Micromorphological study of the tribe Agrimonieae family Rosaceae in Iran

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Abstract

The current study, devoted to leaf micromorphology of tribe Agrimonieae in Iran including three genera (Agrimonia, Aremonia, and Sanguisorba), four species (Agrimonia eupatoria, Aremonia agrimonioideae, 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, verrucate and granulate), and two types of glandular hairs (round to cylindrical head cell with pisilate to echinate surfaces). In all the examined species, epicuticular wax types were either of film (smooth layers and crust), and crystals (granule and platelets), and wax sculpturing which comprises four types of syntipum. In addition, stomata traits were identified especially three types of outer stomatal rim/peristomatal rim, four types of inner stomatal rim, and three types of wax distribution on the stomata rim/pore/epidermal cell. Based on these characters, an identification key is also prepared and presented herewith.

Keywords: Agrimonia, Aremonia, epicuticular wax, leaf blade, Sanguisorba, SEM, trichome

مطالعه خاض بر مطالعه صفات ریزبیجکت شناسی برگ گیاهان طالب‌نامه

مطالعه ریزبیجکت شناسی برگ طالب‌نامه

Agrimonieae از تهیه گل سرخ‌بان در ایران

Agrimonieae

دی‌بهار: ۱۳۹۶/۰۷/۲۷

مریمی بیگم فقیه‌فر

گلنوش شمشیری: دانشجوی کارشناسی ارشد، گروه زیست‌شناسی، دانشگاه گیلان، رشت، ایران

عاطفه مهرمنش: دانشجوی کارشناسی ارشد، گروه زیست‌شناسی، دانشگاه گیلان، رشت، ایران

خلاصه

مطالعه حاضر به مطالعه صفات ریزبیجکت شناسی برگ گیاهان طالب‌نامه

جنس (Agrimonia, Aremonia, and Sanguisorba), four species (Agrimonia eupatoria, Aremonia agrimonioideae, 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, verrucate and granulate), and two types of glandular hairs (round to cylindrical head cell with pisilate to echinate surfaces). In all the examined species, epicuticular wax types were either of film (smooth layers and crust), and crystals (granule and platelets), and wax sculpturing which comprises four types of syntipum. In addition, stomata traits were identified especially three types of outer stomatal rim/peristomatal rim, four types of inner stomatal rim, and three types of wax distribution on the stomata rim/pore/epidermal cell. Based on these characters, an identification key is also prepared and presented herewith.

Keywords: Agrimonia, Aremonia, epicuticular wax, leaf blade, Sanguisorba, SEM, trichome

مطالعه ریزبیجکت شناسی برگ گیاهان طالب‌نامه

Agrimonia, Aremonia, epicuticular wax, leaf blade, Sanguisorba, SEM, trichome

Agrimonieae DC. Agrimoniae


زبان‌های: یکندر

بررسی طالب‌نامه

Agrimonia, Aremonia, Sanguisorba

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, verrucate and granulate), and two types of glandular hairs (round to cylindrical head cell with pisilate to echinate surfaces). In all the examined species, epicuticular wax types were either of film (smooth layers and crust), and crystals (granule and platelets), and wax sculpturing which comprises four types of syntipum. In addition, stomata traits were identified especially three types of outer stomatal rim/peristomatal rim, four types of inner stomatal rim, and three types of wax distribution on the stomata rim/pore/epidermal cell. Based on these characters, an identification key is also prepared and presented herewith.

Keywords: Agrimonia, Aremonia, epicuticular wax, leaf blade, Sanguisorba, SEM, trichome
Introduction

The tribe Agrimonieae (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., Ancistrocarpum J.R. Forst. & G. Forst., Cliforia L., Agrimonia L., Neurada L., Alchemilla L., Aphanes L., and Sibbaldia L., in this tribe. However, circumscription of the tribe (syn.: Sanguisorbeae DC. 1825, Poterioeae 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 Frigaria transferred to the tribe Potentilleae (Eriksson et al. 1998, Sojak 2004, Faghir et al. 2014).


Tribe Agrimonieae, 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 Agrimonieae 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, Polyplepis, Margrylicarpus, Cliforia, 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 Agrimonieae, 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 Agrimonieae, 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).

### Results

In the present study, micromorphological evidences of the tribe *Agrimonieae* 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>
<thead>
<tr>
<th>Tribe <em>Agrimonieae</em></th>
<th>IRAN: Province, Collector, Date</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Subtribe <em>Agrimoniinae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Agrimonia eupatoria</em> subsp. <em>eupatoria</em></td>
<td>Gilan prov.: Lahijan, Faghir, 6.5.2015; West Azarbaijan prov.: 14 km to Ashgholor, Arasvali</td>
<td>5753 (GUH); 55289 (THE)</td>
</tr>
<tr>
<td><em>A. eupatoria</em> subsp. <em>grandis</em></td>
<td>Mazandaran prov.: Noshahr, Darzi kola, Sabeti; Mazandaran prov.: Kelachai, 29.4.2013</td>
<td>2277 (TARI); 26281 (THE)</td>
</tr>
<tr>
<td><em>A. eupatoria</em> subsp. <em>asiatica</em></td>
<td>Mazandaran prov.: Ramsar, 1962 m, Mobaiien; Markazi prov.: Arak, 1900 m, Juliet</td>
<td>401 (TEH); 402 (TEH)</td>
</tr>
<tr>
<td><em>A. agrimonoides</em></td>
<td>Gilan prov.: Asalem to Khalkhal road, 1200 m, Faghir; Mazandaran prov.: Ramsar, Janat Roodbar, 1150 m, Roneh &amp; Massoumi</td>
<td>5754 (GUH); 21625 (TARI)</td>
</tr>
<tr>
<td>II. Subtribe <em>Sanguisorbinae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sanguisorba officinalis</em></td>
<td>Khorasan prov.: Kalat, 1505 m, Shahi; Gilan prov.: Asalem to Khalkhal, 2200 m, Faghir</td>
<td>5302 (GUH); 5303 (GUH)</td>
</tr>
<tr>
<td><em>S. minor</em> subsp. <em>minor</em></td>
<td>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 &amp; Dailamy</td>
<td>24117 (TEH); 19254 (TEH); 5300 (GUH)</td>
</tr>
<tr>
<td><em>S. minor</em> subsp. <em>lasiocarpa</em></td>
<td>Azarbaijan prov.: Mishoodagh, 1840 m, Ghahreman; Kordestan prov.: Sanandaj, Kerdaneh, Shamshiri</td>
<td>9297 (TEH); 5301 (GUH)</td>
</tr>
<tr>
<td><em>S. minor</em> subsp. <em>muricata</em></td>
<td>Kerman prov.: Kohgar, Mirtajedini; Mazandaran prov.: Karaj to Chaloos road, Pol-e Zangooleh, 300 m, Nazarian</td>
<td>29493 (TEH); 33151 (TEH)</td>
</tr>
</tbody>
</table>
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: Echinate 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 I: 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 Agrimonieae 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 Agrimonieae: 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.
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).

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

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


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 stright long hairs and lacking curve trichomes; *Aremonia agrimonoides* possesses stright 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, verucate and granulates. This diversity is also a good tool for identification purpose (Eriksen & Yurtsev 1999), especially at species (echinate in *Aremonia*...
agrimonioides, verrucate in Sanguisorba officinalis), and subspecies levels (transversely elongated papilla in A. eupatoria subsp. eupatoria; both transversely rided 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 Agrimonieae. The current result also supports the diagnostic value of micromorphological characters of trichome and glandulare hairs trichome (Faghir et al. 2010, Eriksen & Yurtsev 1999).

Epicuticular wax data of the Iranian species of the tribe Agrimonieae 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 epicuticulare 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 Agrimoniniae 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 Agrimoniniae (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 sinuolate 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 Agrimonieae. 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.

Acknowledgements

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Key to the species and subspecies of subtribe Agrimoninae 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 Agrimoninae …………………………… 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 vcerucca ………………………………………………………………………………..……. 3
- Trichome stright and curved, with echinate and vcerucca ………………………………………………………………………………..……. 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 scarly 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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