scale bar = 500 μ m; J, K, L, N, O, P, scale bar = 200 μ m.

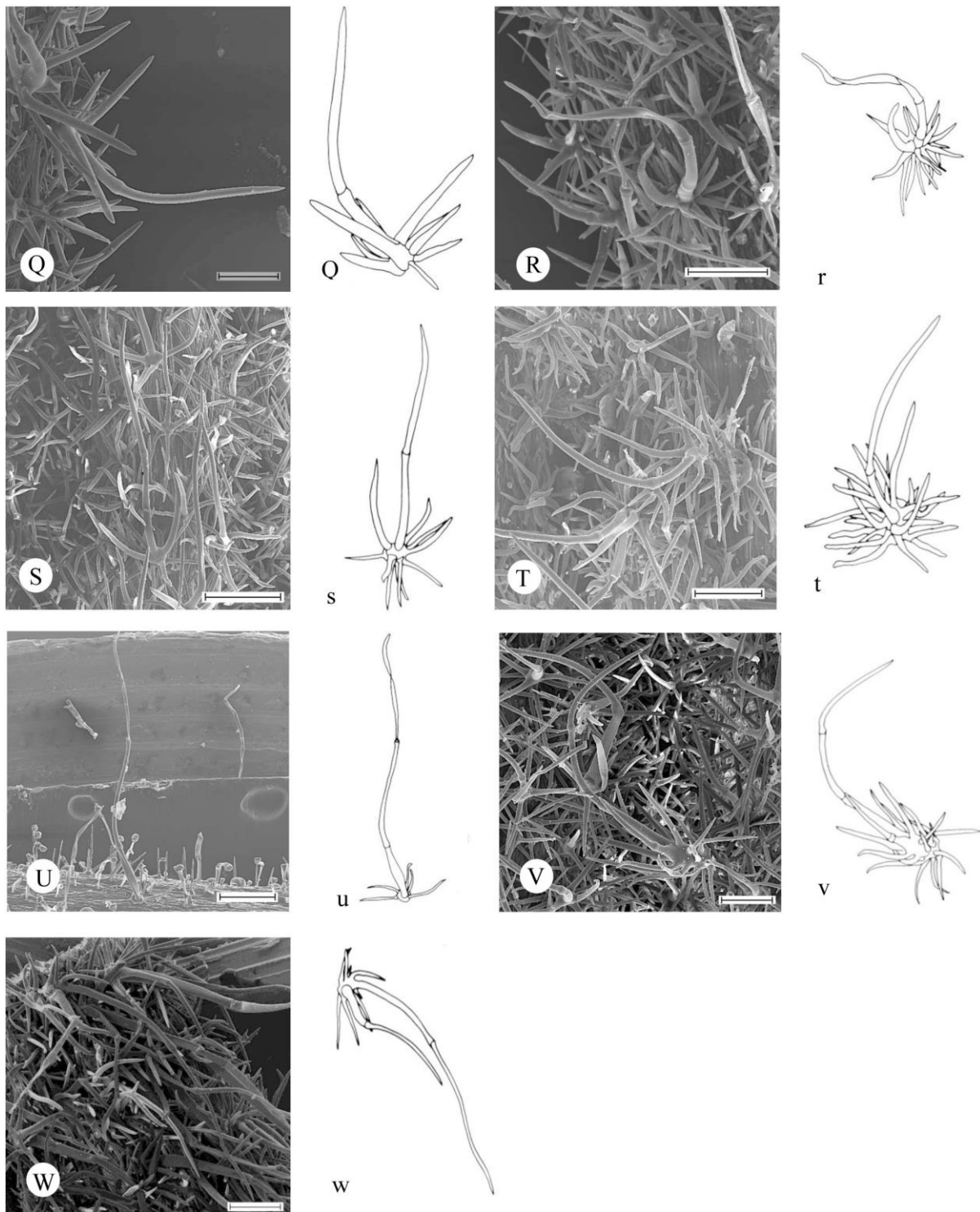


Fig. 1. Continued. *M. crassidens*: Q, q; *M. cuneatum*: R, r; *M. duabense*: S, s; *M. parviflorum*: T, t; *M. propinquum*: U, u; *M. vulgare*: V, v, W, w. In Q, R, T, W, scale bar = 100 μ m; in S, V, X: scale bar = 200 μ m.

Leaf

Upper surface

As with the calyx, leaves of forty two accessions were studied. Two types of glandular trichomes were observed in upper surface of the leaves, but there were fewer capitate or stipitate trichomes. Furthermore, there were mono-cellular simple hairs in some species such as *M. crassidens*, *M. duabense*, *M. persicum*, and *M. vulgare*. Peltate trichomes were observed in all studied species with little differences. From the three types of stellate trichomes present on the calyx, we could observe mainly mono-seriate and bi-seriate stellate trichomes on the leaves. Most of the long branches in stellate hairs are four-celled in the upper surface in populations of *M. astracanicum* (fig. 2 A, a to G, g and I, I to L, l), *M. cordatum* (fig. 2 M, m) and *M. vulgare* (fig. 2 U, u to W, w). *Marrubium astracanicum* has the longest size of the long branches (1818 μ m) and *M. propinquum* (fig. 2 T, t) has the shortest size of the long branches among the studied species (375 μ m). The number of short branches varies from 1 in *M. crassidens* (fig. 2. N, n) and *M. duabense* (fig. 2 R, r) to 23 in *M. astracanicum* (fig. 2. B, b). Images of trichomes of upper leaf surfaces of studied species are presented in fig. 2 A-X and a-w. The measurements and characteristics of trichomes of upper surface of leaves are summarized in table 3.

Lower surface

In the lower surface of leaves, we observed more of the bi-seriate and multi-seriate short branches stellate trichomes. Two types of glandular trichomes were also observed. There were more peltate trichomes than capitate or stipitate ones on the lower surface of the leaves (fig. 2). Most long branches are 5 cellular among population of *M. astracanicum* (fig. 2 C', c') and *M. crassidens* (fig. 2 O', o'). *Marrubium astracanicum* had the longest and *M. crassidens* the shortest size of long and short branches (2616 μ m & 311 μ m respectively). The number of short branches varies from 1 in *M. vulgare* (fig. 2 U', u') to 23 in *M. astracanicum* (fig. 2 D', d'). The measurements and characteristics of trichomes of lower surface of leaf are summarized in table 4.

In conclusion the trichome morphology is useful in circumscription of some species, but not significant at sectional level. In both *Marrubium* and *Microdonta* (Briquet) Seybold sections the trichome types mentioned above are observed.

DISCUSSION

Trichome micro-morphological characters in *Marrubium* species showed that the main hair type is stellate with unequal rays, with one of the rays 3-15 times longer than the others (mono-radial hairs). The short branches are mono-multi-seriate. Stellate and branched hairs are characteristics of some closely related genera within Lamioideae, i.e. *Phlomis* (Azizian &

Cutler 2008) and *Eremostachys* (Seyedi & Salmaki 2015). Stellate and branched hairs are also present in some other genera in Lamioideae i. e. *Stachys* (Salmaki & al. 2009) and Nepetoideae i.e. *Nepeta* (Jamzad 2001) and *Lavandula* (Upson 2004), but with a different shape, and density. The trichome type of *Marrubium* is characteristic of this genus.

The general trichome shapes in the species studied are similar but there are variations in stellate trichomes based on their long branches length, the short branches length and the number of short branches. The variation is partly at species level and can be characteristic of a species, for example in some species there are only mono-seriate stellate trichomes, i.e. in *M. propinquum* and *M. anisodon*. *Marrubium propinquum* is unique in its indumentums type on calyx, with dense stipitate glandular trichomes and lax stellate trichome. Trichome density may be different in different species but mainly these characters are not of taxonomic significance. In contrast, there are variations in one single species, i.e. in *M. astracanicum* which shows variations in number of short branches (from 8 to 18). It seems that there is a correlation between the length of short branches, the number of short branches and some environmental factors, for example, altitude (table 2). At higher altitudes, the number of short branches increases significantly: the number of short branches in stellate hairs is more in specimens from higher altitudes (3500-4000m) than at lower altitude (1350). In *M. crassidens*, the number of short branches increased from 3 to 20 with an altitude increase (from 1700m to 2500m), but at 2600m an exception was observed (15 short branches and long branch-cells with four cells). We can conclude that high altitude may affect the number of short branches and the number of cells in long branches (fig.1). In *M. vulgare* with an altitude increase the number of short branches also increased (from 7 to 15). The maximum size of long branches is 1908 μ m and minimum is 731 μ m (table.2). At higher altitudes we observed the minimum size of long branches was 480 μ m and in lowest altitude size of long branches was 778 μ m. It seems that with altitude increase the size of long branches decreases.

There were more multi-seriate stellate hairs in *M. astracanicum* and *M. crassidens* depending to altitude. The numbers of long branch-cells are 3 to 4 in some accession of *M. astracanicum* with multi-seriate stellate hairs.

Referring to our observations and the previous reports (Azizian & Moore 1982; Seyedi & Salmaki 2015), mono-radial stellate trichome with one very long branch is characteristic of the genus within Lamioideae. The effect of environmental condition i.e. arid climate and altitude are important factors for morphological variations and density of trichomes.

Table 2. Calyx surface indumentum in *Marrubium* species (abbreviation: A, Mono-seriate; B, Bi-seriate; C, multi-seriate; D, number of short branches; E, size of short branch; F, number of cells in short branches; G, size of long branches; H, number of cells in long branches; I, dense; II, very dense).

Species	Simple hair				Stellate hair			Trichome size & number (µm)				
	Peltate		Stipitate		A	B	C	Short branch			Long branch	
	I	II	I	II				D	E	F	G	H
<i>M. anisodon</i> (51460)TARI	+	-	-	+	+	+	-	9	175	1	579	3
<i>M. anisodon</i> (2826)TARI	-	+	+	-	-	+	-	8	802.27	1	2700	4
<i>M. anisodon</i> (53251)TARI	+	-	-	+	-	+	+	14	240	1	1323	4
<i>M. anisodon</i> (76208)TARI	-	+	-	+	-	-	+	13	255	1	1447	4
<i>M. astracanicum</i> (195)TARI	-	+	-	+	-	-	+	9-15	130,146	1	1210	4
<i>M. astracanicum</i> (8968)TARI	+	-	+	-	-	+	-	16	100,57	1	1407	4
<i>M. astracanicum</i> (12948)TARI	+	-	+	-	-	-	+	15	162	1	1039	3
<i>M. astracanicum</i> (12950)TARI	+	-	+	-	-	-	+	15	118	1	1280	3
<i>M. astracanicum</i> (12967)TARI	-	+	+	-	-	+	-	15	192	1	1339	4
<i>M. astracanicum</i> (46176)TARI	-	+	-	+	-	+	-	12-18	116	1	1584	3
<i>M. astracanicum</i> (29562)TARI	+	-	+	-	-	+	-	10	100	1	1295	3
<i>M. astracanicum</i> (51051)TARI	+	-	-	+	-	-	+	16	108	1	731	3
<i>M. astracanicum</i> (60927)TARI	-	+	-	+	-	-	+	8-14	132	1	845	3
<i>M. astracanicum</i> (80198)TARI	-	+	-	+	-	-	+	17	147	1	849	3
<i>M. astracanicum</i> (88994)TARI	+	-	-	+	-	+	-	12	161	1	1908	4
<i>M. cordatum</i> (31446)TARI	-	+	-	+	-	+	-	14	186	1	2899	4,5
<i>M. crassidens</i> (11508)TARI	+	-	+	-	-	+	+	7-3	290	1	501	2
<i>M. crassidens</i> (12637)TARI	+	-	-	+	-	+	+	15	99,112	1	435, 1035	3,4
<i>M. crassidens</i> (25213)TARI	+	-	+	-	-	-	+	20-18	253	1	660	2
<i>M. crassidens</i> (37120)TARI	+	-	+	-	-	+	-	12-7	176	1	397	2
<i>M. cuneatum</i> (14000)TARI	+	-	+	-	-	-	+	18-20	103	1	338	2
<i>M. cuneatum</i> (289)TARI	+	-	+	-	-	-	+	14	197	1	914	3
<i>M. cuneatum</i> (306)TARI	+	-	+	-	-	+	+	10	135	1	597	3

Table 2. Continued.

Species	Simple hair				Stellate hair			Trichome size & number (µm)				
	Peltate		Stipitate		A	B	C	Short branch			Long branch	
	I	II	I	II				D	E	F	G	H
<i>M. cuneatum</i> (3294)TARI	+	-	+	-	-	-	+	16	240	1	971	3
<i>M. cuneatum</i> (12946)TARI	+	-	+	-	-	+	+	11-14	220	1	1022	2,5
<i>M. duabense</i> (85520)TARI	+	-	+	-	-	+	+	10-8	158	1	626	2
<i>M. parviflorum</i> (40363)TARI	+	-	+	-	-	-	+	23	151	1	321	3
<i>M. parviflorum</i> (608)TARI	-	+	-	+	-	+	-	8-9	626,190	1	1743	3
<i>M. parviflorum</i> (72795)TARI	+	-	+	-	-	-	+	14	145	1	879	3
<i>M. parviflorum</i> (80623)TARI	-	+	-	+	-	-	+	11-12	155	1	732	3
<i>M. persicum</i> (34623)TARI	+	-	-	-	+	+	-	-	-	-	15 (mm)	2,3
<i>M. persicum</i> (7285)TARI	+	-	-	-	+	-	-	-	-	-	-	2,3
<i>M. persicum</i> (9898)TARI	+	-	-	+	+	-	-	-	-	-	-	2,3
<i>M. propinquum</i> (2452)TARI	-	+	-	+	+	+	-	5	137	1	990	3
<i>M. propinquum</i> (9848)TARI	-	+	-	+	+	+	-	4	124	1	329, 1573	2
<i>M. propinquum</i> (61638)TARI	-	+	-	+	+	+	-	7,3	250, 312	1	742, 1894	2
<i>M. vulgare</i> (20)TARI	+	-	-	-	-	-	+	15	233	1	1050	3
<i>M. vulgare</i> (444)TARI	+	-	-	-	-	+	+	15	197,145	1	320 ,565	3
<i>M. vulgare</i> (5129)TARI	-	+	-	+	-	+	-	7	199	1	435	2
<i>M. vulgare</i> (13252)TARI	+	-	-	-	-	-	+	15	201	2	1195	2
<i>M. vulgare</i> (22864)TARI	-	+	-	+	-	+	-	8	247	1	1593	2
<i>M. vulgare</i> (90359)TARI	+	-	+	-	-	+	+	9-10	199,164	1	544 ,1503	4,2

Table 3. Continued.

Species	Simple hair				Mono-cellular	Stellate hair			Trichome size & number (µm)				
	Peltate		Stipitate			A	B	C	Short branch			Long branch	
	I	II	I	II					D	E	F	G	H
<i>M. cuneatum</i> (12946)	+	-	+	-	+	+	-	-	3	223	1	638	2
<i>M. cuneatum</i> (14000)	+	-	-	-	-	-	+	-	7	124	1	541	2
<i>M. duabense</i> (85520)	+	-	+	-	+	+	+	-	4-5	300	1	1360	4
<i>M. parviflorum</i> (40363)	+	-	-	-	-	+	-	-	6	195	1	532	2
<i>M. parviflorum</i> (608)	+	-	+	-	-	+	+	-	7	293	1	742	4
<i>M. parviflorum</i> (72795)	+	-	+	-	-	-	+	+	11	152	1	513	2
<i>M. parviflorum</i> (80623)	+	-	+	-	+	-	-	-	-	-	-	970	3
<i>M. persicum</i> (34623)	+	-	-	-	+	-	-	-	-	-	-	15 (mm)	2,3
<i>M. persicum</i> (7285)	+	-	+	-	+	+	-	-	2	-	1,2	1299	2,3
<i>M. persicum</i> (9898)	-	+	+	-	+	-	-	-	-	-	-	406	3
<i>M. propinquum</i> (2452)	+	-	-	-	-	+	-	-	5	139	1	357	3
<i>M. propinquum</i> (9894)	+	-	+	-	-	+	+	-	6	327	1	811	3
<i>M. propinquum</i> (61638)	-	+	+	-	-	+	-	-	3	273	2	427	3
<i>M. vulgare</i> (20)	+	-	-	-	+	+	-	-	2	105	1	623	2
<i>M. vulgare</i> (444)	+	-	-	-	-	+	-	-	4,5,8	141	1	594	2
<i>M. vulgare</i> (5129)	+	-	+	-	-	+	-	-	6	244	1	1323	3,4
<i>M. vulgare</i> (13252)	-	+	+	-	+	-	-	-	-	-	-	977	2
<i>M. vulgare</i> (22864)	+	-	-	-	-	+	-	-	3,5,6	305	1	966	2
<i>M. vulgare</i> (90359)	+	-	+	-	-	+	+	-	2,3,4	298	1	1212	2

Table 4. Indumentum of the lower surface of leaves in *Marrubium* species (abbreviation: A, Mono-seriate; B, Bi-seriate; C, Multi-seriate; D, Number of short branches; E, size of short branch; F, number of cells in short branches; G, Size of long branches; H, number of cells in long branches; I, dense; II, very dense).

Species	Simple hair				Mono-cellular	Stellate hair			Trichome size & number (μm)				
	Peltate		Stipitate			A	B	C	Short branch			Long branch	
	I	II	I	II					D	E	F	G	H
<i>M. anisodon</i> (51460)	-	+	+	-	-	-	+	-	6	294	1	692	2
<i>M. anisodon</i> (2826)	-	+	+	-	-	+	+	-	5	267	1	757	3
<i>M. anisodon</i> (53251)	-	+	-	+	-	-	+	-	5	459	1	1243	4
<i>M. anisodon</i> (76208)	-	+	-	+	-	+	+	-	6,3	349	1	1100	3
<i>M. astracanicum</i> (195)	+	-	-	-	-	+	+	-	12	259	1	311	1
<i>M. astracanicum</i> (8968)	+	-	+	-	-	-	-	+	6	223	1	1110	3
<i>M. astracanicum</i> (12948)	+	-	-	-	-	-	-	+	13	75	1	723	3
<i>M. astracanicum</i> (12950)	+	-	+	-	-	-	-	+	15	118	1	1280	3
<i>M. astracanicum</i> (12967)	+	-	-	-	-	-	+	-	8,9	178	1	1664	2
<i>M. astracanicum</i> (46176)	+	-	-	-	-	-	-	+	11	141	2	1468	4
<i>M. astracanicum</i> (29562)	+	-	-	-	-	-	+	-	5-7	245	1	1125	4
<i>M. astracanicum</i> (51051)	+	-	+	-	-	-	-	+	18,23	269	1	1106	4
<i>M. astracanicum</i> (60927)	+	-	+	-	-	+	-	-	10	252	1	2616	5
<i>M. astracanicum</i> (80198)	+	-	+	-	-	-	-	+	8,10,14	199	1	1247	3
<i>M. astracanicum</i> (88994)	+	-	+	-	-	-	-	+	21	265	1	937	2
<i>M. cordatum</i> (31446)	-	-	+	-	-	-	+	-	6	130	1	991	3
<i>M. crassidens</i> (11508)	+	-	-	-	+	-	+	+	4,6	105	1	1634	4,5
<i>M. crassidens</i> (12637)	+	-	+	-	-	-	+	+	10	170	1	1431	4
<i>M. crassidens</i> (25213)	+	-	+	-	-	+	-	-	6	308	1	1431	4
<i>M. crassidens</i> (37120)	+	-	-	-	-	-	+	+	6	196	1	556	2
<i>M. cuneatum</i> (14000)	+	-	-	-	-	-	-	+	10	145	1	350	3
<i>M. cuneatum</i> (289)	+		+			+	+		6	320	1	905	4
<i>M. cuneatum</i> (306)	+	-	+	-	-	+	+	-	6	149	1	743	4

Table 4. Continued.

Species	Simple hair				Mono-cellular	Stellate hair			Trichome size & number (µm)				
	Peltate		Stipitate			A	B	C	Short branch			Long branch	
	I	II	I	II					D	E	F	G	H
<i>Marrubium cuneatum</i> (3294)	+	-	+	-	+	-	-	-	-	-	-	523	2
<i>M. cuneatum</i> (12946)	-	+	+	-	-	+	-	-	4	217	1	1293	3
<i>M. duabense</i> (85520)	+	-	+	-	-	-	+	-	7	193	1	1008	4
<i>M. parviflorum</i> (40363)	+	-	-	-	-	-	+	-	-	-	-	-	-
<i>M. parviflorum</i> (608)	-	+	+	-	-	+	+	-	3,7	157	1	581	3
<i>M. parviflorum</i> (72795)	+	-	+	-	-	-	+	+	11	144	1	406	2
<i>M. parviflorum</i> (80623)	+	-	+	-	-	-	+	+	8	196	1	871	4
<i>M. persicum</i> (34623)	+	-	+	-	+	-	-	-	-	-	-	15 (mm)	2,3
<i>M. persicum</i> (7285)	-	+	+	-	+	-	-	-	-	-	-	1223	4
<i>M. persicum</i> (9898)	-	+	+	-	+	+	-	-	4	-	-	2560	4
<i>M. propinquum</i> (2452)	-	+	-	-	-	-	+	-	6	154	1	464	2
<i>M. propinquum</i> (9894)	+	-	+	-	-	-	+	+	14	348	1	512	2
<i>M. propinquum</i> (61638)	+	-	+	-	-	-	+	-	7	296	2	463	3
<i>M. vulgare</i> (20)	+	-	-	-	+	-	-	-	0-1	193	1	867	2
<i>M. vulgare</i> (444)	+	-	+	-	-	+	+	-	8	191	1	962	3
<i>M. vulgare</i> (5129)	-	+	+	-	-	+	+	-	14	146	1	865	3
<i>M. vulgare</i> (13252)	-	+	-	-	+	+	-	-	1,2,3,4	365	1	673	2
<i>M. vulgare</i> (22864)	+	-	+	-	-	-	+	-	9	460	1	815	2
<i>M. vulgare</i> (90359)	+	-	+	-	-	+	+	-	6,7	280	1	480	2

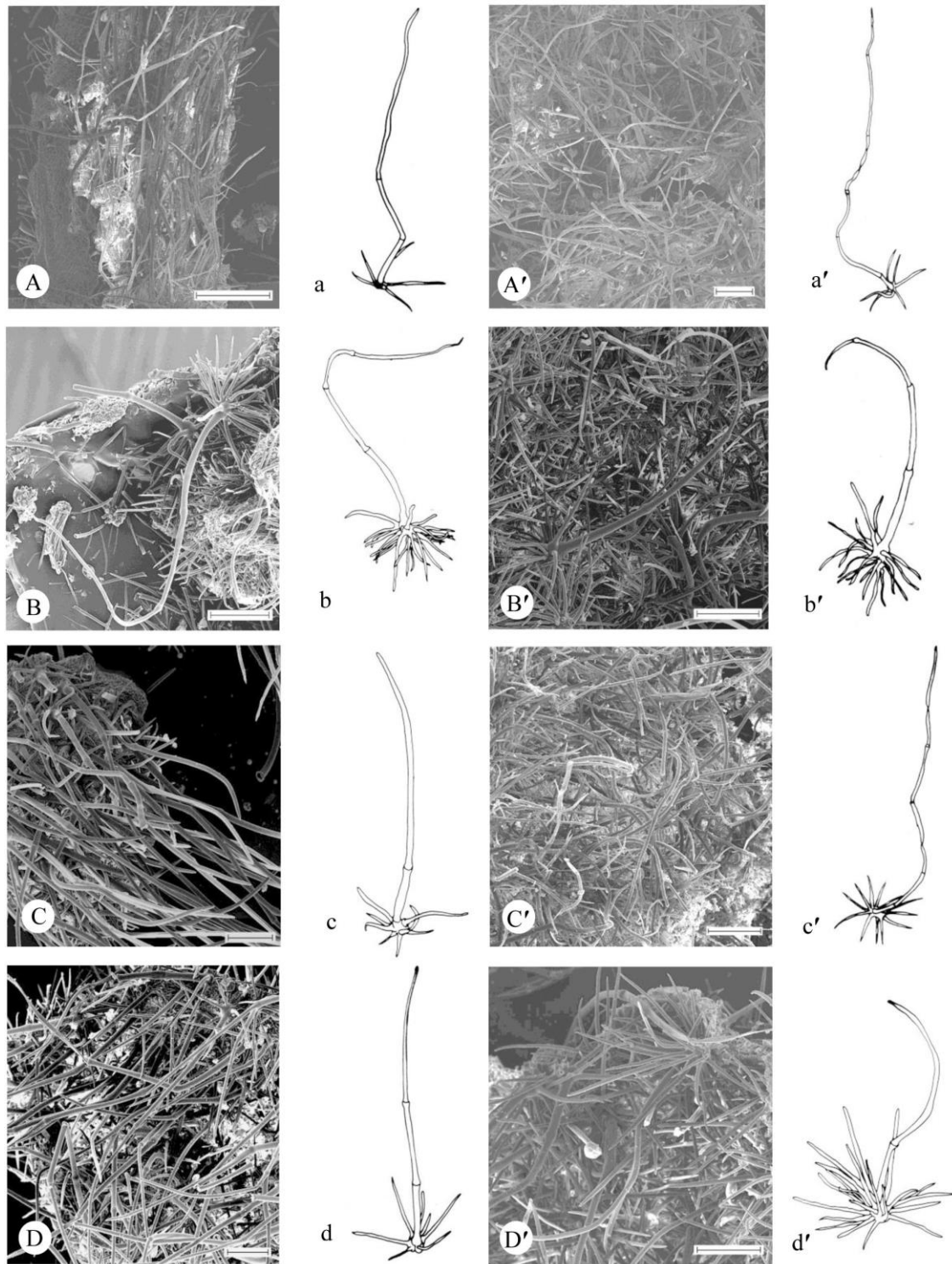


Fig. 2. Scanning electron micrographs of indumentum in upper and lower leaf surfaces in species of *Marrubium*. *M. astracanicum*: A, a; A', a'; B, b; B', b'; C, c; C', c'; D, d; D', d. In A, A', B, B', C, C', D, D', scale bar = 200 μ m. (Pictures in left column are the upper surface and those in the right column are lower surface of the leaves).

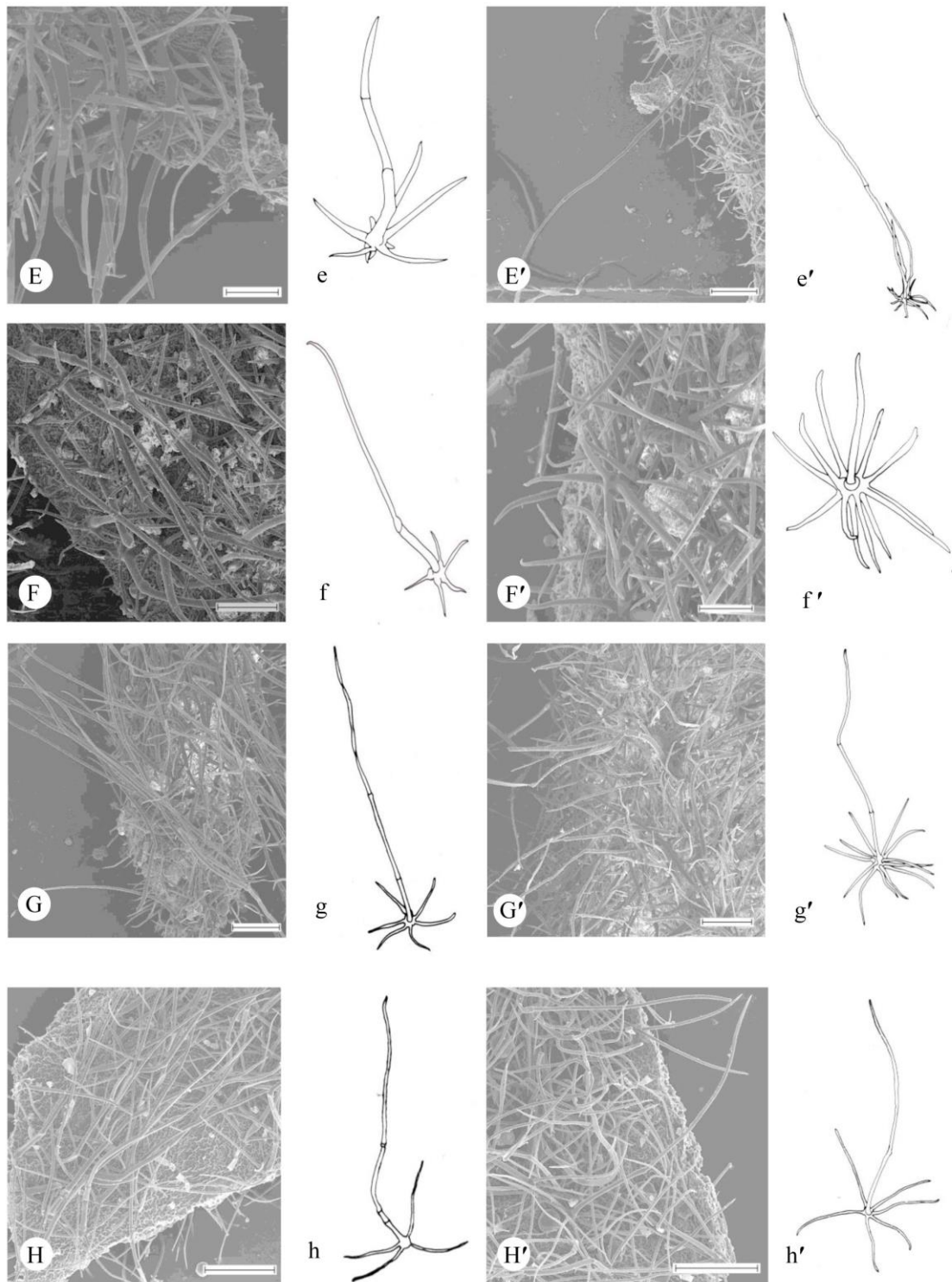


Fig. 2. Continued, *M. astracanicum*: E, e; E', e'; F, f; F', f'; G, g; G', g'; *M. anisodon*: H, h, H', h'. F, G, G', H', scale bar = 200 μ m; E, F'= 100 μ m; H= 500 μ m. (Pictures in left column are the upper surface and those in the right column are lower surface of the leaves).

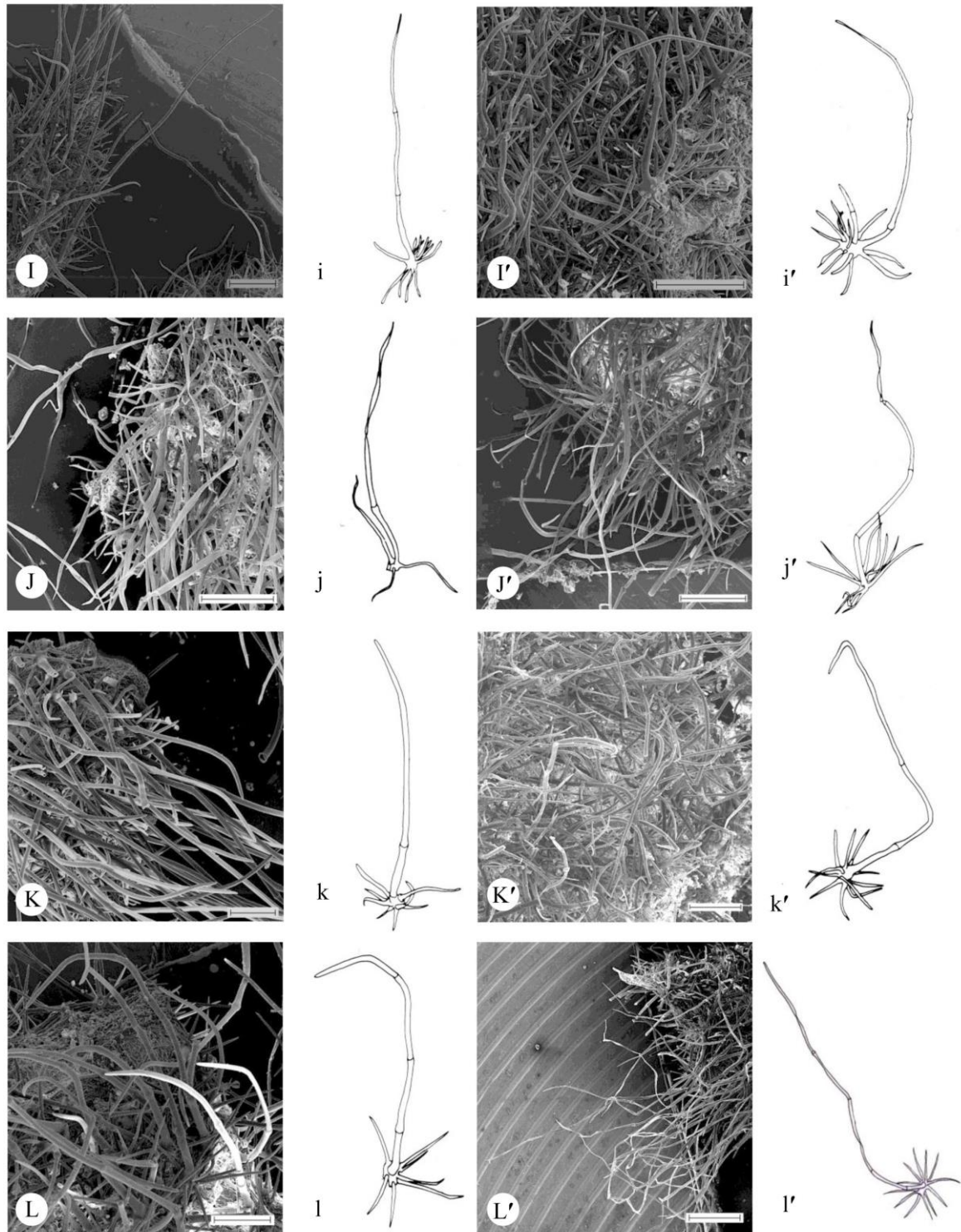


Fig.2. Continued. I, i; I', i' J, j; J', j'; K, k; K', k'; L, l; L', l': *M. astracanicum*. I, I', J, J', K, K', L, scale bar = 200μm; L' scale bar = 500 μm.

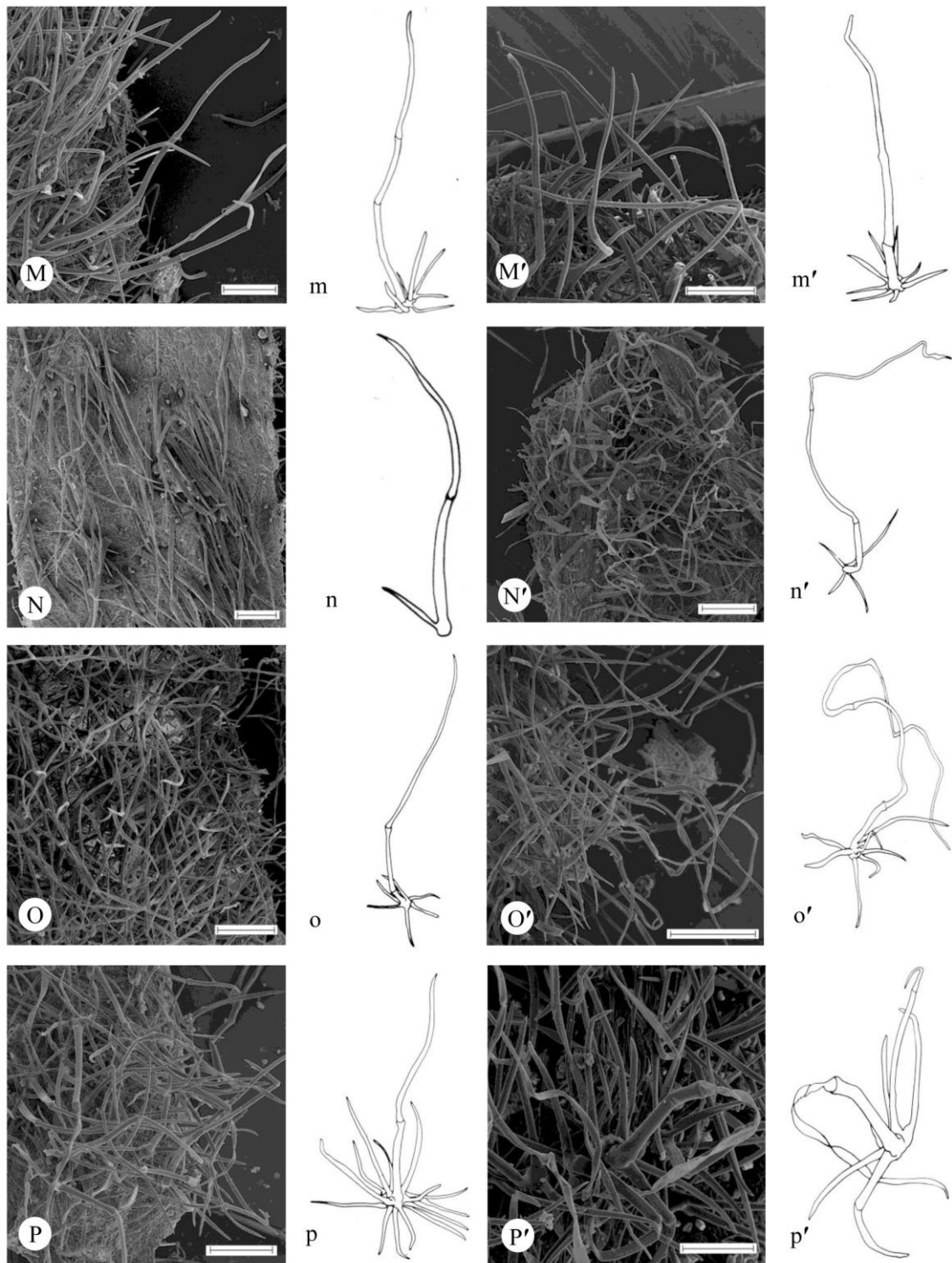


Fig. 2. Continued. M, m; M', m': *M. cordatum*. N, n; N', n'; O, o; O', o'; P, p; P', p': *M. crassidens*. M, M', N, N', O, O', P, P', scale bar = 200µm.

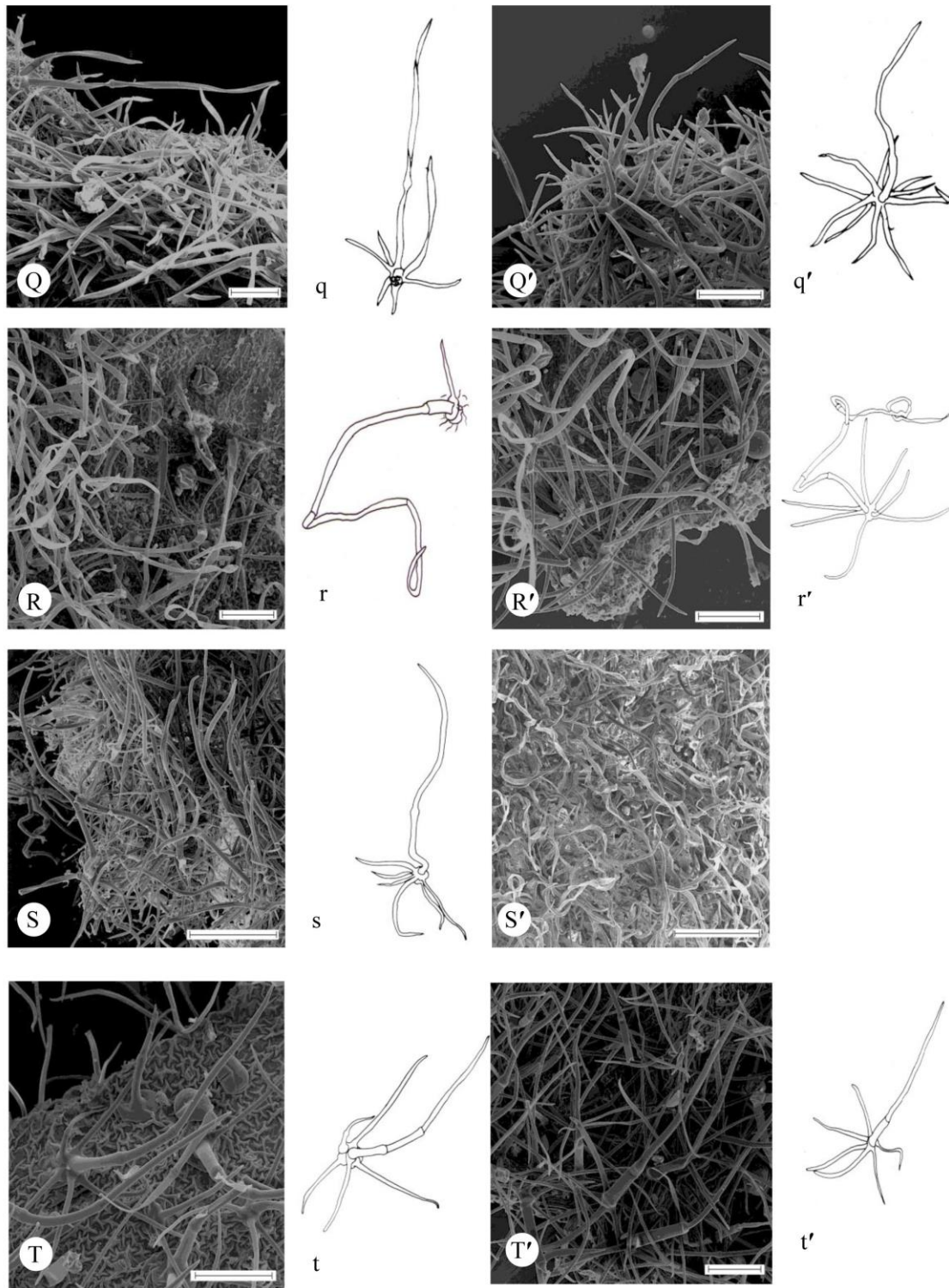


Fig. 2. Continued. Q, q; Q', q': *M. cuneatum*. R, r; R', r': *M. duabense*. S, s; S', s': *M. parviflorum*. T, t; T', t': *M. propinquum*. Q, Q', R, R', T, T', scale bar = 100µm; S, S' scale bar = 200µm.

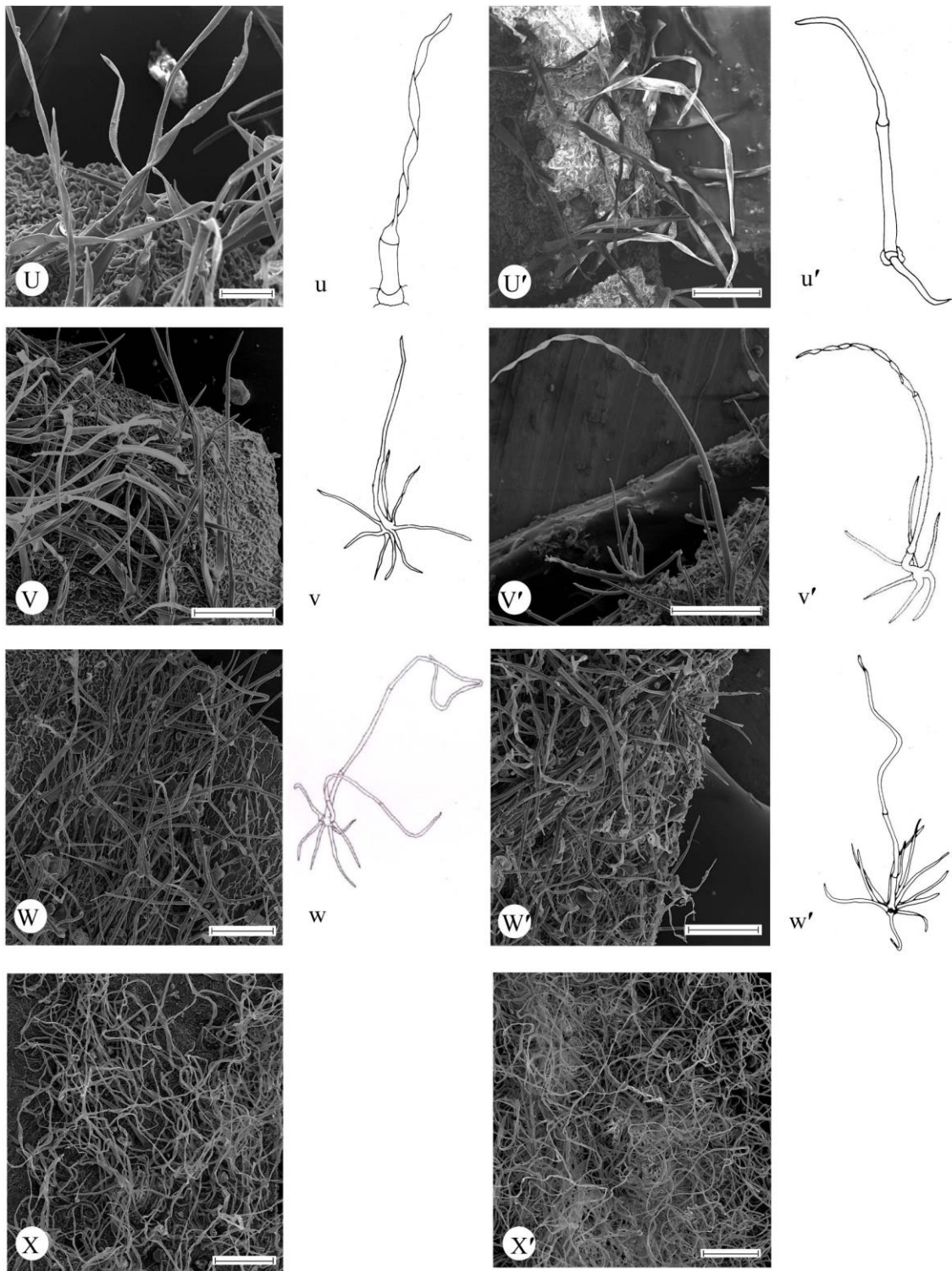


Fig. 2. (Continued), U, u; U', u', V', v', W, w; W', w': *M. vulgare*. X, x; X', x': *M. persicum*. U scale bar = 100µm; U', V', W, W', X, X' scale bar = 200µm.

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