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Pubescence of vegetative organs and trichome micromorphology in some Boraginaceae at different ontogenetic stages

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Summary: The paper presents the ontogenetic investigation of 16 herbaceous (annual, biennial and perennial) representatives of Boraginaceae. Special attention is paid to the pubescence of vegetative organs and to trichome micromorphology. The hairs have been revealed to be formed at early ontogenetic stages. Two hair types, represented by seven forms, as well as six types of pubescence were identified. Trichome surface ultrasculpture is used as an additional trait particularizing the pubescence characters. The correlation between the pubescence of vegetative organs and heteroeremocarpy, common for Boraginaceae, is noted for the first time. Within a species, some seasonal forms can change their pubescence type whereas other ones are stable and keep their types at all ontogenetic stages. Data proving the presence of cystolith-bearing hairs in Boraginaceae are given. Possible ways of and reasons for cystolith body formation are suggested. Pubescence studies in vegetative organs of virginile and definitive plants allowed the authors to reveal a huge diversity of trichomes and their stability not only at the species level but in some cases also within a different taxonomic rank (tribes, sections). Pubescence details can also be used for identifying species at early developmental stages or for recognizing seasonal forms within a species.

Keywords: Boraginaceae, *Asperugo, Symphytum, Borago, Lycopsis*, ontogeny, heterocarpy, pubescence, trichomes, cystoliths, anatomy, morphology, systematics

The sizeable family of Boraginaceae comprises about 115 genera with more than 2 500 species, distributed all around the world, primarily in tropical and subtropical and partly in northern temperate areas (Gürke 1893; Dobrochaeva 1981; Popov 1983). Among their life-forms, there are trees and shrubs as well as perennial and annual herbs. One of their most characteristic traits is a hard setose pubescence of the aerial shoots. Bristly hairs, spines and setae are usually located on tubercles ('rosettes') which are multi-celled epidermal outgrowths. Many species in the family possess other, softer trichomes of various shapes: one- or multi-celled simple or glandular hairs (Dobrochaeva 1981) in addition to the setae.

Starting with the works of Tournefort (1700), Linnaeus (1753) and Bieberstein (1808) up to mid- to late XIX century (De Candolle 1846; Boissier 1849, 1879; Ledebour 1849; Nyman 1854–1855, 1878–1882; Bentham & Hooker 1876), morphological research of Boraginaceae was done mainly for taxonomic purposes. Species diagnoses included, together with structural details of reproductive and vegetative organs, the pubescence of aerial shoots, however, only in mature plants. Thus, Sautkina & Khilko (2008) have compiled a dichotomous key to identify *Myosotis* species based on the pubescence and trichome types. Kruglov & Khanina (2008), who studied leaf micromorphology in some *Pulmonaria* species, have revealed five types of trichomes and their heterogenous location depending on the plant part. They have also discovered a correlation between leaf blade pubescence and a species' attribution to a certain series (*Molles*, *Asperae*). These are just a few examples showing pubescence as an important, taxonomically valuable trait. However, when studying the trichome diversity and location patterns, researchers almost exclusively limit themselves to the adult plant. There is only a rather small number of works concerning morphology of plants at early ontogenetic stages. Fragmentary data for some Boraginaceae can be found in papers of Vasilchenko (1953, 1965, 1979), Miroshnichenko (1954), Golubev (1957), Naida (1998), Nukhimovsky (1997), Pokrovskaya (1960) and Rysin & Rysina (1987). In particular, the authors pointed out the fact that some seedlings were pubescent not only on their first leaves, but also on cotyledons and the hypocotyl. Studying pubescence at early ontogenetic stages is of crucial importance and necessity and allows us to identify the species before the plant enters its reproductive stage.

Brief information on the trichome anatomy in Boraginaceae can be found in Metcalf & Chalk's reviews (1950) as well as in the works of Solereder (1899), Uphof (1962) and Wafaa (2006). They indicate the presence of unicellular hairs in most of the species, often they would be 'sitting' on cystolith-containing props. The latter are represented by 'rosettes' of epidermal cells with their walls impregnated by mineral substances and their protoplasts containing granular, layered or granular-layered groups of calcareous and siliceous compounds. Interesting data on trichome structure in seven species of the genus *Lithospermum* L., differing in their life forms, are presented by Yurison (1965). Leaves of annual monocarpic plants are usually covered by long and narrow hairs, cystoliths are poorly developed and the 'rosettes' are made of only one cell layer. On the contrary, leaves of biennial and perennial polycarpic species possess shorter hairs, well-developed cystoliths and multi-layered (up to 5 layers) 'rosettes'. Hair shapes and cystolith maturity can differ greatly from species to species. Aleykutty & Inamdar (1979) proposed to use these pubescence traits for taxonomic purposes. Lems & Holzapfel (1971) attempted to clarify interspecific connections within the genus *Echium* L. based on trichome types and cystolith structure.

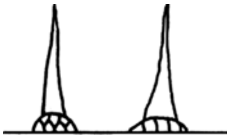
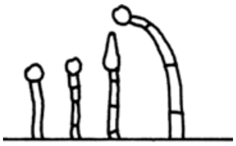
In relation to the above said, further studies on the peculiar pubescence characteristics of most Boraginaceae seem to be very promising. Knowledge of trichome type and shape, their surface ultrasculpture, pubescence of particular plant parts can be used in taxonomic, phylogenetic and pharmacological studies (many Boraginaceae species are known as medicinal plants).

The aim of the present paper is a detailed analysis of pubescence and morphological diversity of trichomes in selected representatives of Boraginaceae at different ontogenetic stages.

Materials and methods

16 herbaceous (annual, biennial and perennial) species of Boraginaceae Juss. belonging to 11 genera were chosen as objects for the present study. These are *Brachybotrys paridiformis* Maxim., *Omphalodes verna* Moench., *Asperugo procumbens* L., *Symphytum cordatum* Waldst. & Kit., *S. tuberosum* L., *S. caucasicum* Bieb., *S. × uplandicum* Nym., *Borago officinalis* L., *Anchusa pseudochroleuca* Desjat.-Shost., *Brunnera sibirica* Stev., *Lycopsis arvensis* L., *Pulmonaria rubra* Schott., *P. mollissima* Kern., *P. obscura* Dumort., *Lithospermum arvense* L. and *Echium vulgare* L. The material for morphological and anatomical research was collected in nature (Moscow, Kaluga, Oryol, Lipetsk and Tambov regions, southern Ukraine, Moldova) as well as in cultivation (Botanic garden of Moscow State (Lomonosov) University, N.V. Tsitsin Main Botanical Garden, 'Belye Nochi' botanic garden of Sochi, Botanical Garden (Institute) of the Academy of Sciences of Moldova). Comparative morphological, anatomical and ontogenetic research techniques were implemented.

Table 1. Types of persistent trichomes (pili persistentes).

Trichome types	Description	Illustration (drawing)
Simple hairs (pili simplices)	Sessile (pili sessiles) Trichomes located directly on the epidermal surface	 *Brachybotrys paridiformis*, *Symphytum* × *uplandicum*, *Echium vulgare*, etc.
	Subtended ('rosette') hairs (pili suffulti) Trichomes located on epidermal elevations (elevations of epidermal cells)	 *Omphalodes verna*, *Asperugo procumbens*, *Symphytum cordatum*, *S. tuberosum*, *S. caucasicum*, *Anchusa pseudochroleuca*, *Brunnera sibirica*, etc.
Glandular (pili glanduliferi)	Trichomes with uni- or multicellular stalk and a head	 *Symphytum cordatum*, *S. tuberosum*, *S. caucasicum*, *Borago officinalis*, *Lycopsis arvensis*, *Pulmonaria rubra*, *P. mollissima*, *P. obscura*, *Echium vulgare*, etc.

For the morphological analysis, the following technique was used. Leaves (cotyledons) were placed in 4% glutaraldehyde for 1 hour and then transferred to 1%OsO_4 for four hours. Afterwards, leaves were dehydrated in the alcohol series of 30, 50, 70, 80, 96 and 100% and then put into absolute acetone (dimethylketone, 2-propanone) and kept for 12 hours in a cold dry place. Metal-coated specimens were studied by means of scanning electronic microscope Hitachi S-405A.

To measure the length, the width and the thickness of hair walls, a screwed eyepiece scale was used. Hair and stomata number was calculated on superficial sections by means of a square diaphragm of certain fixed area, attached to the eyepiece, with subsequent recalculation on 1 mm^2 basis. Epidermal cells were described using the terminology suggested by Zakharevich (1954). For each species, original drawings and microphotographs were made.

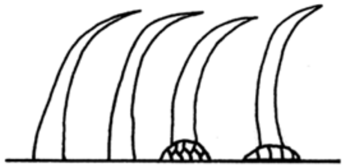
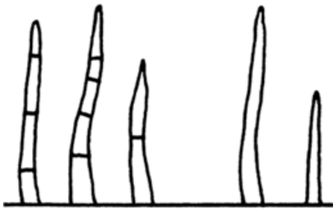
Morphological descriptions of cotyledon and leaf hairs in Boraginaceae were compiled according to the commonly accepted system by Artyushenko & Fedorov (1956), with minor amendments and addenda (Aneli 1962; Miroslavov 1974).

Results

According to their life span, trichomes in studied species are found to belong to the persistent group which comprises two main types (Table 1). Types of simple hairs are shown in Table 2. Surface sculpturing can be a valuable addition to pubescence description; based on that, six types of ultrasculpture were revealed (Table 3) and hair density was also noted (Table 4).

The diversity of hair types and shapes, their growth direction in relation to the surface they grow on (acroscopic or basiscopic) and other peculiarities define the number of pubescence types.

Table 2. Morphological types of simple trichomes.

Trichome type	Trichome shape	Description	Illustration (drawing)
Simple (pili simplices)	Subulate (pili subulati) Unicellular trichomes, swollen at base, with elongated and acuminate tips, with or without subtending 'rosettes'	Straight (pili subulati recti)	 *Brachybotrys paridiformis*, *Omphalodes verna*, *Asperugo procumbens*,*Symphytum caucasicum*, *S.* × *uplandicum*, *Borago officinalis*, *Anchusa pseudochroleuca*, *Brunnera sibirica*, *Echium vulgare*, etc.
		Sickle-shaped (pili subulati falcati)	 *Symphytum cordatum*, *Lycopsis arvensis*, *Pulmonaria rubra*, *Lithospermum arvense*, etc.
		Hooked (pili subulati uncinati)	 *Symphytum cordatum*, *S. tuberosum*, *S. caucasicum*, *Pulmonaria obscura*
	Cylindrical (pili cylindrati) Uni- or multicellular, equal in cross section for their entire height	Straight (pili cylindrati recti)	 *Pulmonaria rubra*

Descriptions of pubescence types revealed are given below (The terms 'minute', 'long', 'short' are descriptive terms for trichomes which should be understood as relative measurements, related to a particular object):

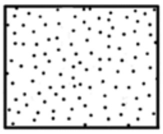
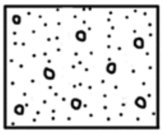
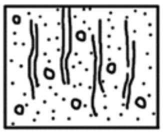
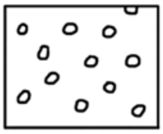
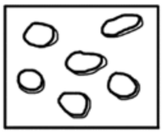
Stiff short hairs (hirtum) – short hairs, more or less tightly connected.

Stiff/coarse hairs (hirsutum) – longer, thicker, elastic sticking out hairs, more or less close, sometimes bent.

Hispid/scabrous (hispidum) – same as previous, yet hairs are not elastic but coarse, usually not densely located, not bent and often of various length.

Setose (setosum) – covered by thick and long, largely acuminated opaque hairs widened at their base.

Table 3. Different types of trichomes' surface ultrasculpture.

Type of ultrasculpture	Description	Drawing
Smooth	Smooth surface of the trichome	 *Lithospermum arvense*, *Pulmonaria rubra*, *P. obscura*
Fine-grained	Small particles, grains, pellets, etc. on the trichome surface	 *Symphytum cordatum*, *S.* × *uplandicum*, *Pulmonaria rubra*
Tuberculous fine-grained	Besides small particles, grains and pellets, bigger tubercules (protuberances) are found on the trichome surface	 *Omphalodes verna*, *Symphytum cordatum*, *Brunnera sibirica*, *Lithospermum arvense*, *Echium vulgare*
Scabrous-plicate	Similar to the previous type, but with small wrinkles, plications, folds and crests along the trichome	 *Lithospermum arvense*, *Pulmonaria rubra*
Tuberculous	Rounded or oblong medium-sized tubercules (knobs) are well visible on the smooth surface of the trichome	 *Asperugo procumbens*, *Symphytum caucasicum*, *S.* × *uplandicum*, *Borago officinalis*, *Anchusa pseudochroleuca*, *Echium vulgare*
Grumous	Similar to the previous type, but the tubercules (knobs) are much bigger	 *Brachybotrys paridiformis*, *Asperugo procumbens*, *Lithospermum arvense*

Strigose (strigosum) – hairs are longer than in the setose type, slightly widened at base and often oriented in the same direction.

Mixed (pubescentia mixta) – hairs of several types.

Pubescence can be more or less uniform or patchy both on the upper (adaxial) and (or) lower (abaxial) leaf surface.

Table 4. Types of pubescence, depending on the density of trichomes.

Types of pubescence	Average number of trichomes per $1\,mm^2$
Rare (scarce)	< 10
Moderate	10–50
Dense	50–100
Heavy (very dense)	> 100

The ontogeny of the studied annual and perennial species has three age-related stages: latent, virginile and reproductive. Even seedlings and juvenile plants have pubescent leaves as well as cotyledons and hypocotyls.

The genus *Brachybotrys* Maxim., belonging to the tribe *Cynoglosseae* M. Gürke, is monotypic, represented by the only species *B. paridiformis* Maxim. This hemicryptophyte possesses a shortened horizontal rhizome and a fringed root system and is largely spread by vegetative propagation. It is known to have di- to tricyclic elongated monocarpic shoots. The plant is found in shady forests of Russian Far East and China (Manchuria) (Gürke 1893; Popov 1953). Germination epigeous (hypocotylar). Seedlings bear two cotyledons, 5 mm long and 5–5.5 mm wide, orbicular-ovate, leaf apex obtuse-acuminate, leaf base obtuse-cuneate gradually continuing into a 4–7 mm long glabrous petiole. Venation pinnate-brochal (Fig. 1 A). Cotyledon blades are pubescent only on the adaxial surface and along the margins (Fig. 2 A, B). Hispid pubescence is present, uniform along the entire surface, however scarce. Hairs very short, barely visible with unaided eye, transparent, one-celled, subulate (straight), sessile (epidermal cells of the 'rosette' are poorly seen at the base of the hairs). Hairs are slightly pressed to the blade surface and directed towards the cotyledon's top (acroscopic). Leaves in juvenile, immature and mature plants, different in shape and size, have indumentum also on their lower surface, petioles (Fig. 6 A), contrary to the cotyledons. It is composed of simple hairs: some of them on the 'rosettes' (pili suffulti) and some sessile. The former are long, unicellular, subulate (straight), thick-walled, filled with cloggy dark green or brown contents; surface grumous (tuberculate). 'Rosette' cells differ from other epidermal cells by their straight, thickened anticlinal walls. Sessile hairs can be long or short (Table 5). Long ones are subulate (sickle-shaped), unicellular, thick-walled, with a little 'beak' on the top, dead (no content inside), with knobby surface. Short sessile trichomes are similar to the previous type but smaller in size and have thinner walls.

The genus *Omphalodes* Mill., belonging to the tribe *Cynoglosseae* M. Gürke, is known as an ancient tertiary relict. We studied *Omphalodes verna* Moench (a representative of the section Omphalodes DC) – a stoloniferous semi-cryptophyte with a short rhizome and semi-rosette-like, dicyclic, monocarpic shoots. The plant occurs in groups in moist broadleaf forests of the lower montane belt, on rocky gorge slopes under canopies of other trees, in river valleys among shrubs and herbaceous plants. The species grows in the southeastern Alps and the Romanian Carpathians (Popov 1953). Germination epigeous, hypocotylar or hypocotylar-cotyledonous. Cotyledon blades broad-elliptical (8–10 mm long, 5–7 mm wide), with slightly acuminated apex, fleshy, dark green, clearly abjoint from the petioles (Fig. 1 B). Indumentum is restricted to the adaxial surface of the cotyledons (Fig. 2 C, D). Scarce hispid (scabrous) pubescence is uniform along the entire

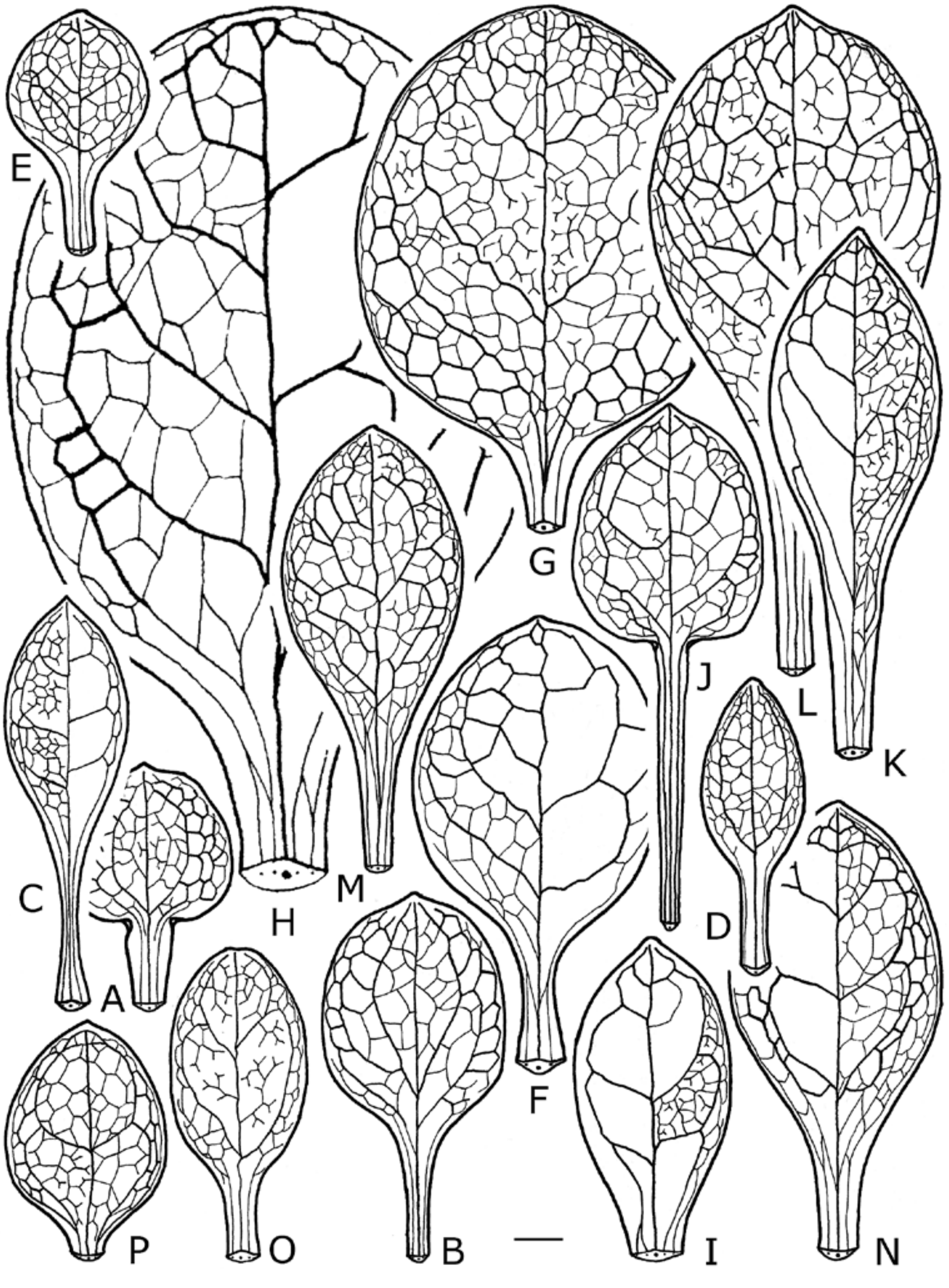


Figure 1. Cotyledons: A – *Brachybotrys paridiformis*; B – *Omphalodes verna*; C – *Asperugo procumbens*; D – *Symphytum cordatum*; E – *S. tuberosum*; F – *S. caucasicum*; G – *S.* × *uplandicum*; H – *Borago officinalis*; I – *Anchusa pseudochroleuca*; J – *Brunnera sibirica*; K – *Lycopsis arvensis*; L – *Pulmonaria rubra*; M – *P. mollissima*; N – *P. obscura*; O – *Lithospermum arvense*; P – *Echium vulgare.* Scale bar = 2 mm.

blade surface. The trichomes, in comparison to those of *Brachybotrys paridiformis*, are longer, transparent, one-celled, subulate (straight), slightly broadened at base. 'Rosette' epidermal cells are poorly expressed. Juvenile leaves petiolate, their blades ovate, margins entire, pubescent on both surfaces (Fig. 6 B). Hairs long on the 'rosettes' and short (Table 5), both being unicellular, subulate (mostly straight, pressed only at the margin), thick-walled with granular dark green

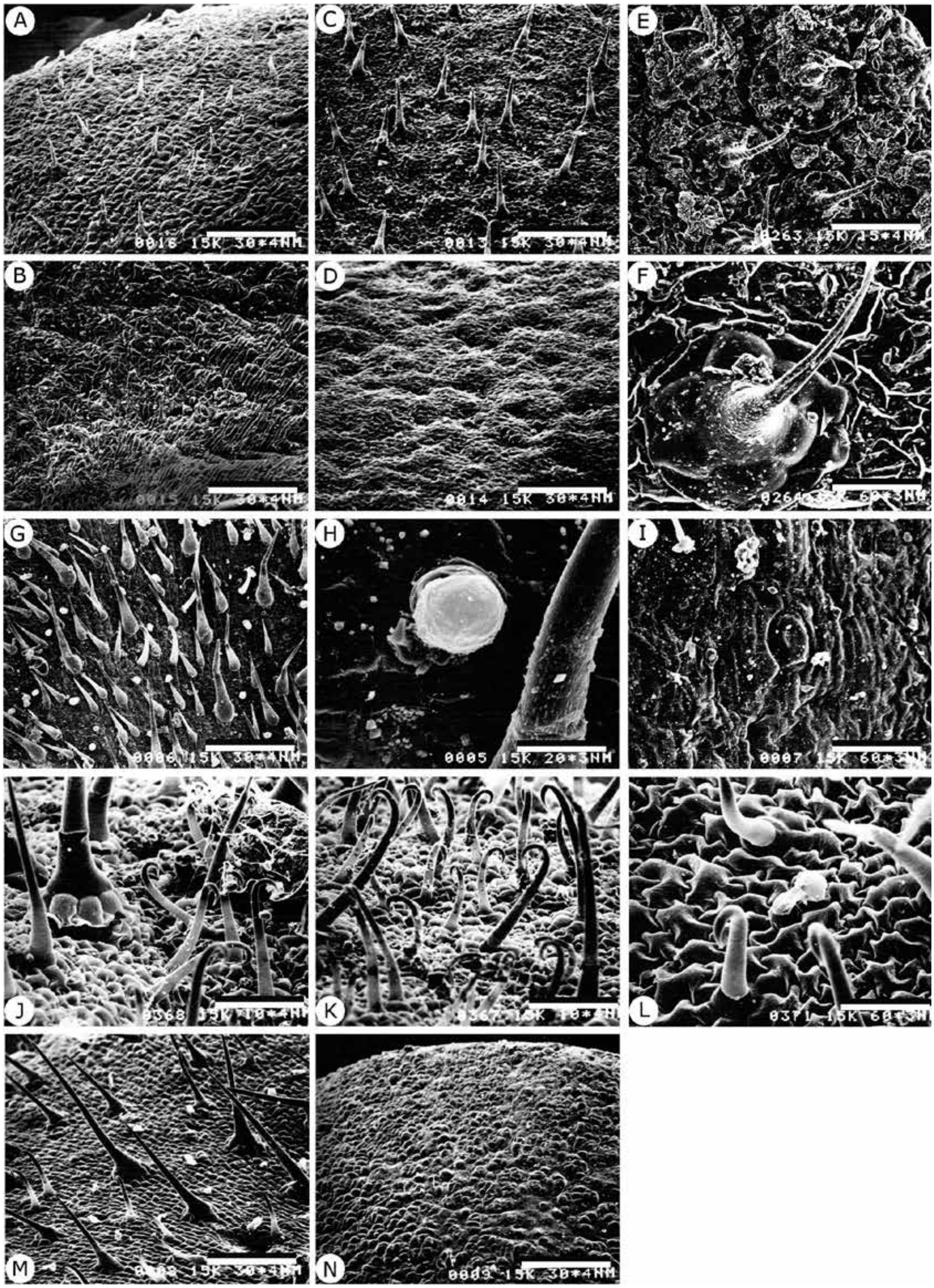


Figure 2. Pubescence of cotyledons: A, B – *Brachybotrys paridiformis*; C, D – *Omphalodes verna*; E, F – *Asperugo procumbens*; G, H, I – *Symphytum cordatum*; J, K, L – *S. caucasicum*; M, N – *S. tuberosum*. A, C, E, G, H, J, L, M – abaxial; B, D, F, I, K, N – adaxial side of the cotyledons. Scale bars: A–D, G, M, N = 300 µm; E = 150 µm; J–K = 100 µm; F, I, L = 60 µm; H = 20 µm.

content. Hair surface tuberculous fine-grained. 'Rosette' (basal) cells with straight thickened anticlinal walls adhering to the hair base and thin wavy anticlinal walls bordering with adjacent epidermal cells. Immature and mature plants are found to develop acuminated ovate and cordate leaves respectively, both petiolate with entire margins, with indumentum of thin short pressed trichomes present on both leaf surfaces.

Asperugo L., a monotypic genus of the tribe *Eritrichieae* Benth. & Hook., is represented by *Asperugo procumbens* L. This is a tap-rooted spring or winter therophyte with a long vegetation period bearing non-rosette or semi-rosette shoots. It is widely spread in the European part of Russia, all parts of the Caucasus, western and eastern Siberia, mountainous and steppe northern areas of Central Asia. Outside of Russia, it is found in Europe, Mediterranean and Indo-Himalayan region. Its common habitats are wasty areas, vegetable gardens, dry slopes, alpine pastures, lower montane belts (under rocks) and river banks (Hege 1972; Popov 1983). Seed germination epigeous, hypocotylar. Seedlings of spring and winter forms have similar shoot structure; once they reach their final size, they possess a well-developed hypocotyl and two cotyledons (6–8 mm long, 3–5 mm wide) (Fig. 1 C). Their blades are acuminate-elliptic, gradually narrowing towards the leaf blade base into a short (4–5 mm long) petiole; indumentum is present on both sides (Fig. 2 E, F). Pubescence uniform, scarce, setose on the upper surface and strigose on the lower. Hairs short, opaque, with tuberculous surface, wedge-like widened and swollen at base. At base, 'rosettes' of 5–7 epidermal cells are present. Juvenile leaves of spring forms opposite, lanceolate, acuminate at tip and attenuate at base (7–8 mm long, 1.5–2 mm wide). The borderline between the petiole and the leaf blade is not pronounced. The length of the petiole is equal to that of the leaf blade. Leaves of winter forms alternate, widely lanceolate, spatulate (14–15 mm long, 2–4 mm wide). Both forms have blades and petioles covered by long unicellular hairs swollen at base (Fig. 6 C; Table 5). Hairs stiff, subulate (straight or sickle-shaped), thick-walled, with tuberculous fine-grained surface and dark green granular contents. Basal 'rosette' cells walls slightly thickened. Immature and mature leaves of both winter and spring forms vary from spatulate-lanceolate to oblong-lanceolate. Upper leaves appeared to have shorter petioles; sessile leaves are formed higher up on the stem. Both surfaces of the blades are pubescent; indumentum uniform, thick (very thick), strigose. On the upper surface, the trichomes are longer, unicellular, subulate (straight), sessile (basal 'rosettes' poorly expressed). Trichome surface grumous (Fig. 8 A, B).

The genus *Symphytum* L. belongs to the tribe *Anchuseae* (DC.) M. Gürke and includes about 40 species which are distributed into 7 sections. Plants of three sections *Tuberosa* Buckn. (*S. cordatum* Waldst. & Kit., *S. tuberosum* L.), *Caerulea* Buckn. (*S. caucasicum* Bieb.) and *Symphytum* Pawl. (*S.* × *uplandicum* Nym.) were studied for the present paper.

Symphytum cordatum – a tuber- and rhizome-forming geophyte with a chain-like shortened rhizome, dicyclic, semi-rosette, monocarpic shoots. It can be found in Ukraine, Romania, Slovakia, Poland (in the Carpathians), most often growing in montane fir forests and less often in broadleaf forests (Pawłowski 1961; Popov 1953). Germination bivariant – epigeous hypocotylar-cotyledonar or hypogeous (Barykina & Alyonkin 2008). In the former case, cotyledon blades are brought out to the surface. They are oblong-elliptic in shape (8 mm long, 4 mm wide), fleshy, with glabrous petioles of 3.5–4 mm long, 1 mm wide, with indumentum only on the adaxial side (Fig. 1 D). Pubescence mixed, composed of two types of trichomes – simple sessile and glandular (Fig. 2 G, H, I). Among sessile trichomes, two morphological subtypes can be

Table 5. Morphological and anatomical characteristics of juvenile leaf trichomes.

Tribe	Species	Upper epidermis			
		Trichome types			Pubescence type and number of trichomes (per 1 mm^2)
		Subtended by 'rosettes'	sessile	glandular (2–5-celled)	
Cynoglosseae	*Brachybotrys paridiformis*	+	+	–	Dense; stiff short hairs, 83
	Omphalodes verna	+	–	–	Moderate; hispid, 14
Eritrichieae	*Asperugo procumbens*	+	–	–	Moderate; strigose, 13
Anchuseae	*Symphytum cordatum*	+	+	Two-celled (along the midrib)	Moderate; mixed, 19
	Symphytum tuberosum	+	+	Two-celled (along the midrib)	Moderate; mixed, 11
	Symphytum caucasicum	+	+	Two- or three-celled (along the midrib and blade margins)	Moderate; mixed, 16
	Symphytum × uplandicum	+	+	–	Moderate; stiff (coarse) hairs, 12
	Borago officinalis	+	–	Two-celled (along large veins)	Moderate; mixed, 12
	Anchusa pseudochroleuca	+	–	Two- or three-celled (along the midrib)	Moderate; mixed, 11
	Brunnera sibirica	+	–	–	Rare (scarce); setose, 4
	Lycopsis arvensis	+	–	Two-celled (along the midrib)	Rare (scarce); mixed, 5
	Pulmonaria rubra	+	+	Three-to five-celled (throughout the entire blade surface)	Heavy (very dense); mixed, 104
	Pulmonaria mollissima	+	+	Two-to five-celled (throughout the entire blade surface)	Dense; mixed, 90
	Pulmonaria obscura	+	+	Three-to five-celled (throughout the entire blade surface)	Dense; mixed, 65
Lithospermeae	*Lithospermum arvense*	+	–	–	Moderate; strigose, 21
Echieae	*Echium vulgare*	+	+	Two- or three-celled (along the midrib and blade margins)	Moderate; mixed, 11

Upper epidermis					
			Trichome sizes		
Presence of well-expressed 'rosettes'	Number of cells per 'rosette'	Presence of mineral contents in 'rosette' cells	Trichome length (μm)	Trichome base diameter (μm)	Trichome base diameter with 'rosette' (μm)
Poorly expressed (only in case of long hairs 'rosette' cell walls are slightly thickened)	10–15	–	long 150–600 short 33–77	long 94–105 short 34–77	126–210
Well-expressed (cell walls adhering to the trichome base are thickened)	6–14	–	129–525	46–87	89–147
Well-expressed, walls slightly thickened	8–12	Grained	245–700	49–100	98–126
Poorly expressed, thin-walled cells	9–15	–	long 300–825 short 70–300	49–122	140–210
Well-expressed, thin-walled cells	7–12	–	long 375–600 short 50–115	28–95	140–210
Well-expressed, thin-walled cells	5–10	–	long 465–900 short 195–345	44–70	94–154
Well-expressed, thin-walled cells	8–14	–	long 465–802 short 140–420	61–87	147–179
Well-expressed, cell walls thickened	6–20	Layered contents only in cells of large 'rosettes'	long 120−975 glandular 49–84	glandular 28–30 simple 63–140	119–350
Well-expressed, cell walls slightly thickened	5–9	–	long 267–600 glandular 54.6–100	glandular 17.5–25.9 simple 56–175	112–255
Well-expressed, thick-walled cells	10–11	–	140–420	70–105	112–175
Well-expressed, cell walls thickened	5–40(50)	Grained, layered and lumpy contents	long 120–600 glandular 86.1–89	glandular 24.5–25 simple 56–105	119–700
Well-expressed, cell walls thickened	6–12	Contents only in cells of large 'rosettes'	long 300–825 short 44.8–280	16.8–91,7	101.5–140
Well-expressed, cell walls thickened	6–10	–	long 675–1200 short 92–210	16.1–102,2	77–122.5
Well-expressed, cell walls slightly thickened	6–8	–	long 31.5–600 short 30–231	23.8–83	112–133
Well-expressed, cell walls slightly thickened	8–10 (up to 20 in winter forms)	Layered contents only in old leaves of winter forms	120–600	52–98	114–175
Well-expressed, cell walls slightly thickened	7–15	–	long 368–1760 short and glandular 26–217	long 66–87 short and glandular 17–56	105–217

identified: relatively long, subulate straight hairs and short hooked hairs; surface fine-grained, tuberculous. Glandular hairs one- or two-celled, with clavate heads and very short stalks. In plants with hypogeous germination, the cotyledons remain covered by the nutlet's coat (pericarp) or partially released. In case of hypogeous germination, pubescence is the same as in aerial (epigeous) cotyledons, i.e., restricted to the adaxial side of the blade, yet it is scarcer and the hairs are very short, ephemerous. Juvenile, immature and mature (vegetative and reproductive) plants are characterized by cordate petiolate leaves; near the inflorescence they are ovate or oblong, with short petioles. Leaf blades have indumentums on both adaxial and abaxial (mainly along protruding veins) sides and along the margins (Fig. 6 D). Trichomes simple (sessile or subtended by 'rosettes' and glandular (Table 5). Sessile trichomes short, unicellular, subulate (hooked or sickle-shaped), thin-walled, with brown content concentrated near the hair's tip; surface fine-grained or tuberculous fine-grained. Subtended ('rosette') hairs long, unicellular, subulate (straight, sometimes sickle-shaped), with thicker walls and dark green content near the top; surface tuberculous fine-grained. Solitary glandular hairs, composed of rounded to oblong heads and unicellular stalks, occur primarily along the veins and leaf margins. The entire petiole surface is covered by simple subulate transparent hairs, spaced apart from each other.

Symphytum tuberosum – a tuber- and rhizome-forming geophyte with a chain-like shortened rhizome, dicyclic, semi-rosette, monocarpic shoots. Often capable of forming hybrids with the previous species described, e.g., *S. ullepitschii* Wettst. (Popov 1953). Distribution areas for *S. cordatum* and *S. tuberosum* largely overlap. *S. tuberosum* is primarily a mountain forest Central European species, commonly found in the Carpathians, sometimes reaching the adjacent plains; unlike *S. cordatum*, it grows mainly in shady moist beech, horn beech and oak forests (Hege 1972; Tikhomirov et al. 1999). Early ontogenetic stages and structure of vegetative organs in *S. tuberosum* are similar to those of *S. cordatum*. Germination unstable – can be hypogeous or epigeous hypocotylar-cotyledonar. Cotyledons petiolate, rounded (5–8 mm long, 3–5 mm wide). Adaxial leaf surface and petioles pubescent (Fig. 1 E). Pubescence moderate, mixed, uniform along the entire adaxial surface (Fig. 2 M, N). Trichomes of two types can be reported: simple (subtended or on 'rosettes' and sessile) and glandular. The former elongated and acuminate, widened at base; 5–10-celled 'rosettes'. Sessile hairs unicellular, hooked, 3–4 times shorter than the 'rosette' ones. Glandular hairs are exactly the same as in *S. cordatum*. Juvenile leaves rounded-ovate or elliptic (1.5–2.3 cm long, 1.5–2 cm wide), slightly acuminate at top, with rounded to cuneate leaf blade base. Petiole long (3–4 cm). Leaf shape changes with the plants age. Immature individuals have rounded-ovate leaves (4–5 cm long, 3–4 cm wide), with acuminate apex and cordate blade base; petioles 4–5 cm long. Mature reproductive plants show pronounced heterophylly. Two to three lower leaves of a flowering annual shoot have small underdeveloped blades, middle three to five leaves, sessile or semi-sessile, are located close together, cuneate at base and with acuminate apex (length 5–10 cm, width 1.5–4 cm), small bracts with decurrent base. Leaf pubescence in juvenile, immature and mature reproductive plants is similar to that of *S. cordatum*; some differences are related only to qualitative characteristics and the presence of scabrous-plicate surface of hairs, subtended by the 'rosettes' (Fig. 6 E, Table 5).

Symphytum caucasicum Bieb., one-headed, tap-rooted, herbaceous hemicryptophyte with semi-rosette, dicyclic, monocarpic shoots, is a typical representative of the section *Caerulea*. Natural distribution area of this species is restricted to the Caucasus, except for southeastern Azerbaijan. The plant grows in meadows and forest margins and is cultivated as forage crop and bee plant

(Gviniashvili 1976; Frolov 1982, 1989, 1997; Tikhomirov et al. 1999). Germination epigeous, hypocotylar. Cotyledons rounded or oval (14–16 mm long, 11 mm wide); petioles 4–5 mm in length, pubescent and widened at base (Fig. 1 F). Blades have indumentums on both sides, pubescence more or less uniform, mixed, dense (Fig. 2 J, K, L). Two types of hairs are present: simple (sessile or subtended by 'rosettes') and glandular. Subtended ('rosette') hairs long, one-celled, subulate (straight), swollen at base, with tuberculous surface. Pronounced 'rosettes' are composed of 6–10 epidermal cells. Sessile hairs 2–2.5 times shorter than the former, unicellular, hooked. Glandular hairs two- to three-celled, with pronounced stalks and clavate heads. Juvenile leaves of rosette shoots possess oblong-ovate petiolate leaves with entire margins and acuminate apex. Immature and mature vegetating plants retain the same leaf shape, whereas their sizes increase (up to 45 cm in length and 22 cm in width) together with the petiole length reduction to 12–13 mm, until sessile leaves are formed. All leaves are covered by trichomes of two types (Fig. 6 F; Table 5). Sessile leaves unicellular, short, subulate (straight or hooked – mostly located along the midrib), thin-walled, with greenish-brown contents near the tip and tuberculous surface. Subtended ('rosette') hairs unicellular, long, subulate (straight ot slightly bent), with thicker walls than in the previous species. Their cell cavities permanently possess lumpy dark green contents; surface tuberculous. 'Rosettes' consist of 5–10 epidermal cells. Two- to three-celled glandular hairs are also filled with green or greenish-brown contents. Glandular hairs are located along large veins and blade margins.

The life form of *S. caucasicum* is similar to that of *S.* × *uplandicum* (section *Symphytum*), a natural hybrid of *S. officinale* and *S. asperum* Lepech. It is widespread in Europe and North America; in Central Russia, it is known only from relatively few localities (Moscow city and region). The species grows in moist meadows, swampy areas, alongside rivers or streams (Pawłowski 1972; Hege 1972; Frolov 1991; Tikhomirov et al. 1999). Germination epigeous, hypocotylar. Cotyledon blades oval-elliptic, almost orbicular, 15–17 mm long and 10–12 mm wide; leaf apex rounded, margins entire, blade base attenuate; indumentum restricted to the adaxial side (Fig. 1 G). Pubescence uniform, scarce, of stiff short hairs (Fig. 3 A, B). All trichomes unicellular, more or less similar in size, relatively short, transparent, sessile, subulate (straight and hooked). Cotyledon petioles long, 13–15 mm, fully (except for basal parts) covered by straight and (or) hooked, short, transparent, upward-directed hairs. Juvenile leaves petiolate, vary from oval-ovate (20 mm long, 11–15 mm wide) to wide-lanceolate (40 mm long, 18–20 mm wide). On the first leaf, the indumentum is restricted to the adaxial side, subsequent leaves of all age groups are pubescent on both leaf surfaces. Only simple trichomes are present – sessile and subtended by 'rosettes' (Fig. 6 G; Table 5). The former are short, one-celled, subulate (straight), thick-walled, with fine-grained surface. The latter are long, unicellular, subulate (straight and (or) sickle-shaped), thick-walled, with tuberculous surface; brown granular content is present inside them. 8–14 'rosette' cells thin-walled, much larger than other dermal cells of the leaf.

The genus *Borago* L. is represented by three species. *Borago officinalis* L. from the section *Borago* has been studied. The plant is a tap-rooted, biennial hemicryptophyte or, more often, a therophyte with a long vegetation period characterized by semi-rosette shoots. Widespread in all parts of Europe, the Mediterranean region, Iran, less often in the Caucasus, Central Asia, western Siberia. Germination epigeous, hypocotylar. Wide-elliptic (20 mm long, 18–20 mm wide) cotyledons with short petioles are covered by stiff hairs solely on the adaxial side (Fig. 1 H). Pubescence uniform, scarce, strigose (Fig. 3 C, D). Trichomes one-celled, from long to short, subulate (straight), originating from multicellular (up to 30 epidermal cells!), one- to three-layered 'rosettes', well visible with unaided eye. The first juvenile leaf spatulate (7 mm long, 2–4 mm wide), petiole short, margin slightly undulate, both leaf surfaces pubescent. Subsequent leaves larger, obovate, with undulate margins and elongated petioles. All leaves are covered by widely spaced, unicellular, subtended ('rosette') trichomes. Rosette leaves of mature vegetative plants oval (30–70 mm long, 20–50 mm wide), with rounded apex, undulate margins and attenuate blade base, gradually transforming into a more or less elongated (15–25 mm) petiole. Both adaxial and abaxial leaf surfaces and the entire petiole are pubescent (Fig. 6 H, Table 5). Trichomes of two types can be found here: simple and glandular. The former are subtended ('rosette'), long or short, single-celled, subulate (straight and (or) sickle-shaped), thin-walled, filled with dark green or brownish-green contents; surface tuberculous. 'Rosettes' one- to two-layered, consisting of 6–20 cells, filled with layered contents. Glandular hairs minute, with brown contents inside, located along major veins.

The genus *Anchusa* L. consists of up to 40 species. Primarily they are perennial herbs with unstable life cycle, which can be shortened to two years, rarely – typical biennials or annuals. *Anchusa pseudochroleuca* Shost. (section *Anchusa*) is a multiple-headed, particulating hemicryptophyte with a short taproot and semi-rosette, monocarpic shoots. It is widespread at calcareous steppe slopes of Moldova, less often in Middle Dnieper and the Black Sea regions (Popov 1953; Heideman 1975). Germination epigeous, hypocotylar. Cotyledon blades are acuminate-elliptic, wide oval in shape (12 mm long, 8.3 mm wide), with short petioles, with more or less uniform adaxial and marginal pubescence (Fig. 1 I). Pubescence setose, mixed, represented by two types of trichomes: simple and glandular (Fig. 3 E–H). Simple hairs sessile or subtended ('rosette'), one-celled, opaque, subulate (straight or bent), with tuberculous surface, swollen at base. 'Rosettes' of 5–7 cells, single-layered. Glandular hairs two-celled, with pronounced stalks and clavate heads, 4–4.5 times shorter than simple trichomes, located along the cotyledon blade margin. Juvenile leaves and those of mature vegetative plants oblong or oblong-lanceolate (50–150 mm long, 10–20 mm wide), narrowed at base, with long petioles. Moderate mixed pubescence is present on both leaf surfaces. Hairs simple and glandular (Table 5). Simple hairs sessile, long, one-celled, subulate (straight or sickle-shaped), thin-walled, filled with dark green lumpy contents. Subtended ('rosette') hairs long, one-celled, subulate (straight), thin-walled. Hairs of both types have tuberculous surface. Glandular hairs are located along the midrib; they are two-celled, with oblong heads containing dark substances inside.

The genus *Brunnera* Stev., belonging to the tribe *Anchuseae* (DC.) M. Gürke contains only two species, their distribution connected with western and eastern Siberia as well as the Caucasus. *Brunnera sibirica* Stev. is an old Pliocene relic of beech-horn, beech and oak forests which had existed in late Tertiary in the Altai, Kuznetsky Alatau and the Western Sayans and became extinct in Pleistocene (Popov 1953). Currently, the phytocoenotic range of *Brunnera sibirica* encompasses the northeastern part of the Western Sayans, where the species grows in pine, birch-pine and birch forests (Samosenko 1999). *B. sibirica* is a vegetatively propagated hemicryptophyte with a short horizontal rhizome, fringed root system and monocyclic, semi-rosette, monocarpic shoots. Germination epigeous, hypocotylar. Fully developed seedlings have two bright green, widely ovate cotyledons (10 mm long, 7.5–8 mm wide) with acuminate apex, entire margin and sinuate blade base. Indumentum restricted to the upper surface (Fig. 1 J). Pubescence uniform, scarce, setose, of only one hair type (simple trichomes subtended by 'rosettes'). The hairs are rather long,

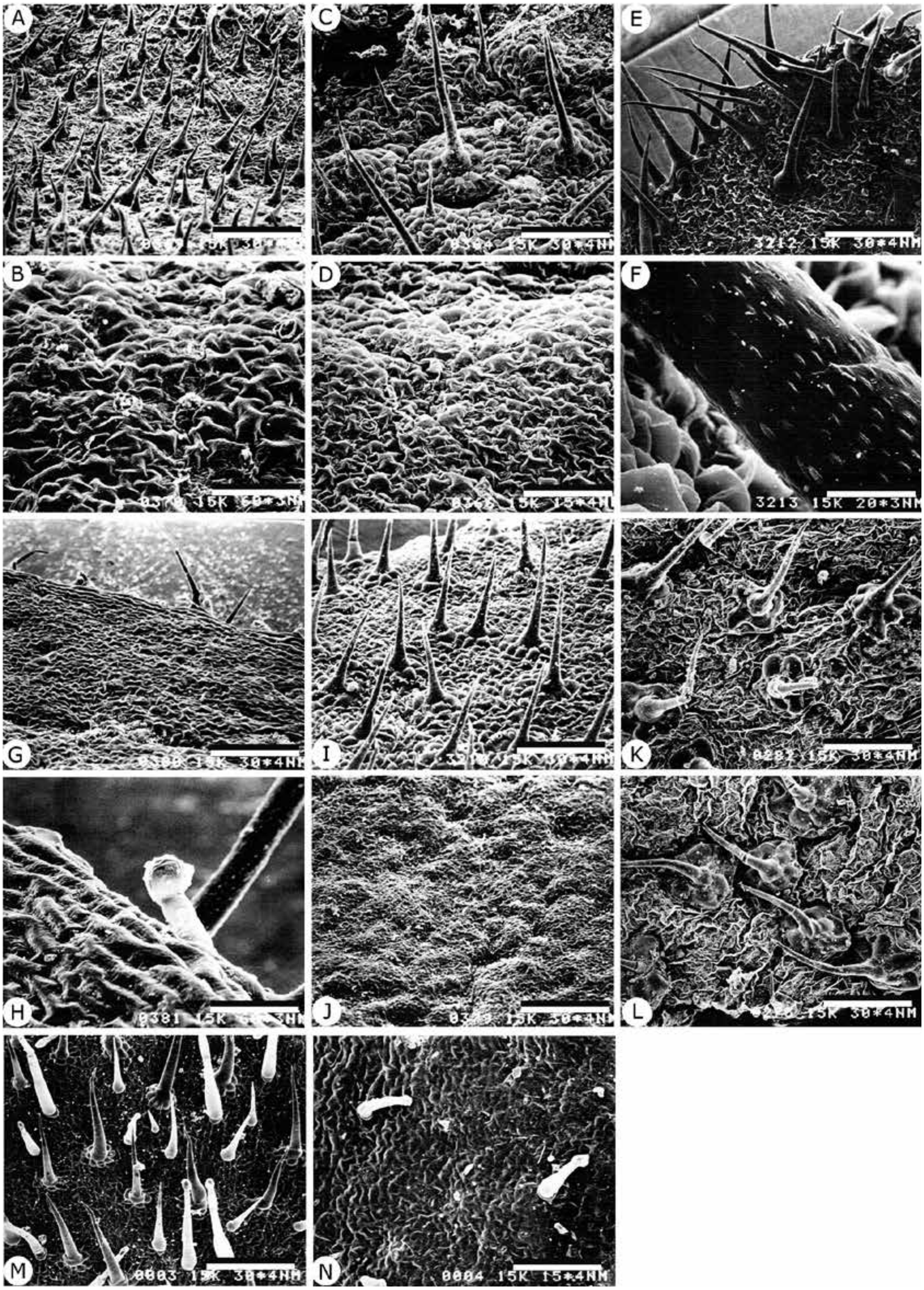


Figure 3. Pubescence of cotyledons: A, B – *Symphytum × uplandicum*; C, D – *Borago officinalis*; E–H – *Anchusa pseudochroleuca*; I, J – *Brunnera sibirica*; K, L – *Lycopsis arvensis*; M, N – *Pulmonaria rubra*. A, C, E, I, K, L, M – abaxial and B, D, F, G, H, J, N – adaxial side of the cotyledons. Scale bars: A, C, E, G, I, J, K, L, M = 300 µm; D, N = 150 µm; B, H = 60 µm; F = 20 µm.

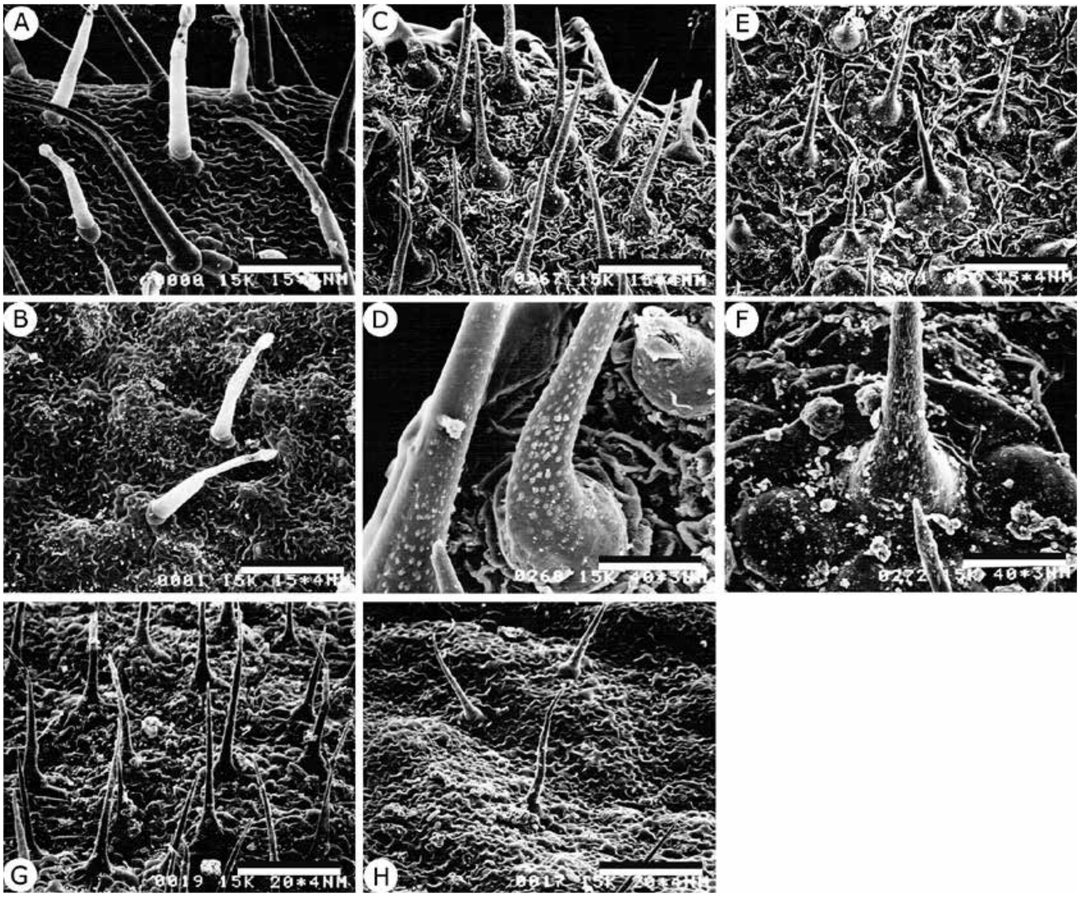


Figure 4. Pubescence of cotyledons: A, B – *Pulmonaria mollissima*; C–F – *Lithospermum arvense* (C, D – spring form, E, F – winter form); G, H – *Echium vulgare*. A, C, E, G – abaxial and B, D, F, H – adaxial side of the cotyledons. Scale bars: G, H = 200 µm; A, B, C, E = 150 µm; D, F = 40 µm.

unicellular, opaque, subulate (straight), swollen at base. 'Rosettes' of 6–10 cells, single-layered. Cotyledon petioles of 12–15 mm in length, grooved. Up-directed transparent hairs occur along the groove margin. In juvenile and immature plants, leaves are oval-cordate, with long petioles. Pubescence on both leaf surfaces (Fig. 7 J). All hairs simple, subtended by 'rosettes', long, one-celled, subulate (straight), with tuberculous surface and no inner contents (Table 5). 'Rosettes' single-layered, 10–11-celled. In mature vegetative and reproductive plants, the petiole length can reach 45 cm and more. Leaf blades cordate to deltoid, with acute apex, undulate margins and reniform base, with their entire adaxial surface covered by short sparse setose hairs, whereas their abaxial side remains glabrous or has hairs only along the veins.

Lycopsis L. is a genus of the tribe *Anchuseae* (DC.) M. Gürke, including 3 species and several varieties. *L. arvensis* L. is a tap-rooted therophyte with a long vegetation period, possessing non-rosette (elongated) or semi-rosette shoots developing according to the spring or the winter type (Barykina & Alyonkin 1999, 2007; Alyonkin & Barykina 2014). The plant occurs throughout the European part of Russia, in Caucasus, Central Asia, Ural region and Bashkortostan, as well as in the Mediterranean area, western Europe and Africa (Ethiopia) (Popov 1953, 1983), growing on dry slopes, meadows, fields, abandoned and wasty territories, forest margins, etc. The initial ontogenetic stages go similarly in spring and winter forms. Germination epigeous, hypocotylar. Cotyledon blades wide-elliptic (15–17 mm long, 5–6 mm wide), acuminate at top, with attenuate base gradually transforming into wide petioles (Fig. 1 K). Just like both sides of the blades, they possess a scarce, uniform strigose indumentum (Fig. 3 K, L). Hairs simple, subtended (by 'rosettes'), unicellular, opaque, subulate (straight or sickle-shaped, widened at base. Surface tuberculous, fine-grained. 'Rosettes' one-layered, consisting of just a few cells (6–7). The hypocotyl is also covered by the indumentum. Seedlings of spring forms develop the first leaf shortly after the emergence of cotyledons. It is 17–18 mm long and 4–5 mm wide, with acuminate top and slightly undulating leaf margin. The leaf blade is gradually narrowed into a decurrent base and transformed into a very short petiole (5–6 mm long, 1–1,5 mm wide). Pubescence on both leaf surfaces stiff, simple, sessile, one-celled hairs. In case of autumn germination (winter forms), the seedling stage is not pronounced, due to the quick emergence of the rosette of overwintering leaves. At the juvenile stage, the first morphological structural differences between spring and winter form show up. The main shoot of the former is elongated, 2–5 cm tall and of the latter it is a rosette shoot of 1.5–2 cm. The elongated shoot bears 3–5 leaves from oblong-elliptic (25–30 mm long, 6–8 mm wide) to oblong-lanceolate, with acute apex and slightly undulating margins; length of petioles decreases by 5–7 mm towards the shoot apex. On the contrary, the rosette shoot of winter forms produces five widely lanceolate or spatulate (25–33 mm long, 6–10 mm wide) leaves with gradually narrowing blades that later on transform into a petiole, shorter in size (3–4 mm long) than that of the spring forms. In both forms, the epicotyl and juvenile leaves' blades (on both surfaces) possess uniform strigose pubescence represented by simple and glandular hairs (Fig. 7 K; Table 5). Simple subtended ('rosette') hairs are long, one-celled, subulate (straight or sickle-shaped), with their walls thickened and cell cavities either empty or filled with dark green (brown) contents. Hair surface scabrous, plicate. 'Rosettes' multicellular (up to 50 cells), multi-layered, bigger and larger (up to 3–4 layers) in the spring forms. Glandular hairs occur very rarely, mostly along the midrib. Elongated shoots of virginile plants, up to 10–15 cm long, with 3–7 leaves structured similarly to those of mature reproductive plans. Blades vary in shape from linear-lanceolate to oblong-lanceolate, with undulate margins, gradually decurrent towards the base. In winter forms, the flowering shoot is of semi-rosette type, bearing 2 or 3 closely located overwintering leaves and one or two newly produced summer-green ones, having developed in spring; 4–5 leaves are formed on the upper part of the flower-bearing shoots, before the inflorescence appears. Along the shoot, a pronounced layered heterophylly can be seen: lower rosette leaves are linear-lanceolate, with entire margins, 35–70 mm long and 6−10 mm wide, mid-stem and upper leaves oblong-lanceolate, with paracladia, undulate and slightly dentate margins, 30–50 mm long and 4–12 mm wide on average. All leaves are found to have scarce mixed pubescence on both leaf surfaces (Fig. 8 C–F). Hairs on the adaxial side are long, sessile, one-celled, subulate (straight), slightly swollen at base (Table 5), with tuberculous or tuberculous fine-grained surface. 'Rosettes' multicellular (up to 30–40 epidermal cells), one- to three-layered, well seen with unaided eye. Glandular hairs concentrated mostly along protruding midrib and lateral veins on the abaxial side.

The genus *Pulmonaria* L. (tribe *Anchuseae* (DC.) M. Gürke consists of approximately 10 species characterized by rather wide environmental range. Most representatives are confined to broadleaf (oak or beech) and mixed forests, forest ravines.

Pulmonaria rubra Schott. (section *Molles*) is a vegetatively propagating hemicryptophyte with a short horizontal rhizome, fringed root system and monocyclic, semi-rosette, monocarpic

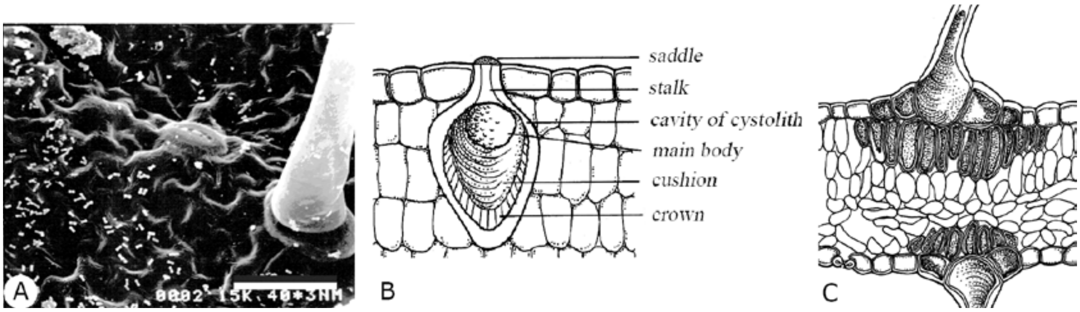


Figure 5. A – Numerous fungal spores on the adaxial surface of cotyledons in *Pulmonaria mollissima*; B – Cystolith structure by Aneli (1975); C – Cross-section of the leaf of *Lappula squarrosa* (biennial form). Scale bar: A = 40 µm.

shoots. The species' range encompasses Eastern Europe, Eastern Carpathians and the Balkan peninsula; the plant is found in mountain beech forests, ascending to fir-spruce forests (Hege 1972). Germination epigeous, hypocotylar. By the moment of transition to the juvenile stage of ontogeny, wide oval, lanceolate cotyledon blades reach their maximum size (25–28 mm long, 10–12 mm wide) (Fig. 1 L). Petioles, 5–6 mm long, are densely pubescent, and so is the hypocotyl. Leaf blades pubescent on both surfaces; pubescence uniform, moderate, mixed on the adaxial and glandular on the abaxial side (Fig. 3 M, N). Hairs simple (sessile and those subtended by 'rosettes') and glandular. The former are unicellular, opaque, subulate (straight or slightly bent), long or of medium length, with scabrous plicate surface. 'Rosettes' of 8–10 cells, one- to two-layered. Glandular hairs three- to five-celled, larger on the adaxial side, with well-pronounced stalk of 2–4 cells and a unicellular clavate head. During the first year of vegetation, 3–5 petiolate juvenile leaves succeed the cotyledons. Their blades are oblong-ovate, with acuminate apex, entire margins and attenuate base, pubescent in the same way as the petioles (Fig. 7 L). Mixed indumentum is present on both surfaces, uniform and very dense, compiled by two types of hairs – simple and glandular. Simple sessile hairs more or less short, one- to four-celled. In the former case, they are subulate (straight), thin-walled, transparent, with fine-grained surface; in the latter they are cylindrical, thin-walled, transparent and smooth. Subtended ('rosette') hairs long, single-celled, subulate (straight or sickle-shaped), thin-walled, lacking contents, with fine-grained surface. 'Rosettes' multicellular, multi-layered. Glandular hairs located throughout the entire leaf surface; they are known to have long stalks (of 2–4 cells) and clavate heads. The pubescence of immature and mature vegetative or reproductive plants, despite some differences in shape, size and life duration, in general remains constant without pronounced ontogenetic changes.

Pulmonaria mollissima A. Kern., just like the previous species, belongs to the section *Molles* and is similar to it in terms of biomorphology. In the territory of the former USSR, this plant can be found in the Ciscarpathian, Volga and Ural regions, throughout the entire taiga part of Siberia up to Lake Baikal and the Lena in the north. Also, this species grows in Caucasus and Central Asia. It is common in primarily coniferous forests, less often in fir and spruce forests (Popov 1953; Hege 1972). Germination epigeous, hypocotylar. Cotyledon blades wide-elliptic, almost spatulate in shape (10–13 mm long, 8–8.5 mm wide), with entire margins and attenuate base gradually transforming into the petiole (Fig. 1 M). The cotyledons in *P. mollissima* have the same pubescence as in *P. rubra*, but the hairs are markedly bigger (Fig. 4 A, B). Hypocotyl 3–4 mm long, pubescent. Juvenile leaves, 3–4, lanceolate, 2–2.5 cm long, 1.7–1.9 cm wide, with acuminate apex and narrowed bases; indumentums on both leaf surfaces. Dense mixed pubescence is identical to *P. rubra*, the differences concerning only some qualitative factors and the absence of four-celled cylindrical hairs (Fig. 7 M; Table 5). After reaching vegetative maturity, the number of rosette leaves increases to 4–6, they become close to adult ones in their shape and size: elliptical, average length 50 cm, average width 8–10 cm, with short petioles. The elongated reproductive shoot developing from the reproductive bud in spring, besides large brown non-pubescent cataphylls, bears 5–7 sessile foliage leaves. Their blades are oblong (3–7 cm long, 1.5–2 cm wide), with almost cordate base, much narrower than leaf blades of rosette leaves in vegetative shoots. Pubescence of virginile and mature reproductive plants greatly resembles that of juvenile leaves.

Pulmonaria obscura (section *Asperae*) is a Central European nemoral (temperate continental) species distributed throughout eastern Europe. It is a characteristic representative of the broadleaf forest flora (Lippmaa 1938; Kleopov 1941; Meusel 1943, 1969; Gorchakovsky 1968, 1972). Biomorphological peculiarities of the lungwort are in many aspects similar to those of other studied species. Germination epigeous, hypocotylar. Cotyledon blades wide elliptic (10–15 mm long, 8–10 mm wide), fleshy, gradually turning into short (4–6 mm long) pubescent petioles (Fig. 1 N). Blades pubescent on both surfaces; pubescence uniform, moderate, of mixed type. Hypocotyl 1.5–2 cm long, rounded, fleshy, covered by sparse simple hairs. During summer, 2–3 juvenile leaves develop on the shortened shoot; their blades elongated-ovate, acuminate at tip, with cuneate base gradually transforming into a long petiole. Just like in petioles, both surfaces are covered by dense mixed indumentums. Trichomes simple and glandular (Fig. 7 N, Table 5). Sessile hairs very short, one-celled, subulate (conical, straight), thin-walled, with dense contents and smooth surface. Subtended ('rosette') hairs long, one-celled, subulate (straight or slightly bent), living, thin-walled, with smooth surface. Glandular hairs of various lengths, with one- to four-celled stalk and one-celled clavate or oblong head; they are spread throughout the entire leaf surface. Leave of other subsequent age groups appeared to have the same pubescence as in previously described species.

The genus *Lithospermum* L. is the largest one (up to 100 species) in the tribe *Lithospermae* (DC.) M. Gürke, it is heterogeneous and distributed throughout the world (except for Australia). In central Russia, it is represented only by seven species (Dobrochaeva 1981; Popov 1983). *Lithospermum arvense* L. (= *Buglossoides arvensis* (L.) I.M. Johnston) is a tap-rooted spring or winter therophyte with a long vegetative period, possessing non-rosette and semi-rosette shoots. It is widespread in the territory of the former USSR and outside of it: Central Europe, the Mediterranean region, the Balkan peninsula, Asia Minor, Iran, China and Japan. The plant grows on rocky slopes, arable lands, abandoned areas, on sands, in steppe or rarely in forest zones and on mountain slopes (Betner 1917; Korsmo 1933; Popov 1983). Germination epigeous, hypocotylar. Seedlings of spring and winter forms are similar in structure. Cotyledons short- or wide-oval, 10–12 mm long, (5)8–9 mm wide, with retuse apex and rounded or slightly cuneate blade base, gradually turning into a short wide petiole (Fig. 1 O). Cotyledon blades and petioles are pubescent. Cotyledons of spring form seedlings have more or less uniform, moderate strigose pubescence on both surfaces (Fig. 4 C, D). It is represented by one-celled, long straight hairs markedly swollen at base, subtended by 'rosettes' as well as by sessile hairs; both trichomes have grumous surface. Unlike the spring forms, the cotyledon blades are covered by sparse hairs in winter forms; they are short, subulate, with well-developed 'rosette' cells (5–6). Hair surface scabrous, plicate (Fig. 4 E, F). Hypocotyl 10–20 mm in length, covered by numerous

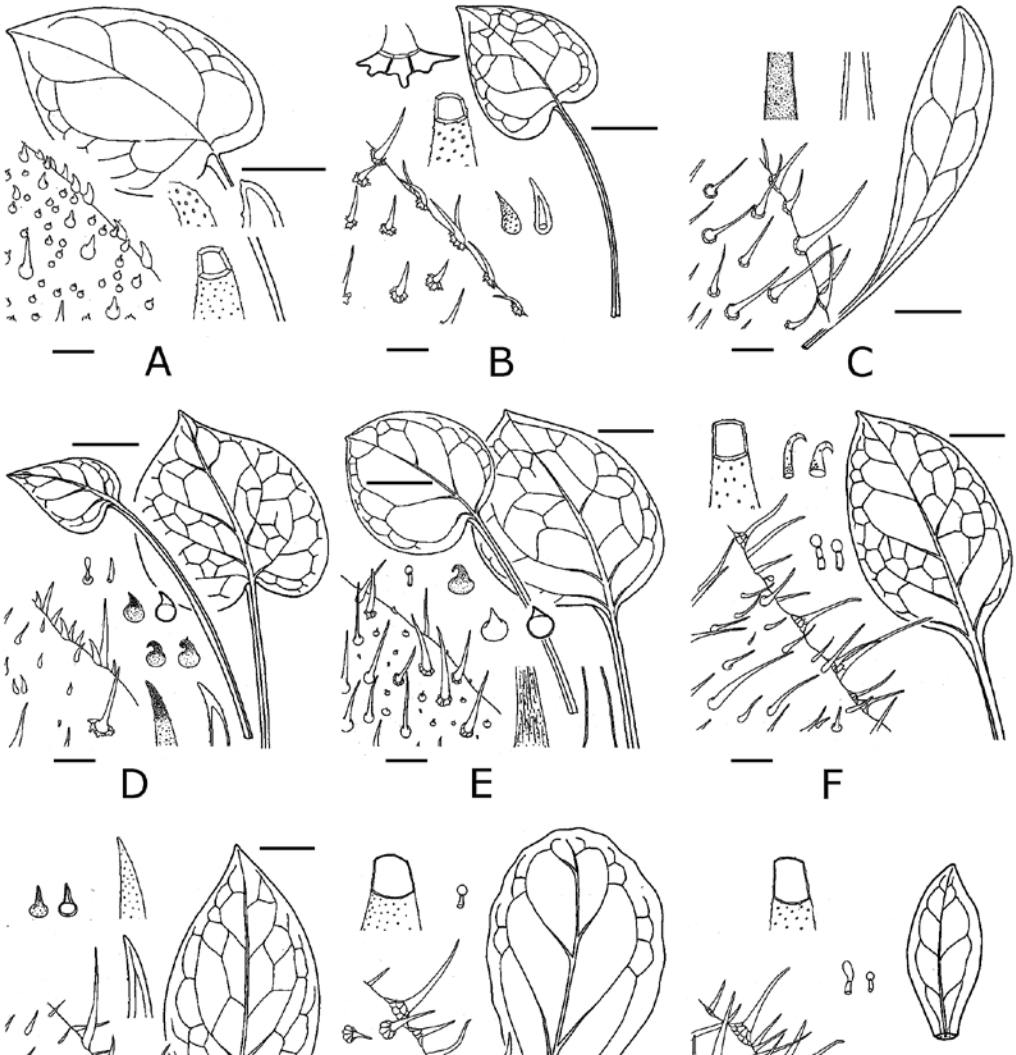


Figure 6. Pubescence of juvenile leaves: A – *Brachybotrys paridiformis*; B – *Omphalodes verna*; C – *Asperugo procumbens*; D – *Symphytum cordatum*; E – *S. tuberosum*; F – *S. caucasicum*; G – *S.* × *uplandicum*; H – *Borago officinalis*; I – *Anchusa pseudochroleuca*. Scale bars: Leaf – A, I = 5 mm, B, C, D, E, F, G, H = 10 mm; Part of leaf with trichomes – A–I = 0.25 mm.

minute, squarrose, sessile and transparent trichomes. Juvenile leaves' blades in the spring forms are lanceolate (13–20 mm long, 3–4 mm wide), with rounded or slightly acuminate apex, narrowed base, smoothly transforming into a petiole equal to one-half of the leaf blade length. Juvenile leaves' blades in winter forms spatulate (8–15 mm long, 3.5–6 mm wide), rounded at tip. Petioles 5–8 mm long, winged. Even though both seasonal forms are known to have moderate strigose pubescence on their leaves, the density of trichomes on the adaxial surface in the latter is higher than on the abaxial one (Fig. 7O; Table 5). Contrary to the former group, all hairs

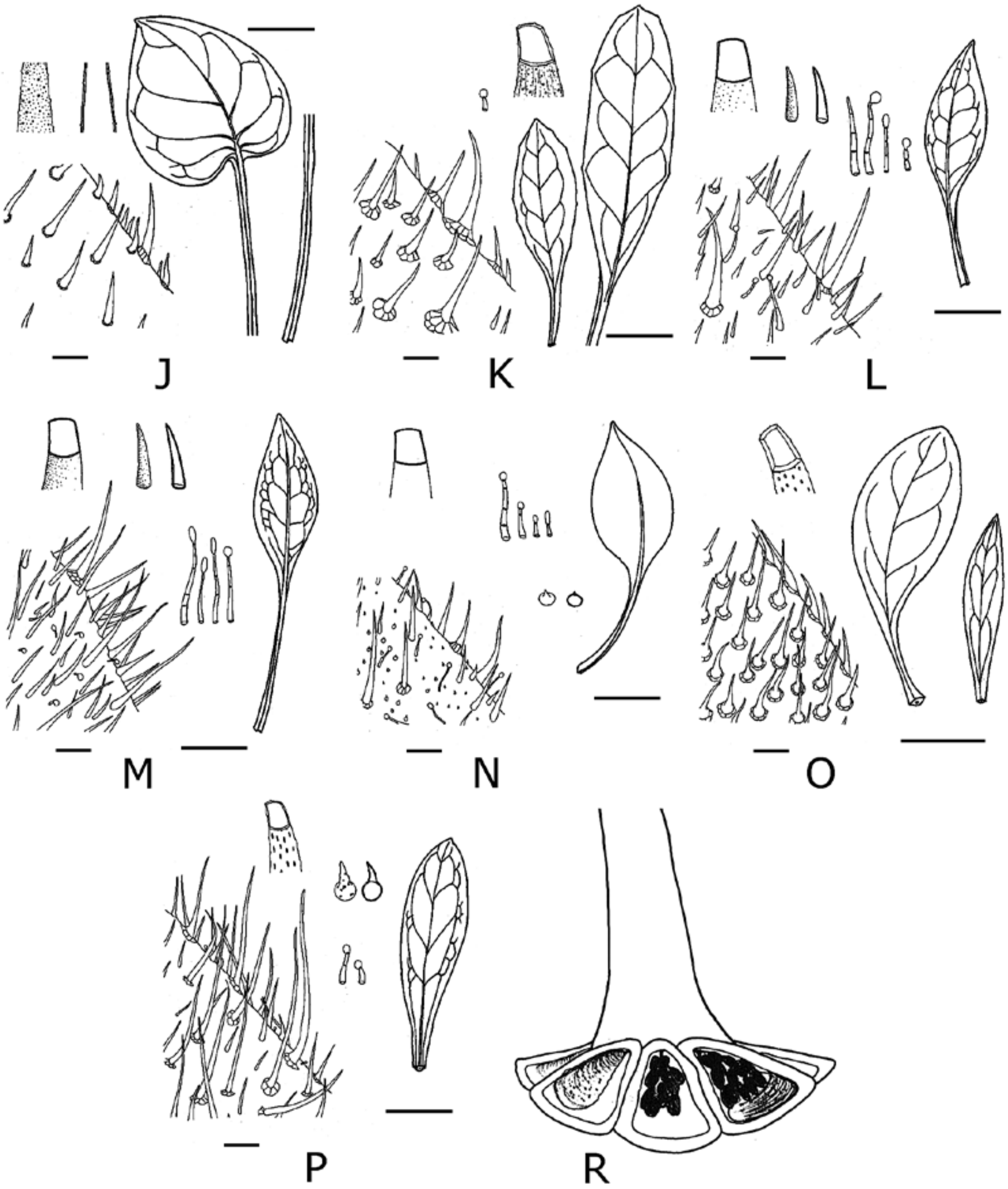


Figure 7. Pubescence of juvenile leaves: J – *Brunnera sibirica*; K – *Lycopsis arvensis*; L – *Pulmonaria rubra*; M – *P. mollissima*; N – *P. obscura*; O – *Lithospermum arvense*; P – *Echium vulgare*; R – Epidermal cells with cystoliths of *Lycopsis arvensis*. Scale bars: Leaf – J–N = 10 mm, P = 5 mm; J–P – Part of leaf with trichomes = 0.25 mm.

subtended by 'rosettes' are long, one-celled, subulate (straight or bent), thick-walled, lacking inner contents and possess a small peculiar ring-shaped thickening near the base. Hair surface grumous. 'Rosettes' are represented by a small number of thick-walled epidermal cells. Mature vegetative and reproductive plants of both spring and winter forms have leaves of similar shape (lanceolate, with bluntly acuminated apex) and similar pubescence types. It is present on both leaf surfaces, denser on the adaxial side, uniform, strigose (Fig. 8 G–K). Trichomes long, subtended by 'rosettes', opaque, subulate (straight), slightly swolled at base; hair surface grumous. 'Rosettes' many-celled (5–15(20) cells).

Species of an ancient Mediterranean genus *Echium* L. (tribe *Echieae*) are mostly herbaceous polycarpic plants and shrubs, widespread in the western 'subregion' of Ancient Mediterranean area. *Echium vulgare* L. is a tap-rooted hemicryptophyte with dicyclic, semi-rosette, monocarpic shoots. The plant is common in steppes, meadows, grassy slopes, abandoned areas, sand dunes near seas, river gravel patches, on mountain and foothill fields and rocky slopes (Popov 1953; Hege 1972). Germination epigeous, hypocotylar. Cotyledons wide-oval (8–15 mm long, 5–10 mm wide), widely rounded at tip, with narrowed blade base and gradually turning into a short petiole, margins slightly undulate, indumentum present on both surfaces but denser on the adaxial side (Fig. 1 P). Pubescence uniform, setose (Fig. 4 G, H). Simple hairs sessile or subtended by 'rosettes'. The former are unicellular, long, subulate (straight or slightly bent), rounded and swollen at base; surface tuberculous or tuberculous fine-grained. Such hairs are, as a rule, confined to the abaxial leaf blade surface. 'Rosette' trichomes are also one-celled, long, subulate (straight), with cuneate widening at base. 'Rosettes' are poorly expressed, 5–7-celled. Juvenile leaves' blades within the limits of a single rosette vegetative shoot vary from oblong-spatulate and oblong-ovate to oblong, with rounded apex and base gradually turning into a short petiole. Foliage leaves and bracts belonging to an elongated reproductive shoot are sessile; their blades being lanceolate and narrow lanceolate, with slightly broadened base. Leaves of both formations as well as juvenile ones, have moderate mixed strigose pubescence on the adaxial and hispid on the abaxial side(Fig. 7 P). Hairs simple and glandular. Sessile simple hairs short, thin, one-celled, subulate (straight), thin-walled, with fine-grained, dark green contents near the tip; their surface is tuberculous. Trichomes subtended by 'rosettes' long, one-celled, subulate (straight and bent), thin-walled, filled with lumpy greenish-brown contents; surface also tuberculous (tubercles oval and oblong). 'Rosette' cells with slightly thickened walls. Glandular hairs possess well-developed stalks and clavate heads, prominent due to its dark green filling. The hairs are located along the midrib and leaf blade margins.

Discussion

The results of the present investigation have shown that most species become pubescent very early in ontogeny and pubescence is present not only in young leaves but also in the cotyledons and hypocotyls. The detailed analysis of pubescence in vegetative organs revealed a great diversity of trichome types (Table 1–4). According to their persistence / duration of existence, trichomes of all studied species belong to the 'persistent' group. Among them there are simple and glandular hairs. They can be unicellular subtended by the 'rosettes' or lack them and they vary in shape (subulate, straight, hooked, sickle-shaped or cylindrical (uni- and multicellular)). Very often, the formation of a simple one-celled trichome is facilitated by neighbouring epidermal cells, which form a 'prop' or 'rosette' at its base. The latter can be composed of few or many cells (up to 30 cells in *Borago officinalis*) and can be one-, two- or multi-layered; their cell walls can be impregnated with mineral substances and protoplasts may contain grained, layered or granular-layered groups of calcareous and siliceous compounds. Glandular trichomes with uni- or multicellular stalks and one-celled heads are not uncommon. An important trait in addition to the pubescence description is the ultrasculpture of trichome surface: smooth, fine-grained, tuberculous fine-grained, scabrous-plicate, tuberculous, grumous (Table 3). Depending on the hair type, their location and direction of growth (in relation to the surface they grow on), the following pubescence types were identified in the studied species: stiff short-haired (hirtum), stiff

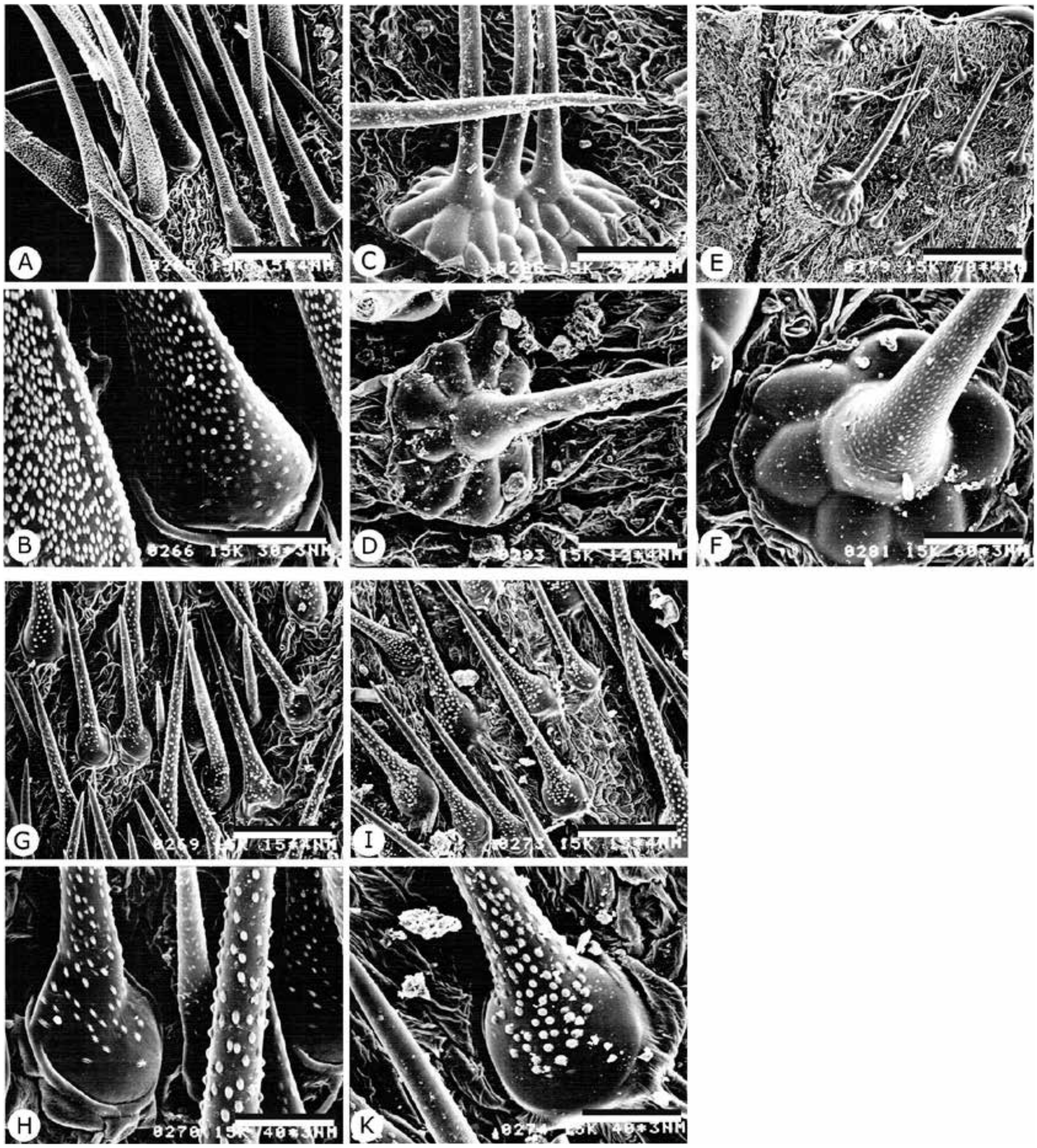


Figure 8. Pubescence of definitive leaves: A, B – *Asperugo procumbens*; C–F – *Lycopsis arvensis*; G–K – *Lithospermum arvense* (A–D, G, H – spring and E, F, I, K – winter forms of plants). Scale bars: E = 600 µm; C = 200 µm; A, G, I = 150 µm; D = 120 µm; F = 60 µm; H, K = 40 µm; B = 30 µm.

(coarse)-haired (hirsutum), hispid (scabrous), setose, strigose and mixed. The pubescence can be more or less uniform or irregular, restricted to the upper (adaxial) and (or) lower (abaxial) leaf blade surface; depending on the trichome density, it can be divided into scarce (rare), moderate, dense or very dense (heavy) (Table 4).

Most of the studied species are characterized by epigeous germination of the nutlets. Cotyledon blades are brought out to the surface due to hypocotyl elongation (up to 35 mm in *Asperugo procumbens*), hypocotylar germination type, or because of the excessive growth of cotyledon petioles (cotyledonar type). In *Symphytum cordatum* and *S. tuberosum*, the germination type is semi-epigeous, when cotyledons appear above the ground but retain the nutlet's coating (fruit wall) fully or partially, release can take place due to cotyledon blades' growth.

Blades of assimilating cotyledons vary in shape, which ranges from widely- , oblong- and acuminate-elliptic to orbicular- or widely ovate or spatulate (Fig. 1). Indumentum can be restricted to only one side of the leaf or can occur on both sides. In case of the latter, the adaxial surface is usually densely pubescent while the abaxial one has only sparse hairs. Assimilating cotyledons with pubescence restricted to one (abaxial) side are common in the tribes Cynoglosseae (*Brachybotrys paridiformis*, *Omphalodes verna*) and Anchuseae (sections Tuberosa, Symphytum, Anchusa, Borago, the genus *Brunnera*). Both surfaces of cotyledon blades are often pubescent in the species of Eritrichieae, Anchuseae (sections Caerulea, Molles, Asperae; the genus *Lycopsis*), Lithospermeae (section Rhytispermum) and Echieae. An important diagnostic trait at the species level is the distribution of various hair types within the blade. Usually, simple trichomes (subtended by 'rosettes' or lacking them) are distributed uniformly throughout the entire surface of a cotyledon; their density can be rare (sparse) or moderate (Table 4). In some species, glandular trichomes are formed on either side of the blades (*Pulmonaria mollissima*, *P. rubra*), while in other ones, they occur only on the abaxial side along large protruding veins (*Symphytum caucasicum*, *Borago officinalis*, *Pulmonaria obscura*); yet another group of species has glandular hairs alongside the blade margins (*Symphytum cordatum*, *S. tuberosum*, *Anchusa pseudochroleuca*). The pubescence of cotyledon petioles as taxonomically valuable trait is not less important. In most species, the cotyledon petioles are covered by trichomes of the same kind as their blades; some representatives of the tribe Cynoglosseae (*Brachybotrys paridiformis*, *Omphalodes verna*) and *Symphytum cordatum* from the tribe Anchuseae are an exception and have glabrous petioles.

In many cases, the pubescence of assimilating organs tends to change significantly as the plant ages. Thus, cotyledon pubescence in half of species investigated is formed solely by simple, sessile, one-celled hairs (subtended by 'rosettes', 'props' or without them) and by uni- or multicellular cylindrical hairs; at the same time, juvenile leaves are known to possess glandular hairs together with simple ones. Some other plants (*Brachybotrys paridiformis*, *Omphalodes verna*, *Asperugo procumbens*, etc.) lack glandular hairs, but their pubescence also varies over age: from uniform rare (scarce), hispid pubescence (cotyledons in *B. paridiformis*, *O. verna*) and setose (cotyledons of *A. procumbens*) it changes to a dense indumentum of stiff, short hairs, moderate hispid and moderate strigose (juvenile leaves), respectively. *Brunnera sibirica* appears to be an exception because its juvenile leaves retain the same type of pubescence as reported for its cotyledons. The difference in the pubescence of juvenile and adult plants is not significant; it is related to qualitative factors. For instance, with aging in *Borago officinalis*, *Anchusa pseudochroleuca*, *Lycopsis arvensis*, *Lithospermum arvense*, *Echium vulgare*, etc., multi-layered (up to 3–4 cell layers) 'props' or 'rosettes' start to form under the simple unicellular trichomes. 'Rosettes' contain well-developed cystoliths. Quite a number of hairs covering old leaves are dead, with thick walls impregnated with silicon. Glandular trichomes are retaining vitality longer than any other kind. In *Pulmonaria* and *Lithospermum*, the density of pubescence increases greatly during ontogeny, the indumentum changes from moderate to dense and heavy in mature reproductive plants.

A certain correlation can be traced between the pubescence of vegetative structures and heteroeremocarpy, characteristic of Boraginaceae. A striking example is the shift of pubescence in winter forms of *Lithospermum arvense*: from scarce strigose in cotyledons to dense in juvenile leaves, whereas in spring forms the pubescence remains unchanged. Therefore, pubescence characteristics can be used not only to identify individual species at early ontogenetic stages, but also to recognize seasonal forms within a species, which has previously been shown in winter and spring forms of wheat (Vasilchenko 1936).

The results of morphological and anatomical studies of trichomes in Boraginaceae by means of various techniques confirmed already existing records (Solereder 1899; Metcalfe & Chalk 1950; Uphof 1962; Yurison 1965; Kochetova & Kochetov 1982) of one-celled hairs, swollen at base, subtended by cystolith-containing basal 'rosettes'. The latter are rosette-like groups of large epidermal cells, with mineralized cell walls and protoplasts containing large calcareous and siliceous cystolith bodies. According to Aneli (1975), a fully formed cystolith is subdivided into a saddle, a stalk, a cavity (of the cystolith), the main body, a cushion and a crown (Fig. 5 B). In *Lycopsis arvensis*, cystolith bodies mineralized by calcium carbonate, which are located in the bases of one-celled hairs and in epidermal 'rosette' cells, are sack-like or cluster-like structures hanging down inside the cell on a very short stalk (Fig. 7 R). Such sack-like (rounded or oval) cystolith bodies can be found in *Pulmonaria rubra*, *P. obscura*, *P. mollissima*, *Lappula squarrosa*, *Echium vulgare*, *Asperugo procumbens*, *Symphytum caucasicum*, *S. × uplandicum*, *Borago officinalis*, *Anchusa pseudochroleuca* and *Lithospermum arvense*. In the leaves of mature reproductive plants of *Lappula squarrosa*, cystolith bodies are formed not only inside the trichomes and epidermal 'rosette' cells, but also in the palisade tissue of the leaf underneath; they are entirely filled with mineralized contents and contain no chloroplasts (Fig. 5 C).

Cystoliths start to occur as early as in cotyledons and young leaves at the bases of one-celled trichomes and in the 'rosette' cells as sack-like cell wall invaginations. Further formation of the layered cystolith body implies the division or branching of the 'main axis' into radial cords penetrating the concentric layers. By the end of the vegetation period, the cystolith body is covered by papillae and mineralized with calcium carbonate. As the leaves age, the mineralization increases. Besides cystoliths, we were able to discover fungal spores around cotyledon hair bases (Fig. 5 A) in *Pulmonaria obscura* and other species.

Studies of cellulose sheaths formed by the infected cell's wall around the hyphae of parasitic and mycorrhizal fungi, producing superficial layers of calcium, has led us to a suggestion that at least some cystoliths can occur as a response to the fungal infection and the formation of the protective sheath by the cell (following Chistikina's (1961) studies of a similar phenomenon in *Ficus elastic*, *F. carica*, *Ruellia formosa*, *Boehmeria macrophylla*, *Pellionia pulchra*, *Pilea grandis*, etc.). However, all in all, the question of formation, structure and chemical nature of cystoliths in Boraginaceae remains open for discussion.

Our ontogenetic morphological and anatomical study of pubescence types in the vegetative organs of Boraginaceae revealed a great diversity of hair types, stability of some of them at the species, or in some cases, at even higher taxonomic level. Thus, the greatest variety of trichomes together with various types of surface ultrasculpture (Table 3) is found in the genera *Symphytum*, *Anchusa* and *Pulmonaria* (tribe Anchuseae). They are also known to have an early occurrence of mixed pubescence. In representatives of Cynoglosseae, Lithospermeae and Anchuseae (sections Anchusa – *Brunnera sibirica* and Symphytum – *S. × uplandicum*) we reported the absence of glandular hairs at any ontogenetic stage. On the contrary, in the species of sections Tuberosa, Caerulea, Anchusa, Molles, Asperae (tribe Anchuseae), glandular pubescence occurs as early as in seedlings and persists in mature reproductive plants. In the course of investigation, the authors were able to identify additional, previously neglected details of early developing hair surface. Together with the shape and size of cotyledons and the first juvenile leaves, pubescence type, trichome types, their macro- and microstructure appear to be a reliable diagnostic trait if applied at early stages of ontogeny. Pubescence traits can be used to identify species at various developmental stages and also allow us to recognize seasonal forms of a species.

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