

## **Trichome morphology of eleven genera of the tribe Alysseae (Brassicaceae) occurring in Bulgaria**

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MINČO ANČEV & VALENTINA GORANOVA

## Trichome morphology of eleven genera of the tribe *Alysseae* (*Brassicaceae*) occurring in Bulgaria

### Abstract

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The leaf and silicule trichomes of 18 species of *Alysseae* were studied by scanning electron and light microscopy. Four trichome types are distinguished: (1) simple, (2) stalked 2- to 5-armed, (3) stellate and (4) dendritic trichomes. (1) Simple trichomes cover the leaves of *Lunaria rediviva* and, mixed with stalked 2- to 3-armed ones, compose the leaf indumentum of *Camelina sativa*. The simple trichomes on the silicules of *Clypeola jonthlaspi* subsp. *microcarpa* are columnar with transverse furrows. (2) Stalked 2-5-armed trichomes are present on the leaves of *Draba korabensis*, *D. muralis*, *D. siliquosa* and *Erophila verna* subsp. *spatulata*. Peculiar stalked trichomes with swollen globular base occur on the lower leaf surface of *D. muralis*. (3) Stellate trichomes cover the leaves of *Alyssoides utriculata* subsp. *bulgarica*, *Aurinia saxatilis* subsp. *orientalis*, *A. uechtritzi* and *Clypeola jonthlaspi* and the leaves and silicules of *Alyssum cuneifolium* s. str., *A. orbelicum*, *A. pirinicum* and *Fibigia clypeata*. (4) Dendritic trichomes co-occur with stellate ones on the silicules (but rarely on the leaves) of *Berteroa* species and *Fibigia clypeata*. The trichomes may be smooth (some stellate trichomes), minutely warty (simple and dendritic trichomes) or tuberculate (most of the stellate trichomes). The trichomes of the morphologically related species *Alyssum pirinicum*, a new combination validated here, and *A. cuneifolium* provide additional reliable features to distinguish them.

Key words: angiosperms, *Cruciferae*, indumentum, micromorphology, scanning electron microscopy.

### Introduction

Trichome morphology has been used by many authors as a character in the classification of the *Brassicaceae* (Hayek 1925, Nyárády 1955, Dudley 1964, Greuter 1974, Rollins & Shaw 1973, Rollins & Banerjee 1976, Ančev 1991, Rollins 1993), especially in the tribe *Alysseae* (Janchen 1942, Al-Shehbaz 1987), where trichomes are widely present. In the Bulgarian flora *Alysseae* are represented by c. 45 species in 11 genera, the largest being *Alyssum* with 23 species. Scanning electron and light microscopy studies of the trichomes of 61 *Alyssum* species, including members of all its six sections (see Dudley 1964), distributed in Europe, SW Asia and the Mediterranean,

demonstrated a large morphological diversity at the species and the section level, as well as the presence of patterns of variability within these groups (Ančev 1991, 2000). The present study aims at expanding the knowledge of the trichome diversity in *Alyssum* and the remaining 10 genera of the tribe *Alysseae* in Bulgaria.

## Material and methods

The morphology of the trichomes on the lower leaf surface of 17 species of the tribe *Alysseae* was studied by scanning electron microscopy (SEM), using a Joel-JSM-35 at 20 kV, and in *Schivereckia doerfleri* by light microscopy. The trichomes were investigated on the basal (rosette) leaves or the lower cauline leaves. The indumentum of the upper leaf surface of all studied species was examined by light microscopy. In addition, the trichomes of the silicles in eight of the species were studied. Trichome description and classification follows the system of Theobald & al. (1979). The investigation is based on herbarium material deposited in BRA, SO and SOM (herbarium abbreviation according to Holmgren & Holmgren 1998-), as listed in Table 1.

Table 1. The examined specimens of the 18 investigated species from the tribe *Alysseae* (*Brassicaceae*); specimens used for SEM are marked by an asterisk (\*).

<i>Alyssoides utriculata</i> subsp. <i>bulgarica</i>	Bulgaria, Slavjanka Mt, 1300 m, 1973, <i>M. Ančev</i> (*SOM A3177)
<i>Alyssum cuneifolium</i>	Italy, Abruzzo, La Maiella, 2400-2800 m, 1899, <i>G. Rigo</i> (*BRA 3909)
<i>Alyssum orbelicum</i>	Bulgaria, Northern Pirin Mt, Banski Suhodol circus, 2400 m, 1995, <i>D. Uzunov</i> (*SOM 155156)
<i>Alyssum pirinicum</i>	Bulgaria, Northern Pirin Mt, Banski Suhodol circus, c. 2600 m, 1996, <i>D. Uzunov</i> & <i>M. Ančev</i> (*SOM 157156); <i>ibid.</i> , 1935, <i>N. Stojanov</i> (SOM 33605); <i>ibid.</i> , El-Tepe, 2915 m, 1939, <i>B. Achtarov</i> (SOM 33610); <i>ibid.</i> , 2500 m, 1980, <i>B. Kuzmanov</i> & <i>M. Ančev</i> (SOM 153913); <i>ibid.</i> , c. 1900 m, 1995, <i>D. Uzunov</i> & <i>M. Ančev</i> (SOM 155679).
<i>Aurinia saxatilis</i> subsp. <i>orientalis</i>	Bulgaria, Northern Struma valley, 1977, <i>M. Ančev</i> (*SOM A746)
<i>Aurinia uechtritziiana</i>	Bulgaria, Black Sea coast, Nesebar, 1980, <i>M. Ančev</i> (*SOM 147375)
<i>Berteroa incana</i>	Bulgaria, Rila Mt, near Semkovo, 1300 m, 1978, <i>M. Ančev</i> & <i>R. Todorova</i> (*SOM 137057)
<i>Berteroa mutabilis</i>	Bulgaria, Vitoša Mt, near Bosnek, 900 m, 1962, <i>N. Vihodcevski</i> (*SOM 105860)
<i>Berteroa obliqua</i>	Bulgaria, Strandja Mt, 1996, <i>S. Bančeva</i> & <i>Č. Gushev</i> (*SOM 155786)
<i>Camelina sativa</i>	Bulgaria, Momino Plato, 2004, <i>S. Stojanov</i> (*SOM 160266)
<i>Clypeola jonthlaspi</i> subsp. <i>microcarpa</i>	Bulgaria, Black Sea coast, between Sv. Nikola and Russalka, 1966, <i>V. Velčev</i> (*SOM 158169)
<i>Draba korabensis</i>	Bulgaria, Northern Pirin Mt, Dolen Kazan circus, 2003, <i>S. Stojanov</i> (*SOM 159929)
<i>Draba muralis</i>	Bulgaria, Zemenska Mt, 1997, <i>M. Ančev</i> (*SOM 154316)
<i>Draba siliquosa</i>	Bulgaria, Rila Mt, Čalovete, 1889, <i>S. Georgiev</i> (*SO 28560)
<i>Erophila verna</i> subsp. <i>spathulata</i>	Bulgaria, Black Sea coast, Nessebar, 1986, <i>M. Ančev</i> (*SOM A9622)
<i>Fibigia clypeata</i>	Bulgaria, Eastern Stara Planina, 1965, <i>V. Velčev</i> (*SOM 154447)
<i>Lunaria rediviva</i>	Bulgaria, Central Stara Planina, 750 m, 1998, <i>M. Ančev</i> (*SOM A9763)
<i>Schivereckia doerfleri</i>	Bulgaria, Central Stara Planina, Jumruk-čal, 1936, <i>B. Kitanov</i> (SO 96401)

Results and Discussion

*Lunaria rediviva* L.

*Leaf trichomes* (Fig. 1A) simple, rather minutely papillose, straight or curved, flattened in the lower part, rounded and tapering above, long on the midrib and veins, otherwise shorter with straight or hooked tips.

*Alyssoides utriculata* subsp. *bulgarica* (Sagorski) Hartvig

*Leaf trichomes* (Fig. 1B, C) stellate, tuberculate, angulate, with mounded, massive, coarse-tuberculate centre. Rays simple or once forked, with 6-7 subpatent, non-appressed, smooth tips.

The stellate leaf trichomes of this taxon are characteristic by their regularly subpatent, non-appressed rays and delimit it clearly from subsp. *utriculata* (Hartvig 2002).

*Alyssum cuneifolium* Ten.

*Leaf trichomes* (Fig. 1D, E) stellate, rounded, smooth or sparsely tuberculate, with narrow, slightly swollen centre. Rays 2(-3)-forked, with 22-23 tips, straight, tapering. *Silicule trichomes* (Fig. 1F, 2A) appressed, stellate, densely tuberculate, with narrow, mounded, coarsely tuberculate or almost smooth centre and massive primary arms at an angle of 35-45°. Rays 2(-3)-forked, with 16-18 tuberculate, mostly straight, tapering tips.

*Alyssum pirinicum* (Stoj. & Acht.) Ančev, **comb. & stat. nov.**

≡ *Alyssum cuneifolium* subsp. *pirinicum* Stoj. & Acht. in Izv. Carsk. Prir. Inst. Sofija 12: 184. 1939. – Lectotype (designated by Ančev 1991: 107): [Bulgaria] “In saxis marmoreis mt. Pirin cacumen El-Tepe” [Vihren], c. 2800 m, 9.8.1938, *N. Stojanov* (SOM 33609!).

Table 2. Trichome types of 18 *Alysseae* species on leaf (L) and silicule surface (S): Stalked = stalked 2-5-armed; Sta-gb = stalked 3-4-armed with globular base; stel-1 = appressed-stellate; stel-2 = stalked-stellate; den-1 = stalked with simple and forked branches, den-2 = terminally stellately branched; den-3 = fasciculately branched. The dominant types are in bold.

Taxon	Trichome types							
	Simple	Stalked	Sta-gb	Stellate		Dendritic		
				stel-1	stel-2	den-1	den-2	den-3
<i>Lunaria rediviva</i>	<b>L</b>	–	–	–	–	–	–	–
<i>Alyssoides utriculata</i>								
subsp. <i>bulgarica</i>	–	–	–	–	<b>L</b>	–	–	–
<i>Alyssum cuneifolium</i>	–	–	–	<b>L, S</b>	–	–	–	–
<i>A. pirinicum</i>	–	–	–	–	<b>L, S</b>	–	–	–
<i>A. orbelicum</i>	–	–	–	<b>L, S</b>	–	–	–	–
<i>Aurinia saxatilis</i>								
subsp. <i>orientalis</i>	–	–	–	–	<b>L</b>	–	–	–
<i>A. uechtritzi</i>	–	–	–	<b>L</b>	–	–	–	–
<i>Berteroa incana</i>	–	–	–	–	<b>L</b>	<b>S</b>	<b>L, S</b>	<b>S</b>
<i>B. mutabilis</i>	–	–	–	–	<b>L</b>	–	<b>S</b>	<b>S</b>
<i>B. obliqua</i>	–	–	–	–	<b>L</b>	–	<b>S</b>	<b>S</b>
<i>Fibigia clypeata</i>	–	–	–	L, S	L, S	L, S	–	–
<i>Clypeola jonthlaspi</i>								
subsp. <i>microcarpa</i>	<b>S</b>	–	–	<b>L</b>	–	–	–	–
<i>Draba korabensis</i>	–	<b>L</b>	–	–	–	–	–	–
<i>D. muralis</i>	<b>S</b>	–	<b>L</b>	–	–	–	–	–
<i>D. siliquosa</i>	–	<b>L</b>	–	–	–	–	–	–
<i>Erophila verna</i>								
subsp. <i>spathulata</i>	–	<b>L</b>	–	–	–	–	–	–
<i>Camelina sativa</i>	<b>L</b>	<b>L</b>	–	–	–	–	–	–
<i>Schivereckia doerfleri</i>	<b>S</b>	<b>L, S</b>	–	–	–	–	–	–

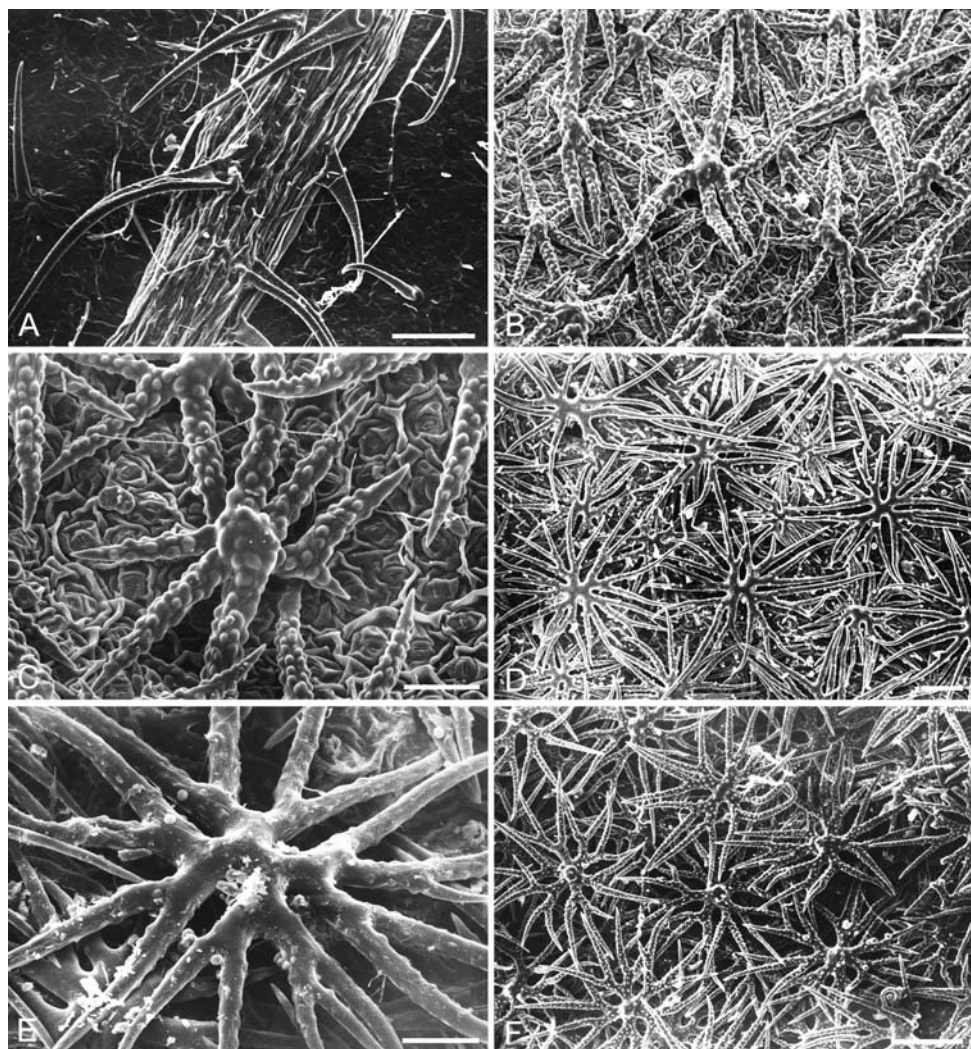


Fig. 1. Trichomes of *Lunaria*, *Alyssoides* and *Alyssum* species – A: *Lunaria rediviva*, simple trichomes on leaves; B-C: *Alyssoides utriculata* subsp. *bulgarica*, stellate trichomes on leaves (B), close up showing mounded centre (C); D-F: *Alyssum cuneifolium*, stellate trichome on leaves (D), close up of swollen centre (E), appressed stellate trichomes of silicules (F). – Scale bars: A, B, D, F = 100  $\mu$ m; C, E = 50  $\mu$ m.

*Leaf trichomes* (Fig. 2B, C) stellate, rounded and angulate, almost smooth to sparsely tuberculate, with narrow flat centre. Rays 1-3-forked, with 20-26 straight or curved and crooked tips, shorter than in *Alyssum cuneifolium*. *Silicule trichomes* (Fig. 2D, E) stellate, not appressed, at least some of them on short stalks, sparsely minutely tuberculate, with narrow flat centre and delicate primary rays at an angle of c. 60-70°. Rays 3-forked, with 16-22 smooth or almost smooth, straight or curved tips.

*Alyssum pirinicum* is a diploid local endemic with  $2n = 16$ , restricted to the alpine area of the N Pirin Mt (Ančev 1991). Stojanov & Achtarov (1939) distinguished it from *A. cuneifolium* at subspecies level, but both taxa are very well separated by their trichome morphology. Besides, *A. cuneifolium* is hexaploid with  $2n = 48$  (Küpfer 1974, for Italy), although Hartvig (2002) reported



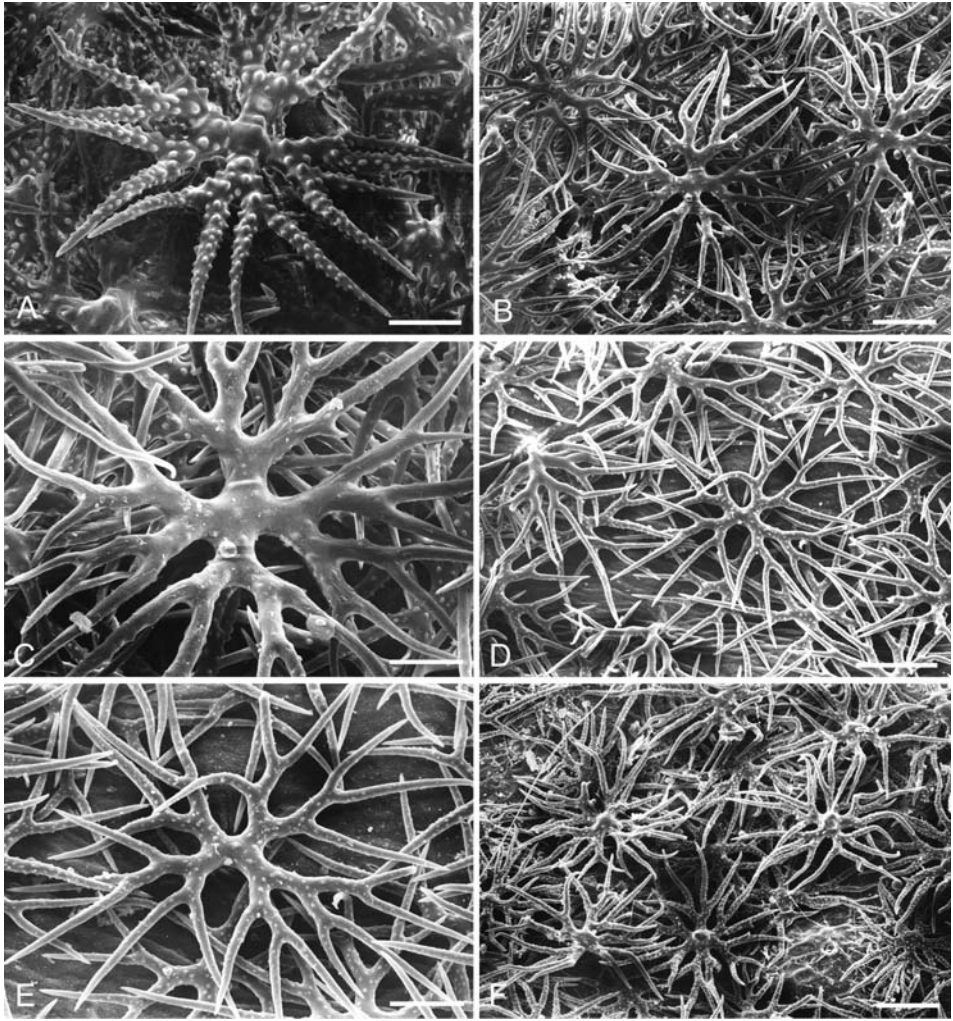


Fig. 2. Trichomes of *Alyssum* species – A: *A. cuneifolium*, stellate trichome on silicule surface, close up of mound, tuberculate centre; B-E: *A. pirinicum*, leaf indumentum of stellate trichomes (B), close up of flat centre (C), indumentum on silicule surface, with stalked, stellate trichome with ascending rays, at upper left (D), close up of stellate silicule trichome with narrow centre (E); F: *A. orbelicum*, leaf indumentum of stellate trichomes. – Scale bars: A, C, E = 50  $\mu$ m; B, D, F = 100  $\mu$ m.

$2n = 32$  with question mark from Greece. Jalas & al. (1996) treated *A. cuneifolium* subsp. *pirinicum* as a synonym of *A. scardicum*, a tetraploid species with  $2n = 32$ . *A. scardicum*, however, differs clearly from the diploid *A. pirinicum* in the indumentum of appressed, symmetric, rounded, stellate, densely tuberculate trichomes with 33-34 ultimate rays (Ančev 2000: fig. 6B).

***Alyssum orbelicum*** Ančev & Uzunov

*Leaf trichomes* (Fig. 2 F, 3A) stellate, rounded, densely minutely tuberculate, with narrow, swollen to umbonate centre. Rays long, often curved or crooked, with 16-23 tips. *Silicule trichomes* (Fig. 3B, C) with coarse, tuberculate, massive centre with large almost smooth umbo. Primary rays 10-12, short, partly fused at the base, with (16-)21-25 long, tapering, smooth tips, densely tuberculate.

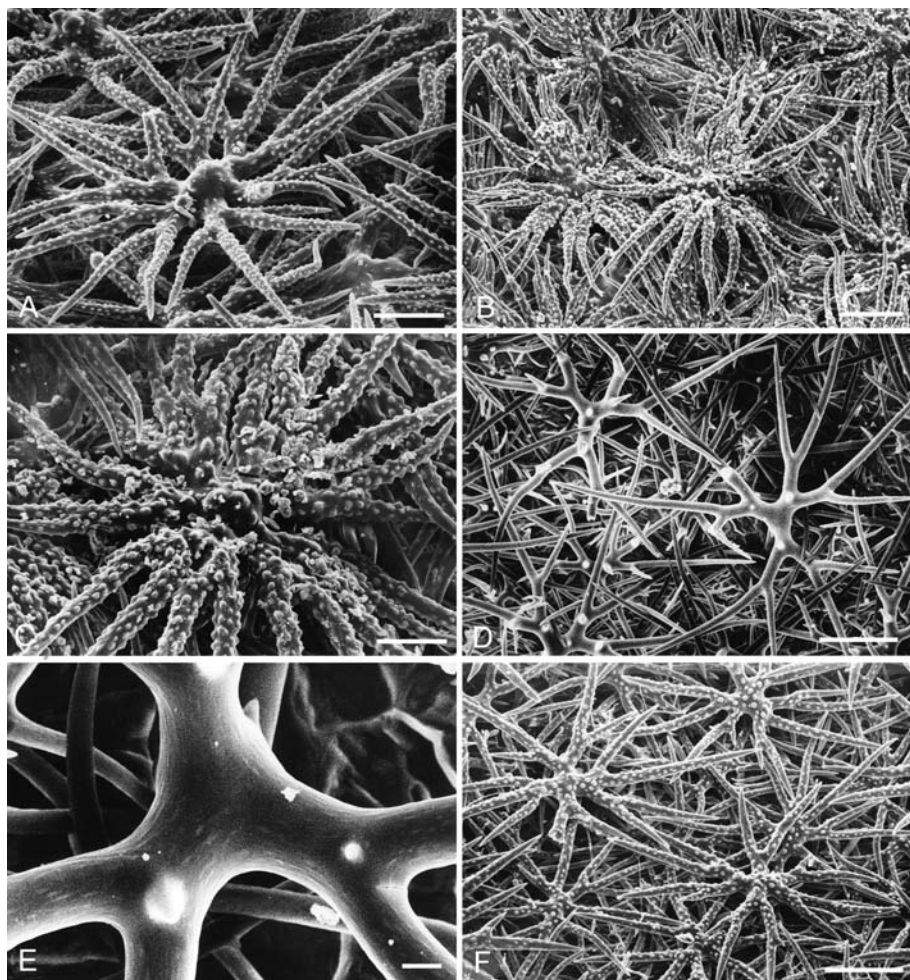


Fig. 3. Trichomes of *Alyssum* and *Aurinia* species – A-C: *Alyssum orbelicum*, stellate leaf trichome with umbonate centre (A), silicule indumentum of appressed, stellate trichomes (B), close up of massive centre of stellate trichome with large umbo and fused primary rays (C); D-E: *Aurinia saxatilis* subsp. *orientalis*, leaf indumentum of stellate trichomes with short, thorn-like side branches (D), close up central portion of leaf trichome (E); F: *A. uechtritiziana*, leaf indumentum of appressed, stellate trichomes. – Scale bars: A, C = 50 µm; B, D, F = 100 µm; E = 10 µm.

The coarse, tuberculate silicule trichomes marked by a massive centre and short, partly fused primary rays well delimit this species, in addition to the inflorescence and flower characters, from the related *A. nebrodensis* and *A. alpestris* (Ančev 2000, Ančev & Uzunov 2002).

*Aurinia saxatilis* subsp. *orientalis* (Ard.) T. R. Dudley

*Leaf trichomes* (Fig. 3D, E) not appressed, stellate, angulate, smooth. Rays slender, 1-2-forked, with short additional side branches and long, tapering tips.

*Aurinia uechtritiziana* (Bornm.) Cullen & T. R. Dudley

*Leaf trichomes* (Fig. 3F, 4A) appressed, stellate, tuberculate, rounded, with narrow, coarsely tuberculate, slightly swollen centre. Primary rays short, with 10-13 smooth tips.



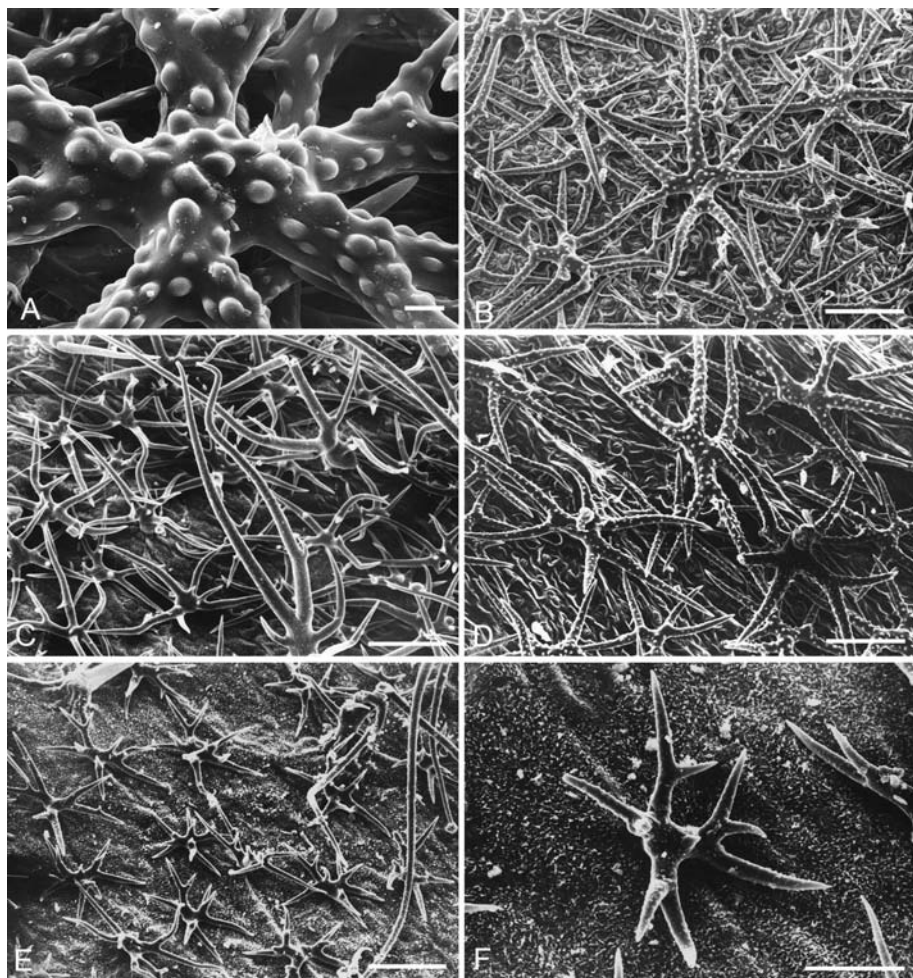


Fig. 4. Trichomes of *Aurinia* and *Berteroa* species – A: *Aurinia uechtritiziana*, close up of central portion of stellate trichome; B-C: *Berteroa incana*, stellate leaf trichomes mixed with smaller, short-stalked, terminally branched trichomes (B), silicule indumentum of branched trichomes with forked and simple branches, mixed with dendritic trichomes (C); D-F: *B. mutabilis*, leaf indumentum of stalked, 5- and 6-armed trichomes (D), silicule indumentum of short-stalked, terminally stellately branched trichomes (E), close up of stalked, stellately branched trichomes (F). – Scale bars: A = 10  $\mu$ m; B, C, D, E = 100  $\mu$ m; F = 50  $\mu$ m.

***Berteroa incana* (L.) DC.**

*Leaf trichomes* (Fig. 4B) stellate and dendritic. Stellate trichomes tuberculate, sessile or almost sessile, with wide, flat or slightly swollen centre; primary rays shortly forked, with 6-7, long, unequal tips, often with short side arms. Dendritic trichomes smaller, with unequal branches. *Silicle trichomes* (Fig. 4C) almost sessile or stalked, branched, with forked and simple branches, mixed with dendritic, minutely warty trichomes with long central and short side branches.

***Berteroa mutabilis* (Vent.) DC.**

*Leaf trichomes* (Fig. 4D) spaced, variable, 5- and 6-armed, tuberculate, stalked or almost sessile, with slightly swollen centre, sometimes approaching the stellate type. Primary arms short, forked, with 5-6 long, unequal tips. *Silicle trichomes* (Fig. 4E, F) stellate, mostly spaced, sessile, ter-



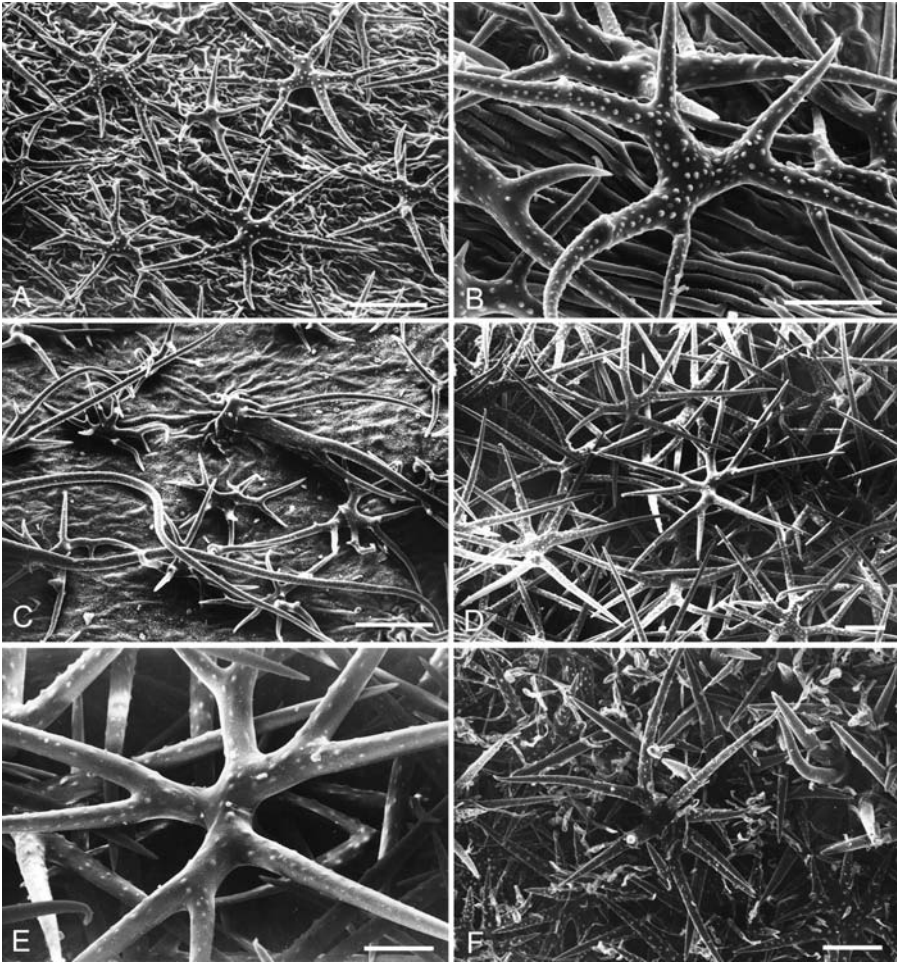


Fig. 5. Trichomes of *Berteroa* and *Fibigia* species – A-C: *Berteroa obliqua*, spaced, stellate trichomes on leaf surface (A), almost sessile stellate trichome with tuberculate surface (B), silicule indumentum of sessile and stalked trichomes (C); D-F: *Fibigia clypeata*, leaf indumentum of stellate trichomes (D), close up of stellate leaf trichome (E), silicule indumentum of simple, stellate trichomes, mixed with stalked ones with ascending rays, and dendritic ones (upper right) (F). – Scale bars: A, C, D, F: 100  $\mu$ m; B, E = 50  $\mu$ m.

minally branched to stellate-like branched with unequal arms and short side branches; among them some single, minutely warty, dendritic trichomes with long central and short side branches.

***Berteroa obliqua* (Sm.) DC.**

*Leaf trichomes* (Fig. 5A, B) stellate, sessile, minutely tuberculate, with slightly swollen centre. Primary rays short, forked, with 6-8 unequal tips, mixed with stalked, terminally branched trichomes with unequal arms. *Silicule trichomes* (Fig. 5C) of two types: almost sessile, terminally branched stellate trichomes with unequal arms, often with short side branches, mixed with single, minutely warty dendritic trichomes with long central and short side branches.

***Fibigia clypeata* (L.) Medik.**

*Fibigia clypeata* has a characteristic, heteromorphic leaf and silicule indumentum, composed of stellate, stellate-like and dendritic trichomes. *Leaf trichomes* (Fig. 5D, E) mostly stellate, moder-

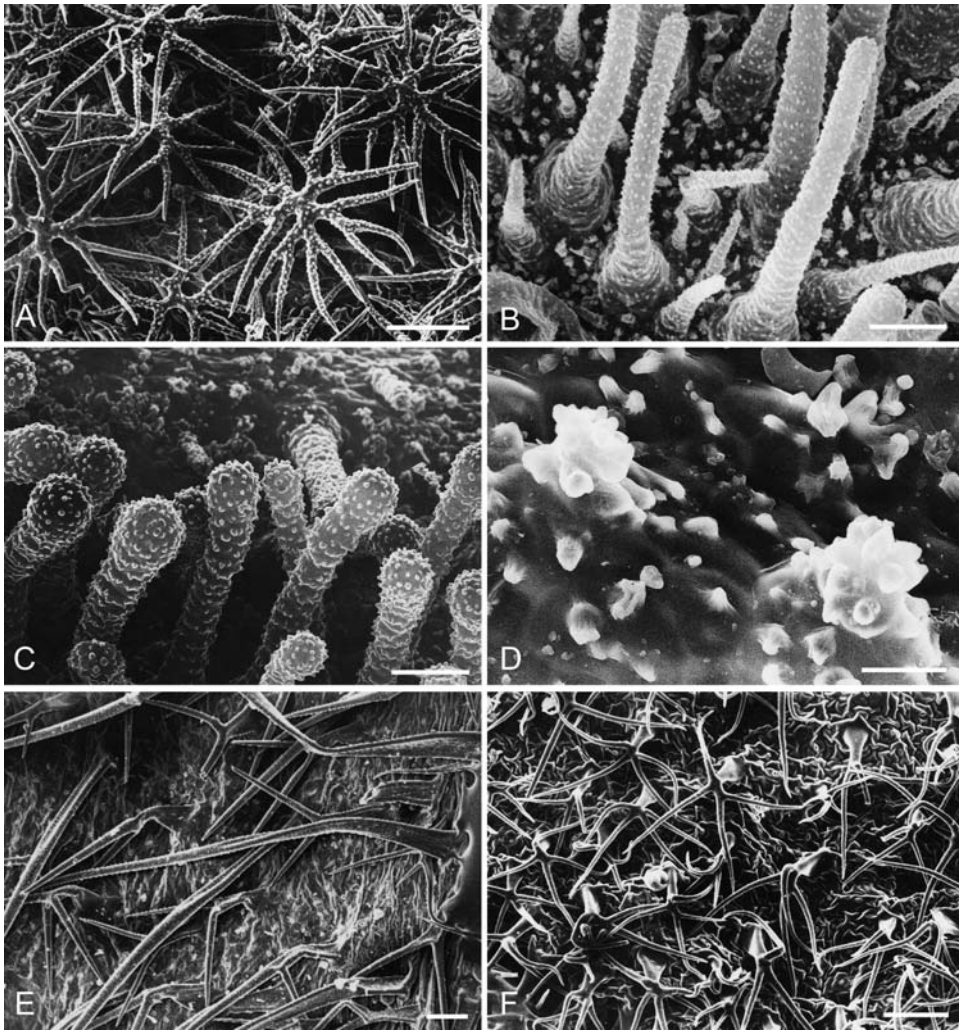


Fig. 6. Trichomes of *Clypeola*, *Camelina* and *Draba* species – A-D: *Clypeola jonthlaspi* subsp. *microcarpa*, stellate trichomes of leaf indumentum (A), simple, long columnar silicule trichomes (B), simple silicule trichomes with swollen tips (C), short papillae and tuberculate outgrowth on silicule surface (D); E: *Camelina sativa*, simple, long trichomes mixed with Y-shaped and stalked, 3-armed ones; F: *Draba korabensis*, stalked, (2-)3-4-armed trichomes on leaf surface. – Scale bars: A, B, F = 100  $\mu$ m; B, C = 50  $\mu$ m; D = 10  $\mu$ m.

ately to scarcely minutely tuberculate, with narrow, slightly swollen centre. Primary rays short, forked, with 6-9 long, unequal tips. Mixed with few dendritic trichomes (Fig. 8A). *Silicule trichomes* (Fig. 5F) simple, stellate, stalked or almost sessile with massive, simple rays and rays forked at a short distance from the centre, mixed with stalked stellate-like trichomes with thick, ascending, unequal, smooth rays and single, dendritic trichomes with smooth branches (Fig. 8B).

***Clypeola jonthlaspi* subsp. *microcarpa* (Moris) Arcang.**

*Leaf trichomes* (Fig. 6A) stellate, tuberculate, with narrow and slightly protruding cental part. Primary rays short, 1-2-forked and with 12-16 straight tips. *Silicule indumentum*: central part (Fig. 6B-D) covered by small papillae and coarse tuberculate outgrowths, mixed with dispersed,



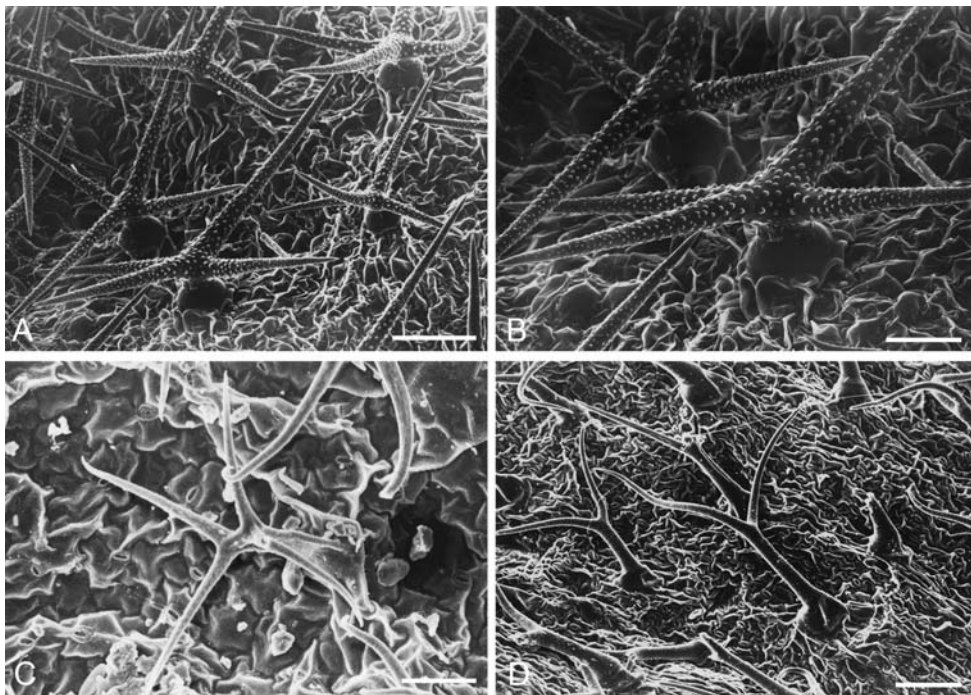


Fig. 7. Trichomes of *Draba* and *Erophila* species – A-B: *Draba muralis*, stalked, 4-armed leaf trichomes with swollen globular base; C: *D. siliquosa*, stalk 4-armed trichome with flattened stalk; D: *Erophila verna* subsp. *spathulata*, stalked Y-shaped trichomes on the leaf surface. – Scale bars: A, C, D = 100  $\mu$ m; B = 50  $\mu$ m.

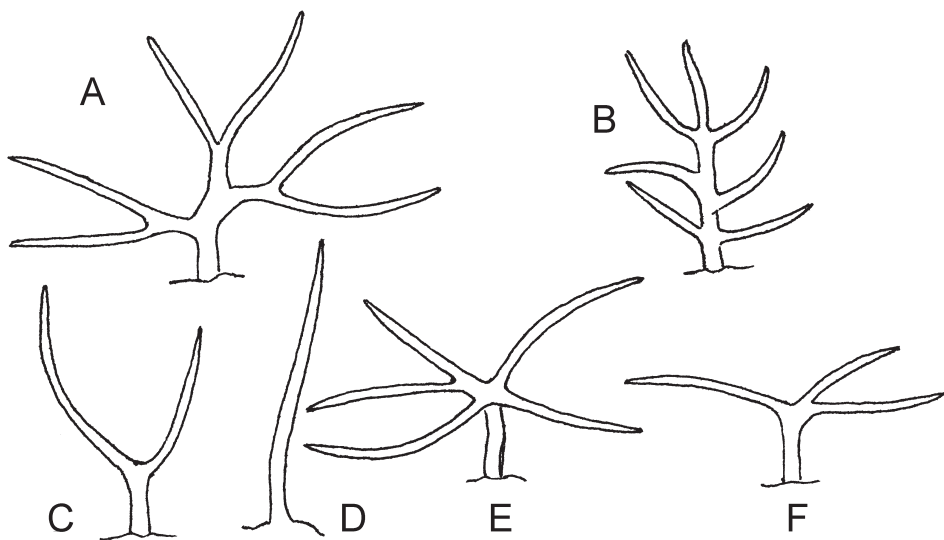


Fig. 8. A-B: *Fibigia clypeata*, dendritic leaf trichome (A), dendritic silicle trichome (B); C-F: *Schivereckia doerfleri*, stalked, Y-shaped trichome (C), simple trichome (D), stalked, 5-armed trichome (E), all from leaf indumentum; stalked, 3-armed silicle trichome (F). – Magnification: x 126.



short, simple trichomes with mounded surface with transversely furrowed appearance and rounded to swollen tips. Silicule wing trichomes long, rounded or clavate at the apex.

The presence of clavate trichomes on the silicules, few or very few on the disc and numerous on the wing, together with the spatulate leaves and smaller silicules, well delimit it from *C. jonthlaspi* subsp. *jonthlaspi*, with silicule disc and wing covered by rounded trichomes or such tapering into a sharp, long, simple point (Runemark 2002).

***Draba korabensis* Kümmerle & Degen**

*Leaf trichomes* (Fig. 6F) stalked (2-)3- to 4-armed, almost smooth or finely tuberculate, stalk flattened at the base, arms unequal, tapering, straight or slightly curved.

***Draba muralis* L.**

*Leaf trichomes of the lower surface* spaced, 3- and 4-armed with long, tuberculate, tapering arms and very short stalk on swollen globular base (Fig. 7A, B). *Trichomes of the leaf margin* simple and 2-armed, Y-shaped, and on *the upper leaf surface* stalked, (2-)3- to 4-armed, of the same type as in *D. korabensis* (Fig. 6F). Stalked trichomes with swollen globular base were not observed on the upper leaf surface.

The stalked trichomes with globular base enlarge the knowledge of the trichome diversity in *Brassicaceae* (Greuter 1974, Theobald & al. 1979, Inamdar & Rao 1983, Hewson 1988, Rollins & Banerjee 1976, 1979, Rollins 1993, Ančev 1991, 2000, Tan & Stevanović 2002). Plants from two other populations had stalked, 3-armed hairs without swollen globular base, the variability and distribution of the trichomes in *Draba muralis* thus requires further study.

***Draba siliquosa* M. Bieb.**

*Leaf trichomes* (Fig. 7C) stalked, 3- to 4-armed, minutely warty, with more or less flattened stalk with thickened margins; arms unequal, tapering, straight or with curved or hooked tips. Some of the trichomes with side branches along the stalk demonstrate a transition to the dendritic type.

***Erophila verna* subsp. *spathulata* (Láng) Walters**

*Leaf trichomes* (Fig. 7D) stalked, 2-armed, Y-shaped, mixed with 3-armed and very few 4-armed trichomes, with long, straight or curved, tapering arms with minutely warty surface.

***Camelina sativa* (L.) Crantz**

*Leaf trichomes* (Fig. 6E) spaced, simple, long, minutely tuberculate, flattened in the lower part, rounded and tapering above, mixed with shorter, stalked, 2-armed, Y-shaped, and stalked, 3-armed trichomes.

***Schivereckia doerfleri* (Besser) Andrž.**

*Leaf trichomes* (Fig. 8C, D, E) stalked, variable, mostly 4-armed, mixed with dispersed 3- or 5-armed ones. *Silicule trichomes* (Fig. 8F) spaced, short, simple, mixed with stalked, 2-armed Y-shaped and few sessile, dispersed, 3-armed trichomes.

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