

Micromorphological study of the tribe *Agrimoniae* family *Rosaceae* in Iran

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The current study, devoted to leaf micromorphology of tribe *Agrimoniae* in Iran including three genera (*Agrimonia*, *Aremonia*, and *Sanguisorba*), four species (*Agrimonia eupatoria*, *Aremonia agrimonioides*, *S. minor*, and *S. officinalis*), and six subspecies (*A. eupatoria* subsp. *eupatoria*, *A. eupatoria* subsp. *grandis*, *A. eupatoria* subsp. *asiatica*, *S. minor* subsp. *minor*, *S. minor* subsp. *lasiocarpa*, and *S. minor* subsp. *muricata*). At first, plant materials were collected and identified and then leaf segments were washed and prepared for Scanning Electron Microscopy (SEM). The result of micromorphological analysis revealed three types of trichomes (curved, *flexuous* and straight), four types of hair surface ornamentations (echinate, transversely elongated papilla, verucate and granulate), and two types of glandular hairs (round to cylindrical head cell with pilate to echinate surfaces). In all the examined species, epicuticular wax types were either of film (smooth layers and crust), and crystalloids (granule and platelets), and wax sculpturing which comprises four types of syntupism. In addition, stomata traits were identified especially three types of outer stomatal rim/peristomatal rim, four types of inner stomata rim, and three types of wax distribution on the stomata rim/pore/epidermal cell. Based on author's achievements, micromorphological characters of studied species of tribe *Agrimoniae*, possess diagnostic value and were capable of separating different taxonomic ranks (subtribes, genera, species, and subspecies). Based on these characters, an identification key is also prepared and presented herewith.

Keywords: *Agrimonia*, *Aremonia*, epicuticular wax, leaf blade, *Sanguisorba*, SEM, trichome**مطالعه ریزریخت‌شناسی برگ طایفه *Agrimoniae* از تیره گل‌سرخیان در ایران***

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خلاصه

مطالعه حاضر به مطالعه صفات ریزریخت‌شناسی برگ گیاهان طایفه *Agrimoniae* (syn.: *Sanguisorbeae* DC.) شامل سه جنس (*Agrimonia*, *Aremonia* و *Sanguisorba*)، چهار گونه (*Agrimonia eupatoria*, *Aremonia agrimonioides*, *S. minor* و *S. officinalis*)، شش زیرگونه (*A. eupatoria* subsp. *eupatoria*, *A. eupatoria* subsp. *grandis*, *A. eupatoria* subsp. *asiatica*، *S. minor* subsp. *minor*، *S. minor* subsp. *lasiocarpa* و *S. minor* subsp. *muricata*) می‌پردازد. ابتدا نمونه‌های گیاهی جمع‌آوری و شناسایی و سپس قطعات برگ شستشو و برای عکسبرداری توسط میکروسکوپ الکترونی (SEM) آماده شدند. نتایج حاصل از آنالیز ریزریخت‌شناسی، سه نوع کرک (خمیده، موجدار و راست)، چهار نوع تزینات سطح کرک (خار مانند، پشته‌های عرضی، زگیل‌دار و گرانول‌دار، دو نوع کرک غده‌ای (سر گرد و استوانه، با سطح صاف تا خاردار)، دو نوع موم روی کوتیکولی لایه نازک (لایه‌ای صاف و پوسته پوسته) و کریستالویدی (گرانول‌دار، صفحات کوچک) و چهار الگوی ترکیبی تزینات موم را آشکار ساخت. به علاوه، در این مطالعه، صفات روزنه بویژه سه نوع الگوی لبه بیرونی/تقریباً درونی و چهار نوع لبه درونی روزنه و سه الگوی پراکنش موم روی لبه درونی روزنه/سلول‌های منفذ/سلول‌های نگهبان شناسایی شدند. براساس یافته‌های این تحقیق، صفات ریزریخت‌شناسی گونه‌های مورد بررسی طایفه *Agrimoniae* حاوی اطلاعات تشخیصی و برای جداسازی سطوح مختلف تاکسونومیک (زیرطایفه، جنس، گونه‌ها و زیرگونه‌ها) کارآمد می‌باشند. براساس این صفات، کلید شناسایی نیز تهیه و ارائه گردید.

واژه‌های کلیدی: پهنک برگ، SEM، کرک، موم روی کوتیکولی، *Agrimonia*, *Aremonia*, *Sanguisorba*

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Introduction

The tribe *Agrimoniaeae* (syn.: *Sanguisorbeae* DC.) is classified in subfamily *Rosoideae* of family *Rosaceae* (Eriksson et al. 2003, Potter et al. 2007). Primarily, Jussieu (1789) included 11 genera including *Poterium* L., *Sanguisorba* L., *Acaena* Vahl., *Ancistrum* J.R. Forst. & G. Forst., *Cliffortia* L., *Agrimonia* L., *Neurada* L., *Alchemilla* L., *Aphanes* L., and *Sibbaldia* L., in this tribe. However, circumscription of the tribe (syn.: *Sanguisorbeae* DC. 1825, *Poterieae* Dumort. 1827) were changed by several authors (Schulze-Menz 1964, Hutchinson 1964, Takhtajan 1997, Kalkman 2004, Potter et al. 2007, Zhang et al. 2017), e.g. *Neurada* was removed from the family *Rosaceae*; *Alchemilla*, *Aphanes*, and *Sibbaldia*, considered as relatives of *Potentilla*, and *Fragaria* transferred to the tribe *Potentilleae* (Eriksson et al. 1998, Sojak 2004, Faghir et al. 2014).

Based on the current classification (Potter et al. 2007, Schulze-Menz 1964, Zhang et al. 2017), the tribe *Agrimoniaeae* comprises the following 17 genera (*Acaena*, *Agrimonia*, *Aremonia* Necker ex Nestle, *Becomia* Webb. & Berth. Svent Marcetella, *Cliffortia*, *Dendriopoterium* Svent, *Hagenia* J.F. Gmel., *Leucosidea* Eckl. et Zeyh., *Marcetella*, *Margyricarpus* Ruiz & Pav., *Polylepis* Ruiz & Pav, *Sanguisorba* (*Poteridium* Spach, *Poterium*), *Sarcopoterium* Spach., *Spenceria* Trimen, and *Tetraglochin* Poepp.) (Potter et al. 2007), arranged in two subtribes i.e. *Agrimoniinae* and *Sanguisorbinae* (Eriksson et al. 2003, Kerr 2004). The first subtribe covers five petalous genera of *Agrimoniaeae* [i.e. *Hagenia abyssinica* (Bruce ex Steud.) J.F. Gmel., *Leucosidea*, *Spenceria*, *Aremonia*, and *Agrimonia*]; while the second subtribe comprises apetaloid flowers genera (including *Sanguisorba*, *Poteridium*, *Poterium*, *Sarcopoterium*, *Becomia*, *Dendriopoterium*, *Tetraglochin cristatum* (Britt.) Rothm, *Polylepis*, *Margyricarpus*, *Cliffortia*, and *Acaena* in the world (Kerr 2004).

Tribe *Agrimoniaeae*, contains two genera (*Sanguisorba* and *Agrimonia*), three species (*Sanguisorba minor* Scop., *S. officinalis* L. and *Agrimonia eupatoria* L.), and seven subspecies [four

subspecies of *Sanguisorba* viz. *S. minor* subsp. *minor* Scop., *S. minor* subsp. *lasiocarpa* (Boss & Hausskn) Nordborg, *S. minor* subsp. *magnolia* (Spach) Briq., and *S. minor* subsp. *muricata* (Spach) Briq.) and three subspecies of *Agrimonia* viz. *A. eupatoria* subsp. *eupatoria* L., *A. eupatoria* subsp. *grandis*. (Andrz. ex Ascherson & Graebner) Bornm, and *A. eupatoria* subsp. *asiatica* (Juz.) Schönbeck-Temesy]] in the area covered by Flora Iranica (Nordborg 1969, Schönbeck-Temesy 1969). In the flora of Iran, Khatamsaz (1993) reported one species of *Sanguisorba* (*S. minor*), and three subspecies (except *S. minor* subsp. *magnolia*), one species of *Agrimonia* (*A. eupatoria*) with its three subspecies as well as a monotypic genus i.e. *Aremonia* [*A. agrimonioides* (L.) DC.] in the above group. Tribe *Agrimoniinae* is distributed mainly in Africa (*Leucosidea* and *Hagenia*), China (*Spenceria*), S. Europe and Asia (*Aremonia* and *Agrimonia*), while tribe *Sanguisorbeae* is widely present in the northern hemispheres, but some genera may occur in southern hemispheres (including *Tetraglochin*, *Polylepis*, *Margyricarpus*, *Cliffortia*, and *Acaena*) also, especially South Africa (Kerr 2004). Only a limited taxonomy and biosystematic studies carried out in *Agrimonia*, which are mainly focused in the family *Rosaceae* at subfamily levels (Metcalf & Chalk 1957, Ritsma, 1966, Hebda et al. 1988, Morgan et al. 1994, Eriksson et al. 1998, Eriksson et al. 2003, Naseri & Tantawy 2003). However, the most outstanding works as the representatives of tribe *Agrimoniaeae*, were based on morphological (Bitter 1911, Weimarck 1934, Simpson 1979, Kessler 1995), cytological (Kaliyera et al. 2014, Kumar et al. 2015), molecular (Mishima et al. 2002), and seed micromorphological studies (Chung et al. 2012).

The main aims of this survey are to provide a detailed account of micromorphological studies of Iranian species of the tribe *Agrimoniaeae*, and also to determine their utility in separating different ranks especially at species and subspecies levels.

Materials and Methods

In the current study, both dried and freshly collected specimens were used. The herbarium

specimens (Table 1) obtained from Research Institute of Forests and Rangelands, Tehran (TARI), Faculty of Pharmacy, Tehran University of Medical Sciences (THE), and Gilan University (GUH) herbaria. The fresh specimens were collected during 2015–16 from different parts of Iran. This resulted to the collection of *Sanguisorba officinalis* from Gilan and N. Khorasan provinces of Iran (Table 1). The voucher specimens of newly collected samples were deposited in Gilan University Herbarium (GUH). For identification purpose, the following references were used: Juzepczuk (1941),

Nordborg (1969), Schönbeck-Temesy (1969), and Khatamsaz (1993).

For SEM observation, the specimens were mounted on the stubs with double-sided cellophane tape and then coated by sputter coater with 25 nm of gold-palladium at an accelerating voltage of 10–15 kv. The micrographs were prepared by Scanning Electron Microscope (Tescan SEM Vega Razi Instrument). The principal references for terminology follows Fehrenbach & Bartholett (1988), Bartholett *et al.* (1998), Erikssen & Yurtsev (1999), and Kumar & Murugan (2015).

Table 1. Plant samples used in the current study along with related data

Tribe <i>Agrimoniae</i>	IRAN: Province, Collector, Date	Accession No.
I. Subtribe <i>Agrimoniinae</i>		
<i>Agrimonia eupatoria</i> subsp. <i>eupatoria</i>	Gilan prov.: Lahijan, Faghir, 6.5.2015; West Azarbaijan prov.: 14 km to Ashgholor, Arasvali	5753 (GUH); 55289 (THE)
<i>A. eupatoria</i> subsp. <i>grandis</i>	Mazandaran prov.: Noshahr, Darzi kola, Sabeti; Mazandaran prov.: Kelachai, 29.4.2013	2277 (TARI); 26281 (THE)
<i>A. eupatoria</i> subsp. <i>asiatica</i>	Mazandaran prov.: Ramsar, 1962 m, Mobaiien; Markazi prov.: Arak, 1900 m, Julliet	401 (TEH); 402 (TEH)
<i>A. agrimonoides</i>	Gilan prov.: Asalem to Khalkhal road, 1200 m, Faghir; Mazandaran prov.: Ramsar, Janat Roodbar, 1150 m, Roneh & Massoumi	5754 (GUH); 21625 (TARI)
II. Subtribe <i>Sanguisorbinae</i>		
<i>Sanguisorba officinalis</i>	Khorasan prov.: Kalat, 1505 m, Shahi; Gilan prov.: Asalem to Khalkhal, 2200 m, Faghir	5302 (GUH); 5303 (GUH)
<i>S. minor</i> subsp. <i>minor</i>	Lorestan prov.: Khoramabad, 1000 m, Vaisian; Mazandaran prov.: Poulor, Lar lake, 1860 m, Vaezi; Gilan prov.: Asalem to Khalkhal road, 2300 m, 11.3.2015, Faghir & Dailamy	24117 (TEH); 19254 (TEH); 5300 (GUH)
<i>S. minor</i> subsp. <i>lasiocarpa</i>	Azarbaijan prov.: Mishoodagh, 1840 m, Ghahreman; Kordestan prov.: Sanandaj, Kerdaneh, Shamshiri	9297 (TEH); 5301 (GUH)
<i>S. minor</i> subsp. <i>muricata</i>	Kerman prov.: Koghar, Mirtajedini; Mazandaran prov.: Karaj to Chaloos road, Pol-e Zangooleh, 300 m, Nazarian	29493 (TEH); 33151 (TEH)

Results

In the present study, micromorphological evidences of the tribe *Agrimoniae* were carefully evaluated and presented (Tables 2 & 3):

- Trichome

The result led to identification of three-hair types as followings: Type I: Curved trichomes in *A. eupatoria* subsp. *eupatoria* (Fig. 1 A-B), *A. eupatoria* subsp. *asiatica* (Fig. 1 C-D), *S. officinalis* (abaxial layer surface), and *S. minor* subsp. *minor* (abaxial layer surface) and *A. agrimonoides* (Fig. 1 E-F

curved from the base); Type II: straight trichomes in *A. eupatoria* subsp. *asiatica* (abaxial layer surface), *A. agrimonoides* and *A. eupatoria* subsp. *eupatoria* (Fig. G-H), *S. officinalis* (adaxial layer surface), and *S. minor* subsp. *minor* (abaxial layer surface); and Type III: Flexuous trichomes in *A. eupatoria* subsp. *grandis* (Fig. 1 I-J), *A. eupatoria* subsp. *eupatoria*, *A. eupatoria* subsp. *asiatica* (adaxial layer surface), and *S. officinalis* (both surfaces).

Table 2. Micromorphological characters of the Iranian species of subtribe *Agrimonieae*

Species/subspecies	Characters		
	Adaxial trichome length	Abaxial trichome length	Glandular hair length
Tribe Agrimonieae			
I. Subtribe Agrimoniinae			
<i>Agrimonia eupatoria</i> subsp. <i>eupatoria</i>	79.09(82.48±2.01)83.9	45.31(46.21±0.54)46.82	26.32(26.84±0.35)27.21
<i>A. eupatoria</i> subsp. <i>asiatica</i>	102.05(102.76±0.45)103.31	108.64(111.13±1.4)112.02	43.55(44±0.25)44.17
<i>A. eupatoria</i> subsp. <i>grandis</i>	71.66(72.22±0.53)73.02	82.17(84.84±2.14)86.48	40.41(40.92±0.57)41.75
<i>A. agrimonoides</i>	68.49(70.41±1.74)72.64	96.70(97.66±1.08)99.45	59.07(61.10±1.4)62.86
II. Subtribe Sanguisorbinae			
<i>Sanguisorba officinalis</i>	114.96(117.8±2.05)120.34	74.50(75.03±0.48)75.63	40.05(41.68±0.93)42.32
<i>S. minor</i> subsp. <i>minor</i>	-	81.3(82.81±1.08)84.20	36.18(37.26±0.64)37.87
<i>S. minor</i> subsp. <i>lasiocarpa</i>	-	-	-
<i>S. minor</i> subsp. <i>muricata</i>	-	-	46.83(45.61±0.61)46.50

Table 2 (contd)

Species/subspecies	Characters	
	Stomata aperture length	Stomata aperture width
Tribe Agrimonieae		
I. Subtribe Agrimoniinae		
<i>Agrimonia eupatoria</i> subsp. <i>eupatoria</i>	7.11(7.48±0.34)7.97	2.38(2.62±0.15)2.81
<i>A. eupatoria</i> subsp. <i>asiatica</i>	7.15(7.58±0.34)8.04	1.03(1.11±0.08)1.22
<i>A. eupatoria</i> subsp. <i>grandis</i>	8.37(8.59±0.18)8.76	2.83(3.16±0.22)3.37
<i>A. agrimonoides</i>	7.42(7.91±0.47)8.71	1.00(1.11±0.1)1.23
II. Subtribe Sanguisorbinae		
<i>Sanguisorba officinalis</i>	10.01(10.3±0.25)10.62	3.65(3.72±0.07)3.84
<i>S. minor</i> subsp. <i>minor</i>	10.11(10.37±0.34)10.98	1.96(2.19±0.18)2.42
<i>S. minor</i> subsp. <i>lasiocarpa</i>	13.63(13.91±0.18)14.15	2.10(2.65±0.4)3.19
<i>S. minor</i> subsp. <i>muricata</i>	5.78(5.91±0.11)6.09	1.05(1.16±0.12)1.36

The straight trichomes were either erect-semierect in *S. officinalis* (Fig. 1 K-L), *S. minor* subsp. *minor*, *A. eupatoria* subsp. *grandis*, *A. eupatoria* subsp. *eupatoria*, and *A. eupatoria* subsp. *asiatica* or erect in *A. eupatoria* subsp. *eupatoria*, and *A. agrimonoides*.

Among the studied species, the longest trichomes were observed in *A. eupatoria* subsp. *grandis* to (102.76 mm and 111.13 mm in both surfaces), *S. officinalis* (75.03 mm at abaxial surface), and *A. agrimonoides* (70.41 mm at adaxial surface). In contrast, the shortest trichomes were measured in *A. eupatoria* subsp. *asiatica* (84.84 mm at abaxial surface), and *A. eupatoria* subsp. *eupatoria* (82.48 mm at adaxial surface).

- Trichome surfaces

The SEM observations revealed four types of trichome surface ornamentations: Type I: Echininate in *Aremonia agrimonoides* (Fig. 2 A); Type II: Transversely elongated papilla in *A. eupatoria*

subsp. *eupatoria* (Fig. 2 B), *S. officinalis* species (Fig. 2 C); Type III: Verucate in *A. eupatoria* subsp. *asiatica*, *A. eupatoria* subsp. *grandis* (Fig. 2 D) and in *A. eupatoria* subsp. *grandis* (Fig. 2 E), and *A. eupatoria* subsp. *asiatica*; and Type IV: Densely granulate in *S. minor* subsp. *minor* (Fig. 2 F).

- Glandular hairs

Two types of glandular hairs were recognized: Type 1: Hairs with rounded head cell (Fig. 2 G-H). This type was observed in *A. eupatoria* subsp. *grandis*, *A. eupatoria* subsp. *asiatica*, *S. minor* subsp. *muricata*, and *S. minor* subsp. *minor*; and Type II: Hairs with cylindrical head cell (Fig. 2 I-J). This type was seen in *Aremonia agrimonoides* and *S. officinalis* which both have first and second types of glandular hairs. The glandular hairs surface, changed from smooth (Fig. 2 G & I) to granular (Fig. 2 H and J), and verucate (or microechinate) (Fig. 2 K-L).

- Epicuticular wax type

Based on SEM observation, epicuticular wax type in tribe *Agrimoniaeae* composed of film (smooth layer and crust) and crystalloides (mainly granule and platelets) (Fig. 3). Wax are either simple (only smooth layer) or syntopism (smooth layer + granule; smooth

layer + granule + platelets; crust + granule; crust + granule + platelet). The irregular platelets were identified in all the studied taxa except *S. minor* subsp. *muricata* which possesses membranous platelets (on the lower side of the leaf surfaces).

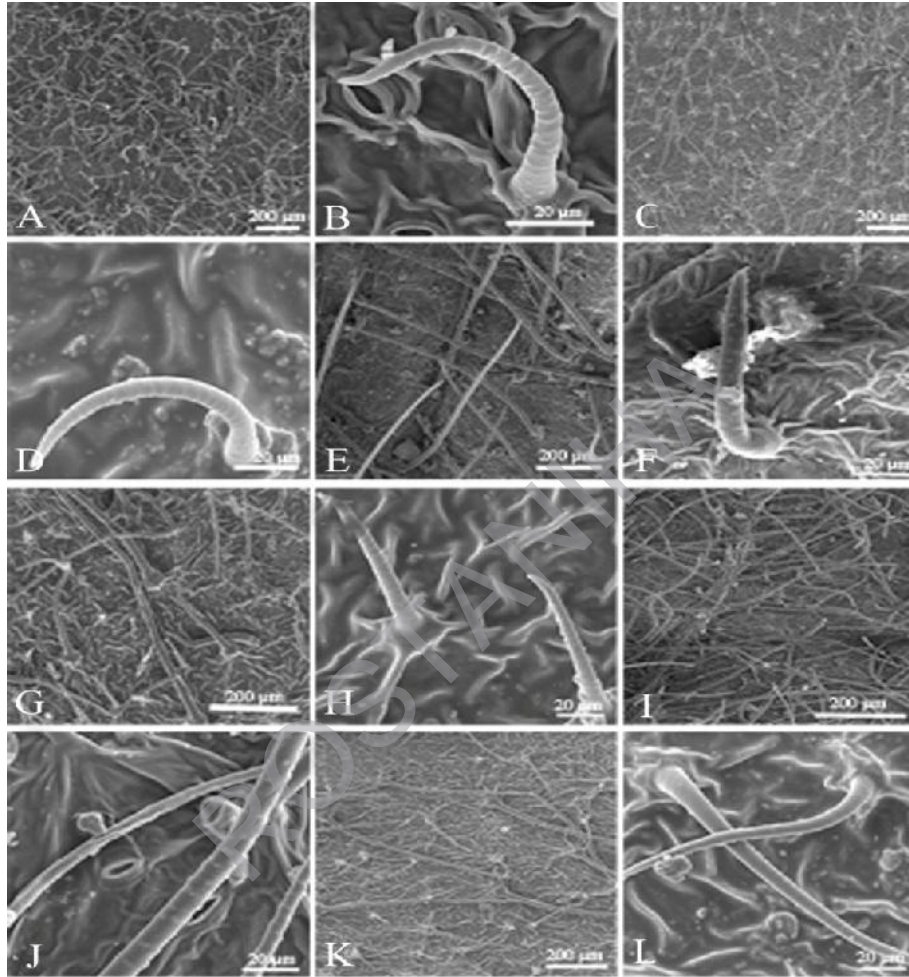


Fig. 1. Trichome types in tribe *Agrimoniaeae*: A-B. *Agrimonia eupatoria* subsp. *eupatoria*, C-D. *A. eupatoria* subsp. *asiatica*, E-F. *A. agrimonoides*, G-H. *A. eupatoria* subsp. *eupatoria*, I-J. *A. eupatoria* subsp. *grandis*, K-L. *Sanguisorba officinalis*.

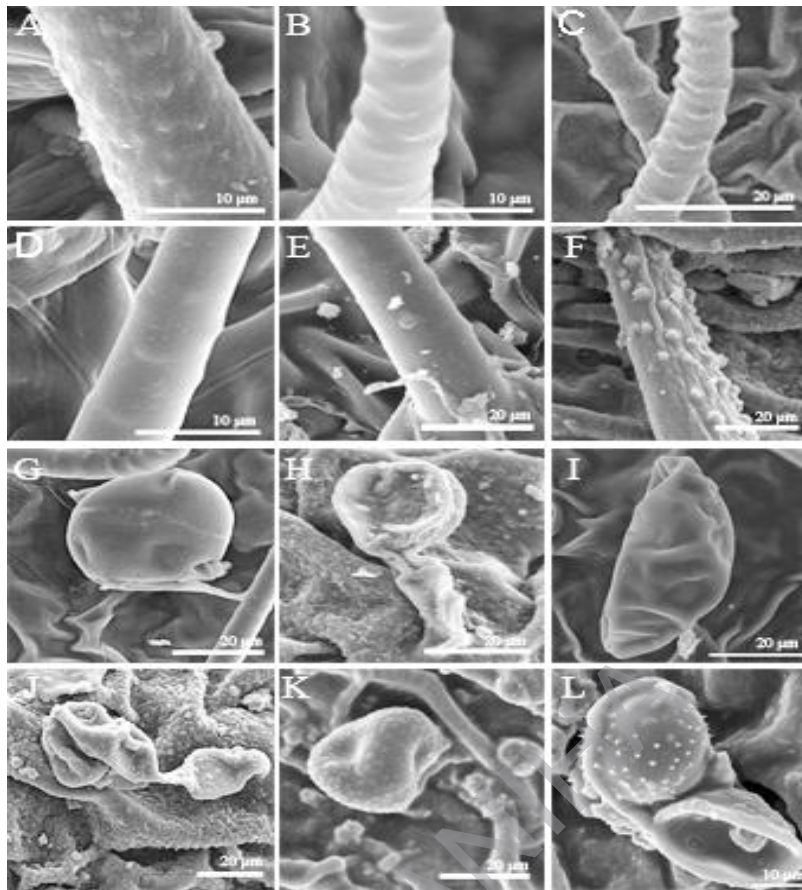


Fig. 2. Trichome surface ornamentation types: A. *Agrimonia agrimonoides*, B. *A. eupatoria* subsp. *eupatoria*, C. *S. officinalis*, D-E. *A. eupatoria* subsp. *grandis*, F. *S. minor* subsp. *minor*, Glandular hairs: G. *A. eupatoria* subsp. *grandis*, H. *Sanguisorba minor* subsp. *minor*, I. *S. officinalis*, J. *S. minor* subsp. *minor*, K-L. *S. minor* subsp. *muricata*.

Based on epicuticular wax sculpturing, six type classes were formed: Type I: Smooth layer in *S. minor* subsp. *lasiocarpa* (Fig. 3 A); Type II: Smooth layer with granule in *A. eupatoria* subsp. *eupatoria* (Fig. 3 B), *A. eupatoria* subsp. *asiatica* (Fig. 3 C) and *S. officinalis*; Type III: Smooth layer with granule and plateletes; *A. eupatoria* subsp. *eupatoria* (Fig. 3 D); *A. eupatoria* subsp. *grandis* (on both sides) (Fig. 3 E); *A. eupatoria* subsp. *asiatica*, and *A. agrimonoides* (lower side of the leaf surfaces); Type IV: Crust with granule in *S. minor* subsp. *lasiocarpa* and *S. minor* subsp. *minor* (on both sides) (Fig. 3 F); Type V: Crust with granule and irregular platelet in *S. minor* subsp. *lasiocarpa* (Fig. 3 G); *S. minor* subsp. *muricata*; and Type VI: Crust with granule and membranous platelet in *S. minor* subsp. *muricata* (Fig. 3 I).

The current micromorphological analysis revealed three types of outer stomatal rim/peristomatal rim characters on both adaxial/abaxial surfaces of the leaves (Table 3):

Type I: Raised/Overlapping-stout in *Agrimonia eupatoria* subsp. *eupatoria* (Fig. 4 A), *S. officinalis* (Fig. 4 B-C), *A. eupatoria* subsp. *asiatica* (Fig. 4 D) and *A. eupatoria* subsp. *grandis* (Fig. 4 E), and *A. agrimonoides* (Fig. 4 F); Type II: Overlapping/Overlapping-stout *S. minor* subsp. *lasiocarpa* (Fig. 4 G-H); and Type III: Overlapping in *S. minor* subsp. *minor* (Fig. 4 I-J), and *S. minor* subsp. *muricata* (Fig. 4 K-L); in the three later subspecies stomata were sunken.

Based on inner stomatal rim variations, four types were identified: Type I: Sinuolate-erose in *A. eupatoria* subsp. *eupatoria* (Fig. 4 A) and *S. officinalis* (Fig. 4 C), and *A. eupatoria* subsp. *asiatica* (Fig. 4 D); Type II: Sinuolate in *S. officinalis* (Fig. 4 B); Type III: Thick sinuolate in *A. eupatoria* subsp. *grandis* (Fig. 4 E) and *A. agrimonoides* (Fig. 4 F); and Type IV: Smooth in *S. minor* subsp. *minor* (Fig. 4 I-J), *S. minor* subsp. *lasiocarpa* (Fig. 4 G-H), and *S. minor* subsp. *muricata* (Fig. 4 K-L).

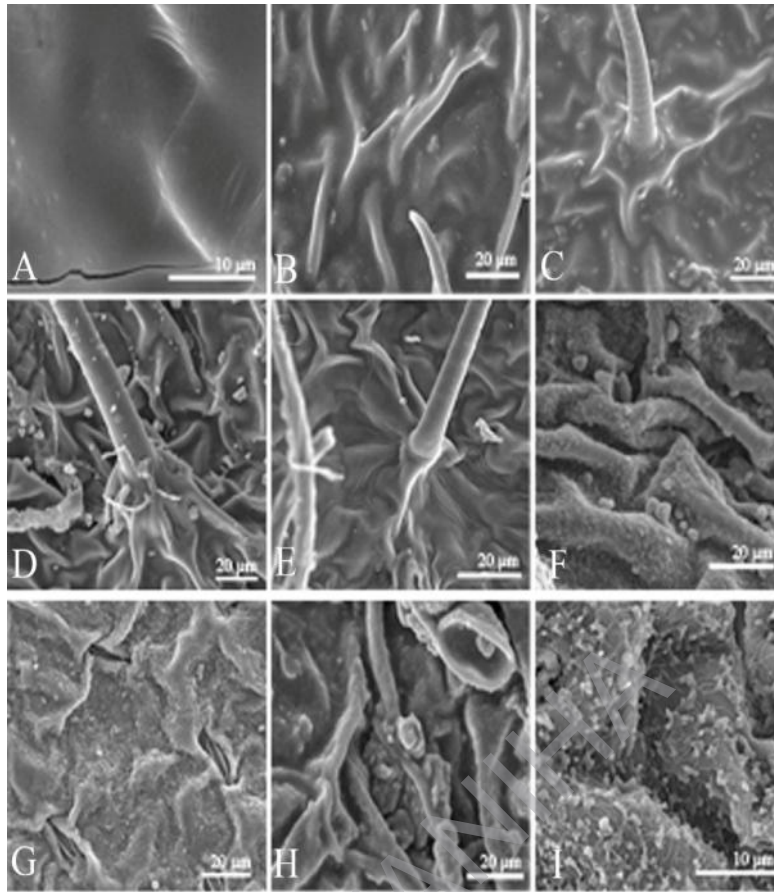


Fig. 3. Epicuticular wax sculpturing: A and G. *Sanguisorba minor* subsp. *lasiocarpa*, B. *Agrimonia eupatoria* subsp. *eupatoria*, C. *A. eupatoria* subsp. *asiatica*, D-E. *A. eupatoria* subsp. *grandis*, F. *S. minor* subsp. *minor*, H-I. *S. minor* subsp. *muricata*.

Table 3. Types of outer stomatal rim, peristomatal rim characters surfaces of the leaves studied representatives of tribe *Sanguisorbeae*

Tribe <i>Agrimonieae</i>	Outer stomatal rim (Ad/Ab)	Peristomatal rim (Ad/Ab)	Inner stomatal rim (Ad/Ab)
I. Subtribe <i>Agrimoniinae</i>			
<i>Agrimonia eupatoria</i>			
subsp. <i>eupatoria</i>	- / raised	- / overlapping-stout	Sinuolate-erose
<i>A. eupatoria</i> subsp. <i>asiatica</i>	- / raised	- / overlapping-stout	Sinuolate-erose
<i>A. eupatoria</i> subsp. <i>grandis</i>	- / raised	- / overlapping-stout	Thick sinuolate
<i>A. agrimonoides</i>	Raised / raised	- / overlapping-stout	Thick sinuolate
II. Subtribe <i>Sanguisorbinae</i>			
<i>Sanguisorba officinalis</i>	Raised / raised	- / overlapping-stout	Sinuolate-erose / sinuolate
<i>S. minor</i> subsp. <i>minor</i> *	- / overlapping	Overlapping	Smooth
<i>S. minor</i> subsp. <i>lasiocarpa</i> *	- / overlapping	- / overlapping-stout	Smooth
<i>S. minor</i> subsp. <i>muricata</i> *	- / overlapping	- / overlapping	Smooth

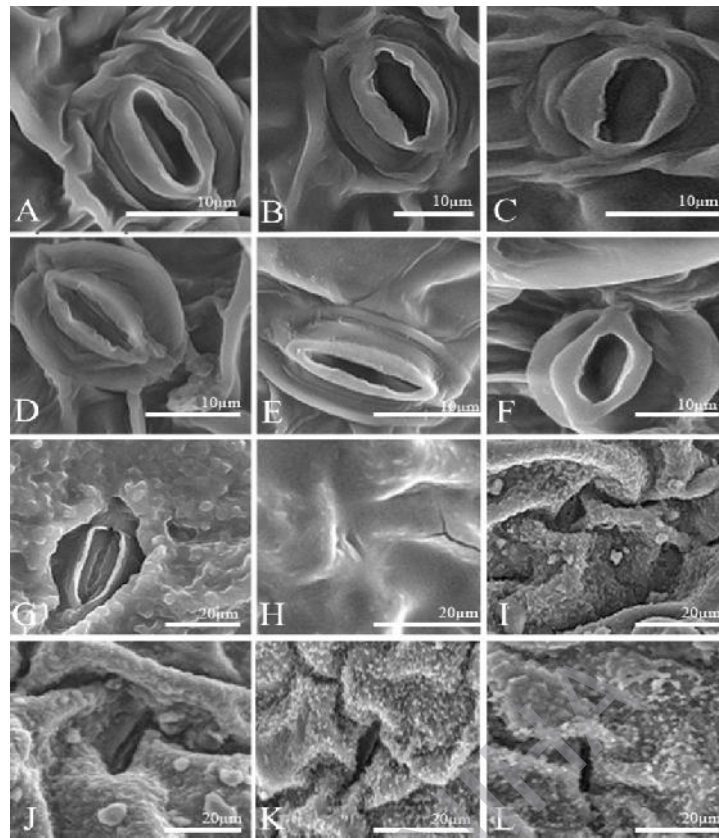


Fig. 4. Stomatal/peristomatal and inner stomatal rims characters: A. *Agrimonia eupatoria* subsp. *eupatoria*, B-C. *S. officinalis*, D. *A. eupatoria* subsp. *asiatica*, E. *A. eupatoria* subsp. *grandis*, F. *A. agrimonoides*, G-H. *Sanguisorba minor* subsp. *lasiocarpa*, I-J. *S. minor* subsp. *minor*, K-L. *S. minor* subsp. *muricata*.

Based on wax distribution on the stomata rims, pore and epidermal cells; three groups were identified: Type I: Stomata rim and guard cell completely covered by wax, pore free. This comprises *A. eupatoria*, *A. agrimonoides*, *S. officinalis* (abaxial side), and *S. minor* subsp. *muricata* (both sides); Type II: Stomata rims and pore free, guard cell covered by wax in *S. officinalis* (adaxial surface); and Type III: Stomata rim and guard cell not completely covered by wax, pore free in *S. minor* subsp. *minor* and *S. minor* subsp. *lasiocarpa*.

Discussion

Several previous authors reported taxonomic importance of leaf and petiole epidermis micromorphology in different genera of the family *Rosaceae* (Neinhuis & Barthlott 1997, Barthlott et al. 1998, Eriksen & Yurtsev 1999, Ganeva & Uzunova 2010, Faghir et al. 2010, Faghir et al. 2014, 2017).

Based on the current findings, all the studied representatives of the tribe *Agrimonieae* have hairy leaves, except two subspecies of *S. minor* (including *S. minor* subsp. *muricata*, and *S. minor* subsp. *lasiocarpa*). Among them, *S. officinalis*, *A. eupatoria* subsp. *eupatoria*, and *A. eupatoria* subsp. *asiatica*, have all the three types (straight to curved and flexuous) of trichomes. While *A. eupatoria* subsp. *grandis* have straight long hairs and lacking curve trichomes; *Agrimonia agrimonoides* possesses straight and curved hairs but do not have flexuous hairs. In addition, *S. officinalis* and *A. eupatoria* subsp. *grandis* have longest trichome within *Sanguisorbinae* and *Agrimoniinae* subtribes, respectively.

Surface ornamentation of trichome varied from smooth to transversely elongated papilla, verrucate and granulates. This diversity is also a good tool for identification purpose (Eriksen & Yurtsev 1999), especially at species (echinate in *Agrimonia*

agrimonioides, verrucate in *Sanguisorba officinalis*), and subspecies levels (transversely elongated papilla in *A. eupatoria* subsp. *eupatoria*; both transversely ridged and scattered verruca in *A. eupatoria* subsp. *asiatica* and granulate in *S. minor* subsp. *minor*).

Glandular hairs were observed in all the studied species. However, they differed on their head-cell shape and their smooth or granular and verrucate surfaces. This character can be used in separating the studied species and subspecies of the tribe *Agrimoniaeae*. The current result also supports the diagnostic value of micromorphological characters of trichome and glandular hairs trichome (Faghir *et al.* 2010, Eriksen & Yurtsev 1999).

Epicuticular wax data of the Iranian species of the tribe *Agrimoniaeae* is typical of the family *Rosaceae* (Fehrenbach & Barthlott 1988, Wissemann 1998, Neinhuis & Barthlott 1997, Faghir *et al.* 2014). These superimposed wax structures (Koch & Barthlott 2009) comprises both films (smooth layers and crusts) and crystalloids (granule and platelets).

Based on author's findings, six types of epicuticular wax sculpturings were recorded. Among them, smooth layer/granules and platelets were recorded in *Agrimonia eupatoria* and *Sanguisorba officinalis*, while, crust/smooth/granules and platelets were observed in *S. minor* and *Aremonia agrimonoides*. In addition, wax sculpturing changes in three subspecies of *S. minor* (*S. minor* subsp. *lasiocarpa* with crust and smooth layer/granules and platelets; *S. minor* subsp. *minor* possess crust/granules and *S. minor* subsp. *muricata* with crust/granules and platelets). Irregular platelets were the most dominant in all the studied representatives. However, membranous platelet occurs only in *S. minor* subsp. *muricata*. Wax sculpturing character can be used for delimitation of species and subspecies.

According to the author's finding, it was revealed that, wax distribution on the stomata rim, pore and guard cell (on both sides of the leaf surfaces) of the subtribe

Agrimoniinae is of Type I. However, it shows variation in subtribe *Sanguisorbinae* (from Type I to III in *S. minor*, and Type II to III in *S. officinalis*). The outer-stomatal/peristomatal- and inner stomatal rim characters of blade were considered as taxonomically informative traits (Ergen Akin 2013, Kumar & Murugan 2015, Faghir *et al.* 2017). Based on the present study, the outer/peristomatal rim of Type I (raised/overlapping stout) is very common among subtribe *Agrimoniinae* (both in *Agrimonia eupatoria* and *Aremonia agrimonoides*) while it changes from Type I (raised/overlapping-stout in *S. officinalis*) to Type II (overlapping/overlapping-stout in *S. minor* subsp. *lasiocarpa*) and Type III (overlapping in *S. minor* subsp. *muricata* and *S. minor* subsp. *minor*) among species of subtribe *Sanguisorbinae*. The inner stomatal rim evidence are diagnostic tool for isolating different species (Type IV in *S. minor*; Type II and I in *S. officinalis*; Type III in *A. agrimonoides*), and subspecies (Type III, thick sinuate in *A. eupatoria* subsp. *grandis*, Type I in *A. eupatoria* subsp. *eupatoria* and *A. eupatoria* subsp. *asiatica*).

The current leaf epidermal survey revealed taxonomic values of the leaf micromorphological characters of Iranian species of the tribe *Agrimoniaeae*. These traits can be use for delimiting the two subtribe and their genera, species and subspecies.

Based on diagnostic micromorphological evidences an identification key is presented herewith.

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Key to the species and subspecies of subtribe *Agrimoniinae* in Iran based on the leaf epidermis features

1. Leaves interruptedly imparipinnate, with small intercalary lobes and hairy; wax distribution on the stomata rims, pore and epidermal cells type I; wax sculpturing in Type II and III 1. Subtribe *Agrimoniinae* 2
 - Leaves pinnate, without small intercalary lobes, hairy or glabrous; wax distribution on the stomata rims, pore and epidermal cells in Type I to III; wax sculpturing in Type I, II, IV-VI (except type III).... 2. Subtribe *Sanguisorbinae* ... 5
2. Trichome stright, flexuous and curved, without echinate and verucca 3
 - Trichome stright and curved, with echinate and verucca 1. *A. agrimonoides*
3. Trichome stright and flexuous (not curved), on both sides of the leaf surfaces, hairs long (102.76 mm on adaxial and 111.13 mm on adaxial sides) 1.2. *A. eupatoria* subsp. *grandis*
 - Trichome stright, flexuous and curved on leaf either sides, hairs both short (82.84 mm) and long 4
4. Stomata edge aperture sinuolate, trichome of abaxial side short (46–46/82 mm) 3. *A. eupatoria* subsp. *eupatoria*
 - Stomata edges aperture thick sinuolate, trichome of abaxial side long (86–82 mm) 1.4. *A. eupatoria* subsp. *asiatica*
5. Leaf lower side with flexuous and curved hairs, trichome surface having alternate linear warts, outer stomatal rime raised 2.1. *S. officinalis*
 - Leaf lower side glabrous or scarsly hairy, trichome surface not veruccose, outer stomatal rime overlapping 6
6. Leaf lower side completely glabrous, trichome surface with or without warts, stomata edges aperture smooth 7
 - Leaf lower side scarsly hairy, trichome surface densely granulate, stomata edges aperture sinuolate 2.2. *S. minor* subsp. *minor*
7. Glandular trichome surface echinate, smooth layer/platelets-granule wax sculpturing on the leaf adaxial/abaxial surfaces 2.3. *S. minor* subsp. *muricata*
 - Glandular trichome surface not echinate, granule/irregular platelets of wax sculpturing on the leaf either sides 2.4. *S. minor* subsp. *lasiocarpa*

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