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

Ahmad Reza Mehrabian¹, Masoud Sheidai¹ & Valeyollah Mozaffarian²

¹ Department of Plant Sciences, Faculty of biological sciences, Shahid Beheshti University, GC, Tehran, Iran, e-mail: a_mehrabian@sbu.ac.ir (corresponding author)
² Department of Botany, Research Institute of Forests and Rangelands, Tehran, 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 “shaggy 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,

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, puberulent or tomentose surface between the setae. The setae occasionally 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 bulbotracha* 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 (Kolarık & 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*.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Locality</th>
<th>Collector</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Onosma rostellata</em> Lehm.</td>
<td>Kurdistan, Bayangan, 1450 m</td>
<td>Mehrabian</td>
</tr>
<tr>
<td><em>Onosma orientale</em> L.</td>
<td>Khuzistan, Masjed Soleyman, Andika, 600 m.</td>
<td>Mozaffarian</td>
</tr>
<tr>
<td><em>Onosma albo-rosea</em> Fisch.</td>
<td>Kermanshah, Kerend-e Gharb, 45 km west of Kerend, Rijab, 900 m.</td>
<td>Lashkar &amp; Hatami</td>
</tr>
<tr>
<td><em>Onosma armena</em> DC.</td>
<td>Azarbijan, Maku to Khoy, 2400 m</td>
<td>Assadi &amp; Mozaffarian</td>
</tr>
<tr>
<td><em>Onosma bilabiata</em> Boiss.</td>
<td>Azarbijan, southern slopes of Mt Sahand, 2300–2600 m</td>
<td>Assadi &amp; Mozaffarian</td>
</tr>
<tr>
<td><em>Onosma dayytricha</em> Boiss.</td>
<td>Gohkilooye va Boyer Ahmad: 11 km from Dogonbadan to Ghoram (VPI), 1400 m</td>
<td>Assadi &amp; Abohamzeh</td>
</tr>
<tr>
<td><em>Onosma hebebulba</em> DC.</td>
<td>Azarbijan, 45 km Kerend, Dalahoo, 2000 m</td>
<td>Assadi</td>
</tr>
<tr>
<td><em>Onosma iranshahrii</em> Gahreman &amp; Attar.</td>
<td>Kurdistan, Marivan to Paveh, Gardan-e Tat, between Dezli and Hanigarmaleh,</td>
<td>Mozaffarian</td>
</tr>
<tr>
<td><em>Onosma kurdica</em> Tepnner</td>
<td>Kurdistan in Mt Hamzehe Arab SE Bijar, 2600 m</td>
<td>Lamond, Termeh &amp; Rechinger</td>
</tr>
<tr>
<td><em>Onosma rasychaena</em> Boiss.</td>
<td>Zanjan, Mahneshan, Angoran Protected Area, Mt Belgehis, 2700 m</td>
<td>Mehrabian</td>
</tr>
<tr>
<td><em>Onosma elwendica</em> Wettst.</td>
<td>Tehran, Lashkarakar, 1900 m</td>
<td>Mehrabian</td>
</tr>
<tr>
<td><em>Onosma Gauvae</em> Bornm.</td>
<td>Tehran, Damavand, Chenar to Tar, 2500 m</td>
<td>Mozaffarian</td>
</tr>
<tr>
<td><em>Onosma nervosa</em> H.Riedl.</td>
<td>Esfahan, Ferydonshah, Mt Venizan, 2500 m</td>
<td>Mozaffarian</td>
</tr>
<tr>
<td><em>Onosma stenosiphost</em> Boiss.</td>
<td>Kerman, Mt Chopar</td>
<td>Kani</td>
</tr>
<tr>
<td><em>Onosma longiloba</em> Bunge.</td>
<td>Semnan, 20 km Mohamad Abad from Firduzkuh,</td>
<td>Pahlevani</td>
</tr>
<tr>
<td><em>Onosma asperrima</em> Bornm.</td>
<td>Fars, Nurabad: Doshman-Zari Region, Ab Zalu village, Kuhe Tasak, 1900–2500 m</td>
<td>–</td>
</tr>
<tr>
<td><em>Onosma dichroantha</em> Boiss.</td>
<td>Golestan, Golestan National Park, 1500 m</td>
<td>Heidari, Ghorbani &amp; Habibi</td>
</tr>
<tr>
<td><em>Onosma microcarpa</em> DC.</td>
<td>Markazi, Arak, Gavar, 2000 m</td>
<td>Mehrabian</td>
</tr>
<tr>
<td><em>Onosma macrophylla</em> Bornm.</td>
<td>Kermanshah, Malavi to Eslam Abad, 1200 m</td>
<td>Mozaffarian</td>
</tr>
<tr>
<td><em>Onosma pachypoda</em> Boiss.</td>
<td>Azarbijan, Yam, Misha Dagh Mt., 1800 m</td>
<td>Mehrabian</td>
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<tr>
<td><em>Onosma platyphylla</em> H.Riedl.</td>
<td>Hamedan, Nahavand, on the road to Nurabad, above Gamasiab, Kuh-e Garin, 2500 m</td>
<td>–</td>
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<tr>
<td><em>Onosma bodeana</em> Bornm.</td>
<td>Tehran, Sohanak, 2200 m</td>
<td>Mehrabian</td>
</tr>
<tr>
<td><em>Onosma bulbotricha</em> DC.</td>
<td>Zanjan to Mahneshan, 2200 m</td>
<td>Mehrabian</td>
</tr>
<tr>
<td><em>Onosma cornuta</em> H.Riedl.</td>
<td>Kurdistan, Bijar to Takab, 1600 m,</td>
<td>Mehrabian</td>
</tr>
<tr>
<td><em>Onosma kiloyensis</em> Boiss. &amp; Hausskn.</td>
<td>Khuzeast, Dehdez, Kuh Sefid, 2700 m</td>
<td>Mozaffarian</td>
</tr>
<tr>
<td><em>Onosma kotschyi</em> Boiss.</td>
<td>Fars, south of Estabbanat, 2200 m</td>
<td>Mozaffarian</td>
</tr>
<tr>
<td><em>Onosma olivieri</em> Boiss.</td>
<td>Kermanshah, Iranshahr &amp; Zargani, 1500 m</td>
<td>Mehrabian</td>
</tr>
<tr>
<td><em>Onosma sabalanica</em> Ponert</td>
<td>Aerdabil, Meshkin Shahr, Mt Sabalan, 2900 m</td>
<td>Mehrabian</td>
</tr>
<tr>
<td><em>Onosma sericea</em> Willd.</td>
<td>Kurdistan, Sanandaj, Abidar, 1730 m</td>
<td>Mehrabian</td>
</tr>
<tr>
<td><em>Onosma straussii</em> H.Riedl.</td>
<td>Markazi, Arak, Gavar, 2178 m</td>
<td>Mehrabian</td>
</tr>
<tr>
<td><em>O. subsericea</em> Freyn</td>
<td>Arzabil, Khalkhal to Givi, 1800 m,</td>
<td>Wendelbo &amp; Assadi</td>
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</tbody>
</table>


<table>
<thead>
<tr>
<th>Species</th>
<th>Setae type (A)</th>
<th>Setae status (B)</th>
<th>Setae length (mm) (C)</th>
<th>Setae color (D)</th>
<th>Setae density (E)</th>
<th>Tubercle pili length (mm) (F)</th>
<th>Tubercle pili density (G)</th>
<th>Tubercle diameter (mm) (H)</th>
<th>Small pili density (I)</th>
<th>Setae ornamentation diameter (μm) (J)</th>
<th>Nectar pili (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Onosma rostellata</em> Lehm.</td>
<td>I</td>
<td>Pa</td>
<td>1.37</td>
<td>white</td>
<td>M</td>
<td>0.27</td>
<td>M</td>
<td>11.96</td>
<td>–</td>
<td>–</td>
<td>3.42–7.77</td>
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<td></td>
<td></td>
<td></td>
<td>1.01–2.36</td>
<td></td>
<td></td>
<td>0.10–0.45</td>
<td></td>
<td>9.32–15.44</td>
<td>–</td>
<td>–</td>
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<tr>
<td><em>Onosma orientale</em> L.</td>
<td>I</td>
<td>Pa</td>
<td>0.64</td>
<td>white</td>
<td>M</td>
<td>0.09</td>
<td>M</td>
<td>4.45</td>
<td>–</td>
<td>–</td>
<td>3.42–7.77</td>
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<td></td>
<td></td>
<td></td>
<td>0.50–0.72</td>
<td></td>
<td></td>
<td>0.06–0.15</td>
<td></td>
<td>3.42–7.77</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
<td><em>Onosma longiloba</em> Bunge</td>
<td>I</td>
<td>Pa</td>
<td>1.59</td>
<td>white</td>
<td>M</td>
<td>0.14</td>
<td>D</td>
<td>12.67</td>
<td>+</td>
<td>–</td>
<td>8.81–13.97</td>
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<td></td>
<td></td>
<td></td>
<td>0.98–1.90</td>
<td></td>
<td></td>
<td>0.10–0.20</td>
<td></td>
<td>8.81–13.97</td>
<td>–</td>
<td>–</td>
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<tr>
<td><em>Onosma platyphylla</em> H. Riedl.</td>
<td>I</td>
<td>Pa</td>
<td>1.26</td>
<td>white</td>
<td>M</td>
<td>0.53</td>
<td>–</td>
<td>9.31</td>
<td>–</td>
<td>–</td>
<td>5.61–13.15</td>
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<td></td>
<td></td>
<td></td>
<td>0.74–2.13</td>
<td></td>
<td></td>
<td>0.35–0.76</td>
<td></td>
<td>5.61–13.15</td>
<td>–</td>
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<tr>
<td>Species</td>
<td>Setae type</td>
<td>Setae status</td>
<td>Setae length (mm)</td>
<td>Setae color</td>
<td>Setae density</td>
<td>Setae ornamentation diameter (μm)</td>
<td>Nectar pili</td>
<td>Small pili density (μm)</td>
<td>Tubercle diameter (mm)</td>
<td>Tubercle pili density (μm)</td>
<td>Tubercle pili length (mm)</td>
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<tr>
<td>Onosma bulbotricha DC.</td>
<td>I Pa</td>
<td>1.32</td>
<td>white</td>
<td>M</td>
<td>0.37</td>
<td>M</td>
<td>8.99</td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>Onosma dichroantha Boiss.</td>
<td>I Pa</td>
<td>1.09</td>
<td>white</td>
<td>M</td>
<td>0.28</td>
<td>M</td>
<td>7.04</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onosma microcarpa DC.</td>
<td>I Pa</td>
<td>1.15</td>
<td>White–yellow</td>
<td>M</td>
<td>0.30</td>
<td>M</td>
<td>8.15</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onosma sabalancia Ponert</td>
<td>I Pa</td>
<td>0.95</td>
<td>White–yellow</td>
<td>D</td>
<td>0.07</td>
<td>M</td>
<td>5.06</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Onosma katschyi Boiss.</td>
<td>I Pa</td>
<td>1.11</td>
<td>white</td>
<td>M</td>
<td>0.17</td>
<td>M</td>
<td>9.85</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onosma asperrima Bornm.</td>
<td>I Pa</td>
<td>0.97</td>
<td>white</td>
<td>M</td>
<td>0.44</td>
<td>–</td>
<td>20.60</td>
<td>–</td>
<td></td>
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<tr>
<td>Onosma corruita H.Riedl.</td>
<td>I Pa</td>
<td>1.51</td>
<td>grey</td>
<td>D</td>
<td>1.22</td>
<td>M</td>
<td>13.62</td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>Onosma sericea Willd.</td>
<td>I Ad</td>
<td>0.77</td>
<td>grey</td>
<td>M</td>
<td>0.08</td>
<td>M</td>
<td>5.36</td>
<td>–</td>
<td></td>
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<tr>
<td>Onosma subsericea Freyn</td>
<td>I Ad</td>
<td>1.21</td>
<td>grey</td>
<td>M</td>
<td>0.17</td>
<td>D</td>
<td>8.05</td>
<td>–</td>
<td></td>
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<tr>
<td>Onosma bodeana Bornm.</td>
<td>I Ad</td>
<td>0.77</td>
<td>grey</td>
<td>M</td>
<td>0.93</td>
<td>–</td>
<td>absent</td>
<td>–</td>
<td></td>
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<tr>
<td>Onosma pachypoda Boiss.</td>
<td>I Ad</td>
<td>0.90</td>
<td>white</td>
<td>M</td>
<td>0.10</td>
<td>M</td>
<td>13.26</td>
<td>–</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Onosma kilouyensis Boiss.</td>
<td>I Pa</td>
<td>1.59</td>
<td>white</td>
<td>M</td>
<td>0.37</td>
<td>L</td>
<td>11.34</td>
<td>–</td>
<td></td>
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<td></td>
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<tr>
<td>Onosma gauiae Bornm.</td>
<td>I Pa</td>
<td>1.04</td>
<td>white</td>
<td>M</td>
<td>0.19</td>
<td>D</td>
<td>12.24</td>
<td>–</td>
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<tr>
<td>Onosma macrophyllum Bornm.</td>
<td>II Ad</td>
<td>0.16</td>
<td>white</td>
<td>M</td>
<td>0.25</td>
<td>D</td>
<td>6.85</td>
<td>–</td>
<td></td>
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<td></td>
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<tr>
<td>Onosma ehwatica Wettst.</td>
<td>II Ad</td>
<td>1.18</td>
<td>white–yellow</td>
<td>D</td>
<td>0.50</td>
<td>D</td>
<td>10.20</td>
<td>–</td>
<td></td>
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<tr>
<td>Onosma oliveri Boiss.</td>
<td>II Ad</td>
<td>1.07</td>
<td>yellow</td>
<td>M</td>
<td>0.39</td>
<td>M</td>
<td>7.41–13.96</td>
<td>–</td>
<td></td>
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<tr>
<td>Onosma nervosa H.Riedl.</td>
<td>II Ad</td>
<td>0.87</td>
<td>yellow–white</td>
<td>M</td>
<td>0.26</td>
<td>D</td>
<td>6.19</td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>Onosma stenosiphon Boiss.</td>
<td>II Ad</td>
<td>1.05</td>
<td>white</td>
<td>M</td>
<td>0.37</td>
<td>M</td>
<td>12.31</td>
<td>–</td>
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<tr>
<td>Onosma paboti H.Riedl.</td>
<td>II Ad</td>
<td>0.92</td>
<td>white</td>
<td>M</td>
<td>0.58</td>
<td>–</td>
<td>8.52–11.64</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onosma straussi H.Riedl.</td>
<td>II Ad</td>
<td>1.34</td>
<td>white</td>
<td>M</td>
<td>0.11</td>
<td>M</td>
<td>10.44</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onosma dasytricha Boiss.</td>
<td>III Ad</td>
<td>1.30</td>
<td>grey</td>
<td>D</td>
<td>0.10</td>
<td>M</td>
<td>8.90</td>
<td>+</td>
<td></td>
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<tr>
<td>Onosma albo–rosea Fisch.</td>
<td>III Pa</td>
<td>1.36</td>
<td>grey</td>
<td>D</td>
<td>0.18</td>
<td>L</td>
<td>16.47</td>
<td>–</td>
<td></td>
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<tr>
<td>Onosma armena DC.</td>
<td>III Pa</td>
<td>1.13</td>
<td>white</td>
<td>D</td>
<td>0.52</td>
<td>M</td>
<td>19.38</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onosma rasychaena Boiss.</td>
<td>III Pa</td>
<td>1.51</td>
<td>white</td>
<td>M</td>
<td>0.24</td>
<td>D</td>
<td>14.13</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Onosma kurdica Teppner</td>
<td>III Pa</td>
<td>1.10</td>
<td>white</td>
<td>M</td>
<td>160.72</td>
<td>M</td>
<td>absent</td>
<td>–</td>
<td></td>
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<td></td>
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<tr>
<td>Onosma bilabita Boiss.</td>
<td>III Pa</td>
<td>1.17</td>
<td>white</td>
<td>M</td>
<td>0.18</td>
<td>M</td>
<td>8.81</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Onosma transshahri Ghahreman &amp; Attar.</td>
<td>III Pa</td>
<td>0.99</td>
<td>white</td>
<td>M</td>
<td>0.13</td>
<td>D</td>
<td>6.64–10.40</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onosma hebebulba DC.</td>
<td>III Pa</td>
<td>1.30</td>
<td>white</td>
<td>M</td>
<td>0.19</td>
<td>M</td>
<td>9.32</td>
<td>–</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Note:** The data includes species names, setae characteristics such as length, density, color, and ornamentation, and additional indices like tubercle and small pili diameters, as well as nectar pili information.
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 tubercle base of some taxa. In the nectar ring, there are short and white pili only. Setae vary between adpressed 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 glabrous tubercules at the base. Dense or sparse papilae cover the setae surface. This type occurs in sect. Protonosma, sect. Podonosma and sect. Onosma subssect. Haplotricha. 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 adpressed (O. sericeum, bodeana, O. pachypodum) to patent (O. longiloba, O. microcarpa).

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

Setae consist of glabrous tubercles at the base and dense or sparse papilae on their surface. The mentioned type occurs in sect. Onosma subssect. 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. pabotii) 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. pabotii). 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 adpressed.

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 subssect. Asterotricha. Type III represents sparse (O. armena, O. rascheyana), adpressed 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). Pili predominately 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.

1-3: O. elwendica

4-6: O. bulbotricha

7-9: O. rostellata

10-12: O. orientalis
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.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Large setae with simple base (absence of short trichomes at base)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Large setae with sparse and asteroidal pilies at base</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Setae hooked</td>
<td>sect. Podonosma</td>
</tr>
<tr>
<td></td>
<td>Setae unhooked</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Anthers connected along the side</td>
<td>sect. Protonosma</td>
</tr>
<tr>
<td></td>
<td>Anthers connected at base or free</td>
<td>sect. Onosma Subsect. Haplotricha</td>
</tr>
<tr>
<td>4</td>
<td>Large setae with small sparse pilies</td>
<td>sect. Onosma Subsect. Heterotricha</td>
</tr>
<tr>
<td></td>
<td>Large setae with asteroidal pilies</td>
<td>sect. Onosma Subsect. Asteroricha</td>
</tr>
</tbody>
</table>

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

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Setae glabrous at base (absence of short pilies at base)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Large setae with sparse or asteroidal pilies at base</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>Setae hooked and loose (Sect. Podonosma)</td>
<td>O. orientale</td>
</tr>
<tr>
<td></td>
<td>Setae unhooked</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Anthers connected along the side</td>
<td>O. rostellata</td>
</tr>
<tr>
<td></td>
<td>Anthers connected at base or free</td>
<td>(subsect. Haplotricha)</td>
</tr>
<tr>
<td>4</td>
<td>Setae adpressed and grey</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Setae patent and white or whitish–yellow</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>Nectar ring with small pilies</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Glabrous nectar ring</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>Dense pilies, tubercles small. Corolla violet; corolla lobe up to 5 mm</td>
<td>O. longiloba</td>
</tr>
<tr>
<td></td>
<td>Scattered pilies, tubercles large. Corolla pale–yellow; corolla lobe up to 2 mm</td>
<td>O. bulbiflora</td>
</tr>
<tr>
<td>7</td>
<td>Setae whitish–yellow</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Setae white</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>Setae dense. Leaves lanceolate–linear. Calyx 15–17 mm long</td>
<td>O. sabalantica</td>
</tr>
<tr>
<td></td>
<td>Setae sparse. Leaves spatulate. Calyx 8–12 mm long</td>
<td>O. microcarpa</td>
</tr>
<tr>
<td>9</td>
<td>Small hairs on leaf surface present</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Small hairs on leaf surface absent</td>
<td>11</td>
</tr>
<tr>
<td>10</td>
<td>Corolla campanulate–tubular, 25–40 mm.</td>
<td>O. dichroantha</td>
</tr>
<tr>
<td></td>
<td>Corolla tubular, 8–11 mm.</td>
<td>O. kotsyi</td>
</tr>
<tr>
<td>11</td>
<td>Tubercles large. Anthers exserted</td>
<td>O. platyphylla</td>
</tr>
<tr>
<td></td>
<td>Tubercles small. Anthers inserted</td>
<td>O. asperrima</td>
</tr>
<tr>
<td>12</td>
<td>Pilies absent. Corolla up to 30 mm long</td>
<td>O. bodana</td>
</tr>
<tr>
<td></td>
<td>Pilies present</td>
<td>13</td>
</tr>
<tr>
<td>13</td>
<td>Setae patent. Nectar ring with pilies, levaes obovate–lanceolate</td>
<td>O. cornuta</td>
</tr>
<tr>
<td></td>
<td>Setae adpressed</td>
<td>14</td>
</tr>
<tr>
<td>14</td>
<td>Very dense pilies. Calyx linear. Leaves linear</td>
<td>O. subsericca</td>
</tr>
<tr>
<td></td>
<td>Pilies of medium density. Calyx wide. Leaves lanceolate</td>
<td>O. serica</td>
</tr>
<tr>
<td>15</td>
<td>Tubercles with sparse pilies</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Tubercles with asteroidal pilies</td>
<td>22</td>
</tr>
<tr>
<td>16</td>
<td>Nectar ring with pilies. Setae yellow</td>
<td>O. olivieri</td>
</tr>
<tr>
<td></td>
<td>Nectar ring glabrous</td>
<td>17</td>
</tr>
<tr>
<td>17</td>
<td>Setae yellow. Inflorescence paniculate</td>
<td>O. nervosa</td>
</tr>
<tr>
<td></td>
<td>Setae white</td>
<td>18</td>
</tr>
<tr>
<td>18</td>
<td>Setae of medium density. Corolla yellowish–blue</td>
<td>O. elwendica</td>
</tr>
<tr>
<td></td>
<td>Setae dense. Corolla yellow to red</td>
<td>19</td>
</tr>
<tr>
<td>19</td>
<td>Dense tubercle pilies. Corolla purple to pink. Inflorescens with apical cyme</td>
<td>O. straussii</td>
</tr>
<tr>
<td></td>
<td>Tubercle pilies of low density</td>
<td>20</td>
</tr>
<tr>
<td>20</td>
<td>Radial hairs of low density. Leaves spatulate. Corolla clavate– campanulate</td>
<td>O. paboti</td>
</tr>
<tr>
<td></td>
<td>Tubercle pilies of medium density</td>
<td>21</td>
</tr>
<tr>
<td>21</td>
<td>Annulus pilose. Inflorescence capitulate. Corolla violet, 7–9 mm</td>
<td>O. stenosiphon</td>
</tr>
<tr>
<td></td>
<td>Annulus glabrous. Corolla yellow. Inflorescence laxe paniculate. Corolla yellow, 17–19 mm</td>
<td>O. macrophylla</td>
</tr>
</tbody>
</table>
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 pilus 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 ≤0.64 mm), while they are longer in other sections (setae length ≥ 0.92 mm). 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 pilus 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 adpressed to patent and can be used for species differentiation in some similar species. For example, in O. dasytricha (adpressed 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 iso-

Table 4. Continuation.

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<tbody>
<tr>
<td>22</td>
<td>Setae grey</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Setae adpressed. Leaves obovate–lanceolate</td>
<td>O. dasytricha</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Setae sparse. Leaves lanceolate–obleng</td>
<td>O. albo–rosea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Piles absent. Corolla 16–20 mm. Leaves spathulate</td>
<td></td>
<td>O. armena</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Piles of medium density. Corolla 20–25 mm long. Leaves linear to lanceolate</td>
<td>O. kurdica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Dense tubercle pilus. Annulus pilose. Corolla 25–30 mm long</td>
<td>O. iranshahri</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Corolla 20 mm. Calyx elongated into fruit, up to 27 mm long</td>
<td>O. bilabiata</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corolla up to 15 mm. Calyx not elongated.</td>
<td>O. hebebulba</td>
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</table>
tion between *O. rostellata* and the other *Onosma* taxa. Analysis of the pollen characters in some Iranian *Onosma* (Mehrabian & al. 2012) has shown high differentiation between *O. rostellata* and the other *Onosma* species. However, trichome studies (recent study) did not show obvious differentiation 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 subsection. *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; Vouillamo 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*. Kolarčik & 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.

**Acknowledgement.** The authors would like to thank Prof. Dr. Vitek and the personnel of the Botany Department of the Natural History Museum of Wien for their help. The authors are also very grateful to Prof. Dr. Till, Head of the Wien University Herbarium, and to Dr. Amini Rad and Mr. Pahlevani from the Iran Herbarium.

**References**


