

Propagation modes in Boraginaceae: Biomorphological and anatomical analyses

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Summary: 14 species of Boraginaceae were studied to reveal the diversity of vegetative propagation modes, connected with adaptation to habitats and different strategies of exploring, expanding to and populating new sites of growing space ('attached' and 'creeping' forms). The main one is sarmentation, accompanied by the formation of sprouts originating from roots (soboles) or shoots. A certain correlation between sarmentation types and the plant's life form, shoot structure and root system type is shown. Tap-rooted woody biormorphs are known to propagate by means of sobole formation. Perennial herbaceous plants, in the vast majority with short tap root or fibrous root systems, appear to demonstrate a huge variation in sarmentation types. It is performed by means of sobole formation as well as through the separation of parts of underground epigeogenous, hypogeogenous and epigeogenous-hypogeogenous rhizomes, aerial rhizomes, specialized procumbent stolon-like biennial shoots as well as by tubers. The ability to form vegetative diaspores is determined by the microstructure of metamorphosed organs of reproduction, i.e. marked parenchyma development, extensive supply of ergastic substances (starch, aleurone), poor lignification and lack of supporting tissues and cork (excluding *Ehretia*, *Mertensia*); the protective function is performed by metacutinized peripheral bark layers and wide dead leaf bases. Specialized organs of vegetative propagation are also characterized by accelerated differentiation and development, short-term activity of the vascular cambium, their fast renewal and additional increased ability to produce adventitious buds and roots. The revealed diversity of vegetative dispersal and propagation ways facilitated the successful expansion of Boraginaceae into various ecological niches in the vegetation of subtropical and tropical areas.

Keywords: Boraginaceae, life forms, vegetative propagation, sarmentation, particulation, biomorphology, anatomy, *Ehretia*, *Symphytum*, *Omphalodes*, *Brunnera*, *Brachybotrys*, *Mertensia*

Materials and methods

The present study encompasses 14 Boraginaceae species, represented by both woody and herbaceous life forms, growing in various environmental conditions in the temperate zone of European Russia, Asia and in the subarctic floristic kingdom: *Symphytum caucasicum* M. Bieb., *S. × uplandicum* Nyman, *S. cordatum* Waldst. & Kit. ex Willd., *S. tuberosum* L., *Pulmonaria rubra* Schott, *P. obscura* Dumort., *P. mollissima* A. Kern., *Omphalodes verna* Moench, *Brunnera sibirica* Steven, *Anchusa pseudocholeuca* Desjat.-Shost., *Ehretia acuminata* R. Br., *E. corylifolia* C.H. Wright, *Brachybotrys paridiformis* Maxim. ex Oliv., *Mertensia maritima* (L.) Gray. The material for comparative biomorphological and anatomical analyses was collected in the wild (Russian Federation: Moscow, Oryol, Tambov and Murmansk region; Moldova and Ukraine) as well as in cultivation (Botanic Garden of Lomonosov Moscow State University and N.V. Tsitsin Main Botanic Garden). For better biomorphological characterization of certain species, herbarium specimens from D.P. Syreishchikov's herbarium collection [MW] (Biological Faculty, Moscow State University) were examined. The anatomy of vegetative organs in juvenile and definitive (mature) plants was studied in longitudinal and cross sections (transverse

sections); temporary freehand sections were made using a razor blade. The presence of ergastic substances (starch, aleurone, etc.) was identified according to the reference book on microscopic techniques (BARYKINA et al. 2004). Plants were photographed with a smartphone (iPhone X) camera, anatomical preparations showing the structure of axial organs – by means of the light microscope Axioplan-2 and AxioCam MRc camera. Suitable photographs were edited using Adobe Photoshop CS6 software.

Abbreviations used in Figures. *adb* – adventitious bud; *adr* – adventitious root; *as* – anastomosing strip; *asrz* – ascending rhizome; *axb* – axillary bud; *btc* – bud trace; *ck* – cork; *cmb* – cambium; *ct* – cortex; *cvb* – collateral vascular bundle; *dllb* – dead leaf bases; *elid* – elongated internode; *elsh* – elongated shoot; *end* – endoderm; *epd* – epiderm; *eph* – external phellem; *eprz* – epigeogenous rhizome; *exd* – exoderm; *fl* – foliage leaves; *flb* – foliage leaf base; *gsh* – generative shoot; *hgrz* – hypogeogenous rhizome; *hp* – hypopodium; *hr* – horizontal root; *ifcmb* – interfascicular cambium; *ifl* – inflorescence; *iph* – internal phloem; *irzb* – isolated rhizome branches; *lc* – lacuna; *lp* – leaf primordia; *lr* – lateral root; *lrp* – lateral root primordium; *ltb* – lateral tuber; *ltc* – leaf trace; *mray* – medullary ray; *pbsh* – parenchymal bundle sheath; *pdm* – periderm; *ph* – phloem; *pp* – parent plant; *prc* – pericycle; *prz* – parenchymal zone; *pt* – pith; *ptb* – parent tuber; *ray* – primary parenchymal ray; *rnb* – renewal bud; *rtc* – root trace; *rxac* – rhexigenous air cavity; *rz* – rhizome; *rzd* – rhizoderm; *sb* – sobole; *scl* – scalariform leaf; *sclt* – scalariform leaf trace; *sg* – starch grains; *shid* – shortened internode; *shmst* – shoot meristem site; *shsh* – shortened shoot; *sm* – stoma; *sphc* – suberized phellem cells; *st* – stele; *stbch* – sympodial tuber chain; *stl* – stolon; *tb* – tuber; *tr* – tap root; *vcmb* – vascular cambium; *vsh* – vegetative shoot; *xl* – xylem; *xlv* – xylem vessel.

Results and discussion

Vegetative propagation holds a key position in the somatic evolution of flowering plants. It is particularly important, if the sexual (seed) reproduction is reduced or temporarily completely suppressed. The vegetative propagation is “the most reliable tool, providing a species with the insurance against extinction” (LEVINA 1981: 11). Structurally and physiologically, it is based on high regenerative activity, connected not only with the presence of apical and lateral meristems, but also with the ability of living cells to dedifferentiate in permanent tissues (BUVAT 1950), i.e. the possibility to return to a state close to the embryonic, under certain conditions. This would lead to the emergence of new meristematic sites, which subsequently would give rise to adventitious buds and root primordia, to protective and other tissues.

The classification of vegetative propagation types is based on morphology of the organ producing vegetative diaspores, the degree of ‘mobility’ shown by the new primordial plants, the sequence of their rooting and the separation from the parent plant, the degree of rejuvenation and the vegetative offspring. Considering all these traits together, three main types can be identified, namely: particulation, sarmentation and vegetative diaspore production.

Numerous literature sources point out the correlation between the reproduction mode and the plant’s life form, its shoot structure, root system type and anatomy of vegetative organs. The most pronounced regenerative abilities as well as a great variety of organs specialized in vegetative propagation is found in haloxial families, in particular, Ranunculaceae (BARYKINA 1995). Data on vegetative propagation in the family of interest, Boraginaceae, are rather scarce; mostly they are related to herbaceous taxa from central Russia.

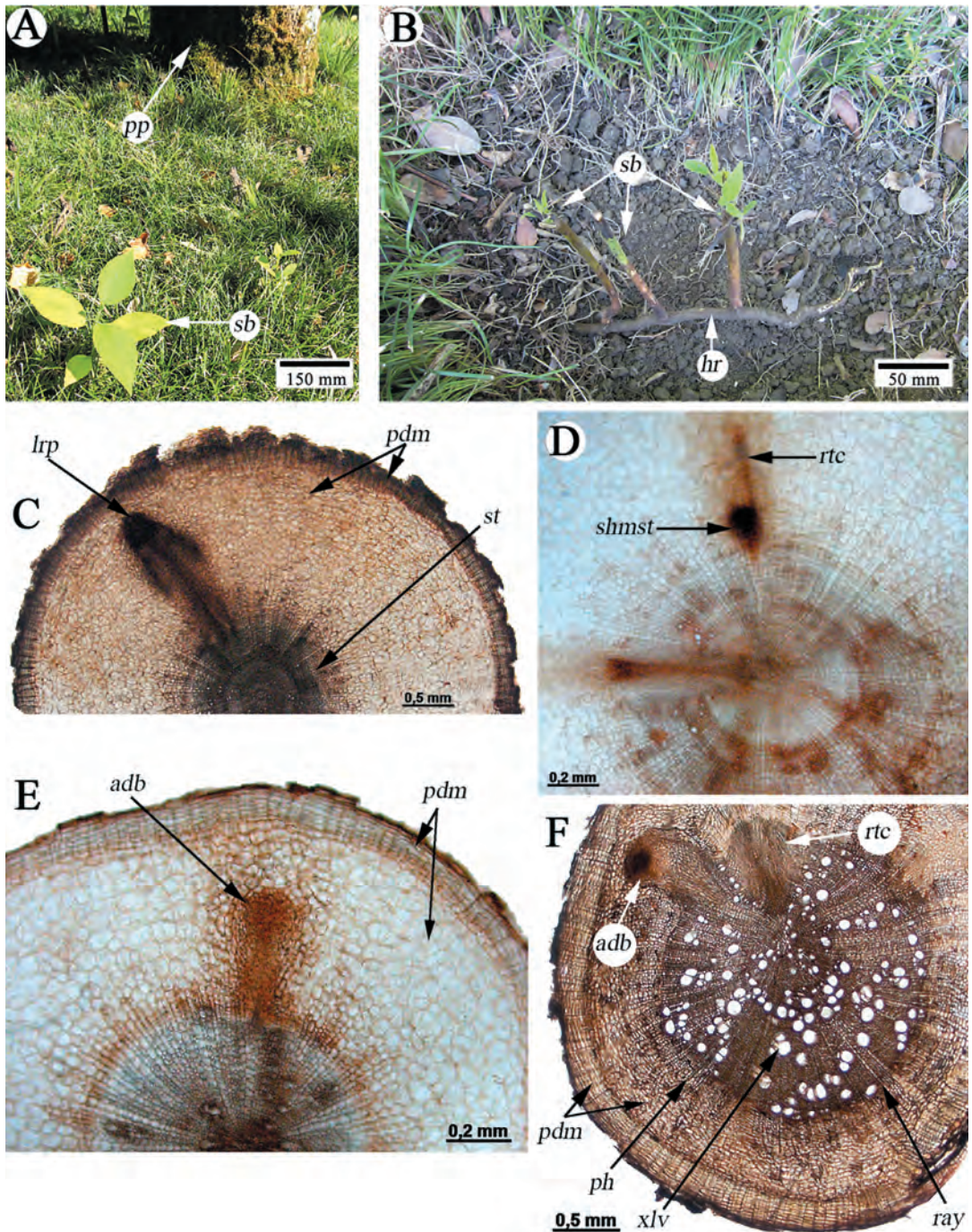


Figure 1. *Ehretia acuminata*. A – emerged soboles 3–5 m apart from the parent tree; B – a part of bare root with three soboles covered by leaves; C – a developed lateral root primordium; D – a fragment of the root cross section with a shoot meristem site at the base of the root trace; E, F – initiation of adventitious buds at the level of cambium and soft bast.

The current study presents the results of comprehensive morphological and anatomical research aimed to investigate the vegetative reproduction in the representatives of two subfamilies, Ehretioideae (woody species of *Ehretia*) and Boraginoideae (herbaceous polycarpic plants from Central Europe, Asia, the Far East and subarctic (circumboreal) areas) as well as the data on the

vegetative reproduction in Heliotropoideae taken from the publications by VASILEVSKAYA & AKYEVA (1967). The authors provide information on the development of soboles on horizontal and vertical roots of *Heliotropium arguzioides* Kar. & Kir. and *Tournefortia sogdiana* Popov – herbaceous psammophytes from Kara-Kum desert, where the sand blown away by the wind causes the naked (exposed) roots to develop numerous shoots which would form a powerful clone at the age of 3–4 years.

As inferred from literature sources and personal observations, the vegetative propagation in Boraginaceae mostly occurs in the form of sarmentation, i.e. through the formation of sarments (offshoots) of shoot origin and soboles of root origin. Soboles formation is found in both woody and herbaceous representatives. According to RAUH's (1937) classification, this ability is compulsory in both groups. In *Ehretia acuminata* and *E. corylifolia*, roots do not start producing adventitious buds until at least the 6th year of the seedling's life (BARYKINA 2012). In mature individuals, the soboles or root suckers (shoots originating from roots) most frequently develop on horizontal roots, 4–6-year-old, 5–10 mm thick, located 7–12 cm below the soil surface within 2.5–3 m apart from the mother plant's trunk (Fig. 1A, B). The minimal diameter of the roots capable of producing soboles is 3 mm. The growing offshoots greatly influence the mother root, causing its marked thickening from the place of the shoot formation upwards. The particularly visible root thickening zone is 8–10 cm long; there, the root becomes 3–5 times thicker. Macronutrients, drained from the offshoot leaves, stimulate the development of new lateral roots nearby (Fig. 2A). Our microscopic study of differently aged roots in Ehretioideae, carried out for the first time ever, revealed the pattern of emergence and further growth of secondary lateral roots and adventitious buds, from which the soboles emerge (Fig. 1C–F). High regenerative ability of roots is connected with the extensive production of parenchyma of phloem and xylem origin, differentiation of the wide parenchymal zone of phellodermal origin and abundant presence of storage starch, while supporting tissues appear to be rather poorly developed.

Most often, adventitious buds originate in the soft bast near the cambium, rarely at some distance from it (Figs 1E; 2B, C), usually close to the secondary or primary medullary ray or directly within it. When the meristematic site enlarges, the ray greatly expands in width, becoming 6–18 cell-layer-wide at the cambial border. Newly emerging cells, mostly belonging to the xylem part of the ray, somewhat elongate radially and gradually transform into tracheidal parenchyma; cell cavities fill in with secondary starch. The above located meristematic site develops into a bud with the apex and the first leaf primordia. Cambial strands differentiating in it form tracheal elements and sieve tubes, which adhere directly to the vascular system of the mother root's last annual ring. Sometimes, adventitious buds originate in the ray parenchyma outside the cambial border. As a rule, this would be one of multiple secondary rays. Often, meristematic bud primordia emerge nearby the thin, ephemeral, mycorrhizal sucking, lateral branches of the parent root (Fig. 2D), where tissues are in more favourable conditions in terms of water supply, nutrition and aeration. Transformations of small callus-like meristematic sites, emerging directly in the pericyclic or cambial zone of the absorbing lateral root traces (before they reach the surface of the parent root Fig. 1D), into bud primordia have been recorded. This fact has been pointed out in relevant literature (VASILEVSKAYA & AKYEVA 1967) in some other sobole-producing Boraginaceae, e.g. *Heliotropium arguzioides* and *Tournefortia sogdiana*. Fully formed buds develop into leaf-bearing shoots 2–3 years after originating.

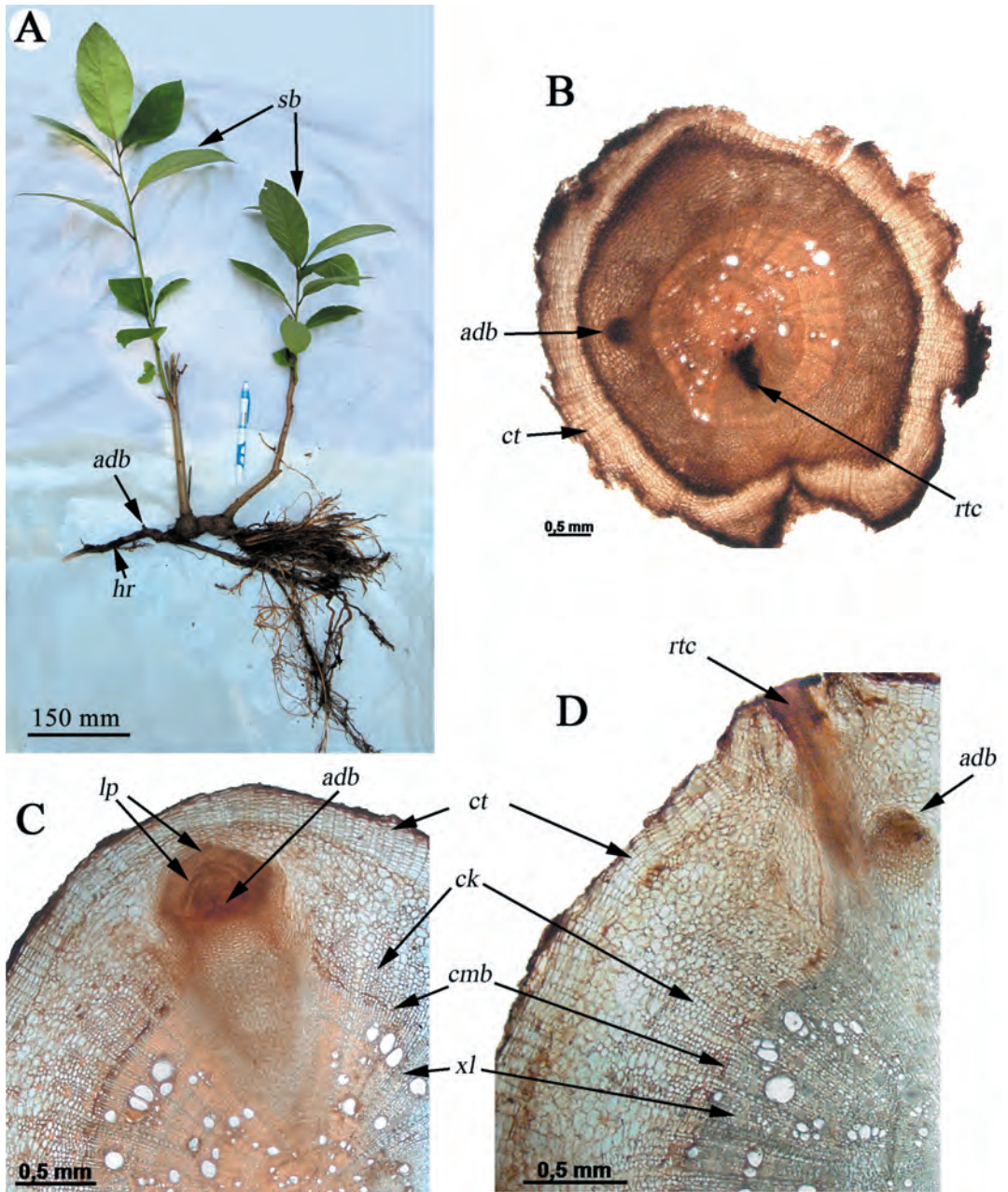


Figure 2. *Ehretia corylifolia*. A – a branching root with adventitious buds and soboles, dug out from a depth of 12 cm; B – parent root with a primordially adventitious bud and a withered ephemeral lateral root trace; C – fully formed bud in the pith part of the medullary ray near the cambium in a 4-year-old root; D – the formation of a bud primordium tightly adherent to the functioning lateral root trace in the bast of 4–5-year-old tetrarch parent root.

The described above pattern of emergence and further development of adventitious buds on the roots of *Ehretia* species is very similar to that in plants we have studied before, such as woody *Robinia pseudoacacia* (BARYKINA 1958), *Malus*, *Hippophae rhamnoides* (LYARSKAYA 1957, 1958) or perennial herbaceous Boraginaceae, in particular, *Symphytum caucasicum* and *S. × uplandicum* (Fig. 3A–E). But in herbaceous forms, contrary to the woody ones, adventitious buds appear at earlier age. Thus, usually horizontally prostrate lateral and adventitious skeletal roots developing

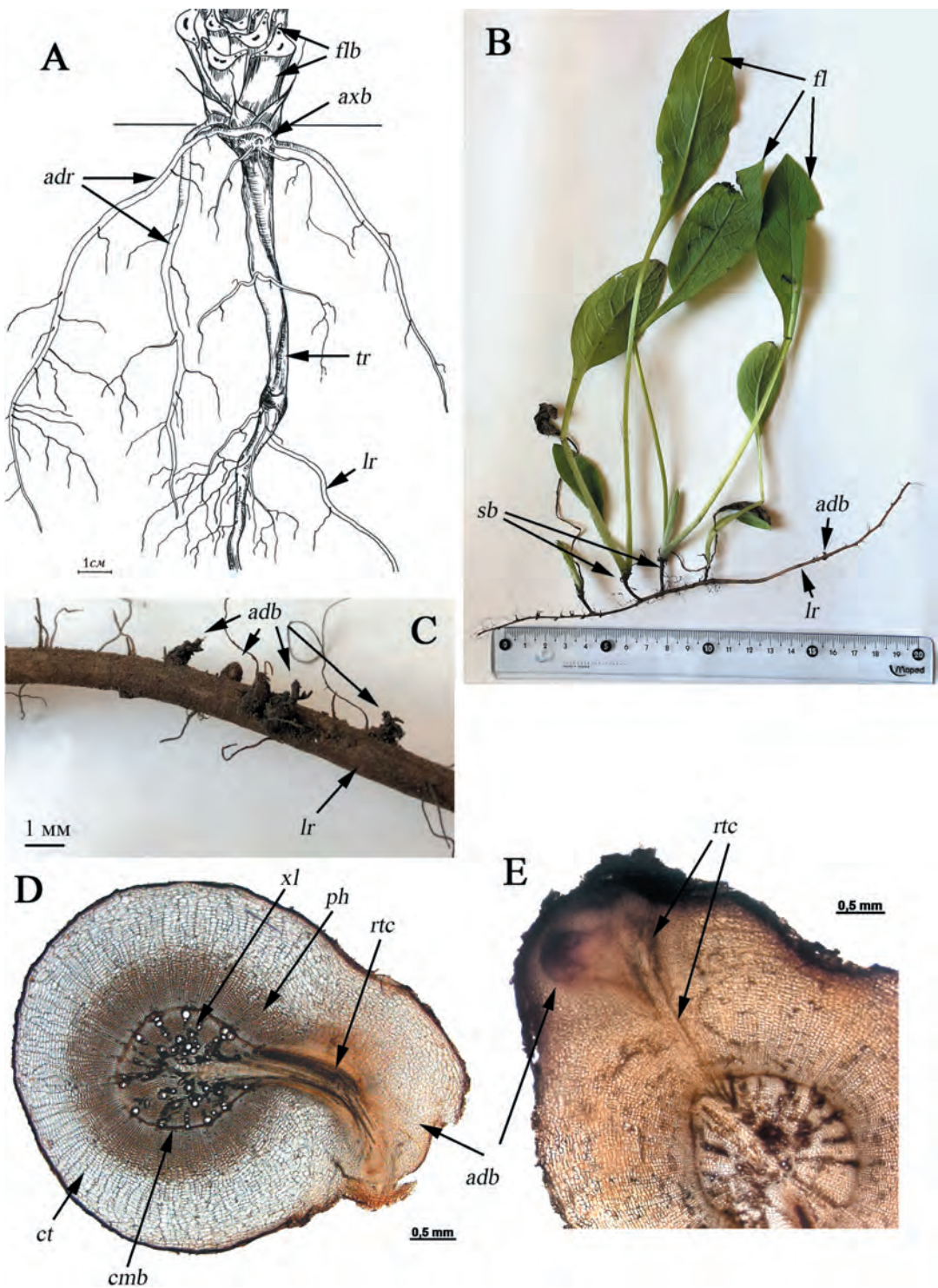


Figure 3. *Symphytum caucasicum*. A – annual mature vegetative plant with allo-homorhiz root system and bases of cut-off rosette leaves; axillary bud primordia in the basal zone of the tap root are visible; B – soboles on the parent plant's horizontal lateral root; C – series of adventitious buds on the adaxial root surface; D – transversal section of a diarch root with an adventitious bud developed adhering to the root trace; E – adventitious bud, initiated in the superficial parenchymal zone; its vascular tissues have connected to the parent root's stele by means of the ephemeral root's vascular system.

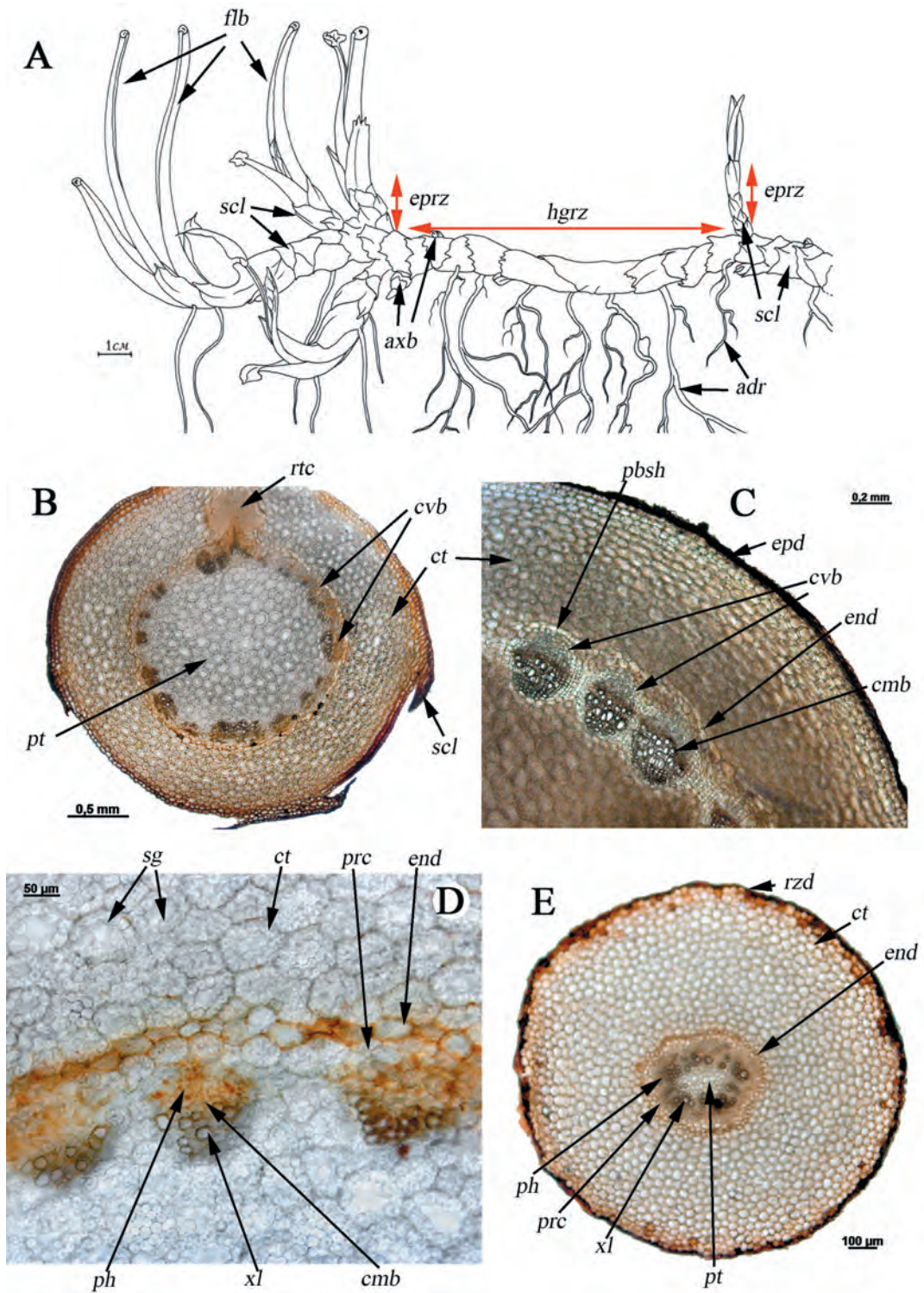


Figure 4. *Brunnera sibirica*. A – dimorphic structure of the underground rhizome in the reproductive stage; B – cross section of the basal internode in an annual epigeogenous rhizome near the scalariform leaf insertion and the adventitious root origination area; C – a segment of a cross section of a 2-year-old hypogeous scaled rhizome with vascular bundles; D – a fragment of an annual epigeogenous rhizome at the stele-cortex border; E – cross section of a 2-year-old pentarch adventitious root.

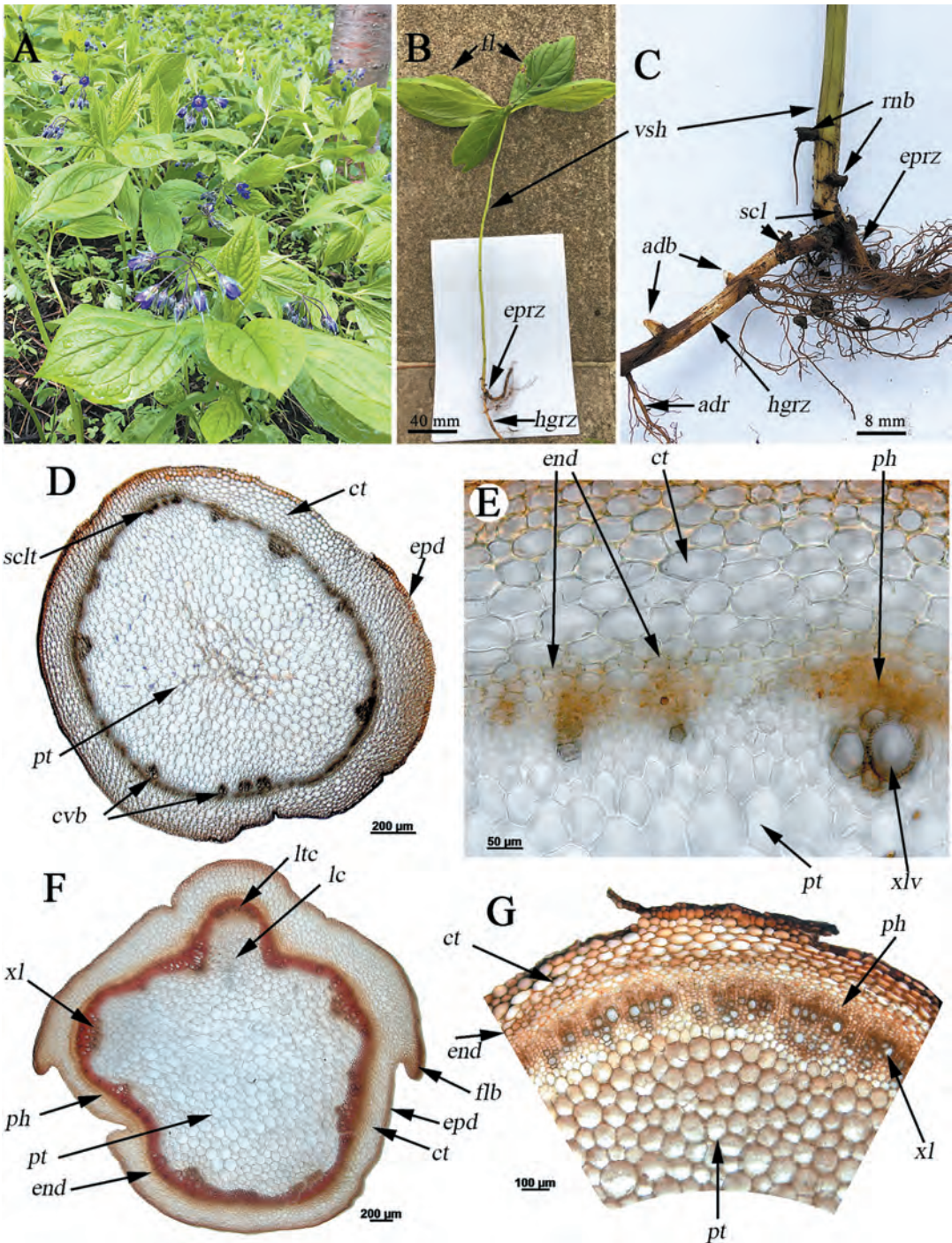


Figure 5. *Brachybotrys paridiformis*. A – flowering and vegetative plants in MSU Botanic Garden; B – mature vegetative plant with small parts of the dimorphic rhizome; C – ibid., at higher magnification; zones of an annual vertical epigeogenous rhizome with renewal buds and the horizontal hypogeogenous rhizome with adventitious buds and nodal adventitious roots are clearly visible; D – anatomical structure of an annual epigeogenous rhizome near closely located nodes bearing scalariform leaves; E – cross section of an annual epigeogenous rhizome at high magnification, with arising vascular bundles and pronounced endodermis with Casparian stripes; F – cross section of an epigeogenous rhizome at the level, where vascular bundles of a foliage leaf are inserted into its stele; G – part of a 2-year-old horizontal hypogeogenous rhizome.

on basal metameres of the rosette in 2-year-old juvenile *S. caucasicum* plants become the site of adventitious buds origination (Fig. 3B, C). They arise acropetally, as the root elongates. Eventually, at the bases of developing soboles, their own root system forms – in future, it will determine their independent existence and the formation of new partial bunches.

Intense vegetative propagation and expansion is characteristic of Boraginoideae species with long rhizomes, primarily boreal and meadow plants, growing in marshy areas along rivers and streams. Their branched, plagiotropic, hypogeogenous rhizomes are found to occasionally form thick, often occupying large space clusters of aerial shoots with their own root system. Subsequently, when the older linking parts of the rhizome die off, these shoots separate and become autonomous individuals. Hence, life-forms with long rhizomes, in contrast to those with short ones (as in *Pulmonaria obscura*, *P. rubra*, *P. mollissima*), which are, in general, less vegetatively mobile, are able to increase quickly their population in number and actively explore, capture and occupy new areas.

In the long-rhizome group, there are life-forms showing pronounced structural and functional rhizome dimorphism, e.g. very common in *Brunnera sibirica* and *Brachybotrys paridiformis* (Figs 4A; 5A). These are shortened vertical epigeogenous rhizomes, emerging first from the renewal buds and possessing bundles of adventitious contractile roots, serving as so-called ‘centers of fixation’ as well as anisotropic relatively long-branched, sympodially growing, hypogeogenous ‘communication’ (according to SMIRNOVA’s terminology (1974)), rhizomes with a fringe-like root system, developing from the basal, deeply located axillary buds and serving for quick and efficient capture of new growing sites within the area populated by the species. Later on, near the soil surface, the terminal bud of such an anisotropic hypogeogenous rhizome forms a leaf-bearing, rooting, rosette-like shoot – the first link of the next newly developing epigeogenous rhizome. Vegetative propagation in plants with long rhizomes often starts late into their virginile stage and results in only partial rejuvenation of the vegetative offspring. As far as the microstructure of underground hypo- and epigeogenous rhizomes with different functional loads is concerned, in general, they retain the same species-specific pattern of organization, typical of a particular species and adapted to the habitat.

Minor differences between two rhizome types appear to be the consequence of the correlation between elements of the developing modified shoot (different leaf formations, node type, growth direction, shoot type and size, root formation, etc.). Thus, in particular, 2–3-year-old, hypogeogenous, horizontal, underground scalariform rhizome of *Brunnera sibirica* (Fig. 4B, C), rounded in cross section, is characterized by the presence of abundant piths (up to 50 cells in diameter). A relatively narrow cortex (14–20-layered) with its peripheral layer stained by tannins, functioning as protective covering, while the inner light one (lacking starch grains but with Casparian stripes) is the endodermis (Fig. 4D). The cells of one-layered pericycle are directly bordered by the endodermis. Adherent to the pericycle, there are relatively short medullary rays of different width (3–10 rows), which is due to different density and location of rounded vascular bundles. At some sites, they are spread far apart, at the other ones they are tightly connected (in groups of 6–8), as if they are fused in a single mass of tissues. The bundle sheaths are made of minute parenchymal cells, which form the peripheral part of the perimedullary zone from the large-celled pith’s side. Near the nodes, traces of diverging adventitious roots and lateral buds can be seen. The adventitious roots are pentarch, with a central parenchymal cord (Fig. 4E). The root epidermis consists of cells with tanniferous outer tangential walls. The root cortex of 17–18

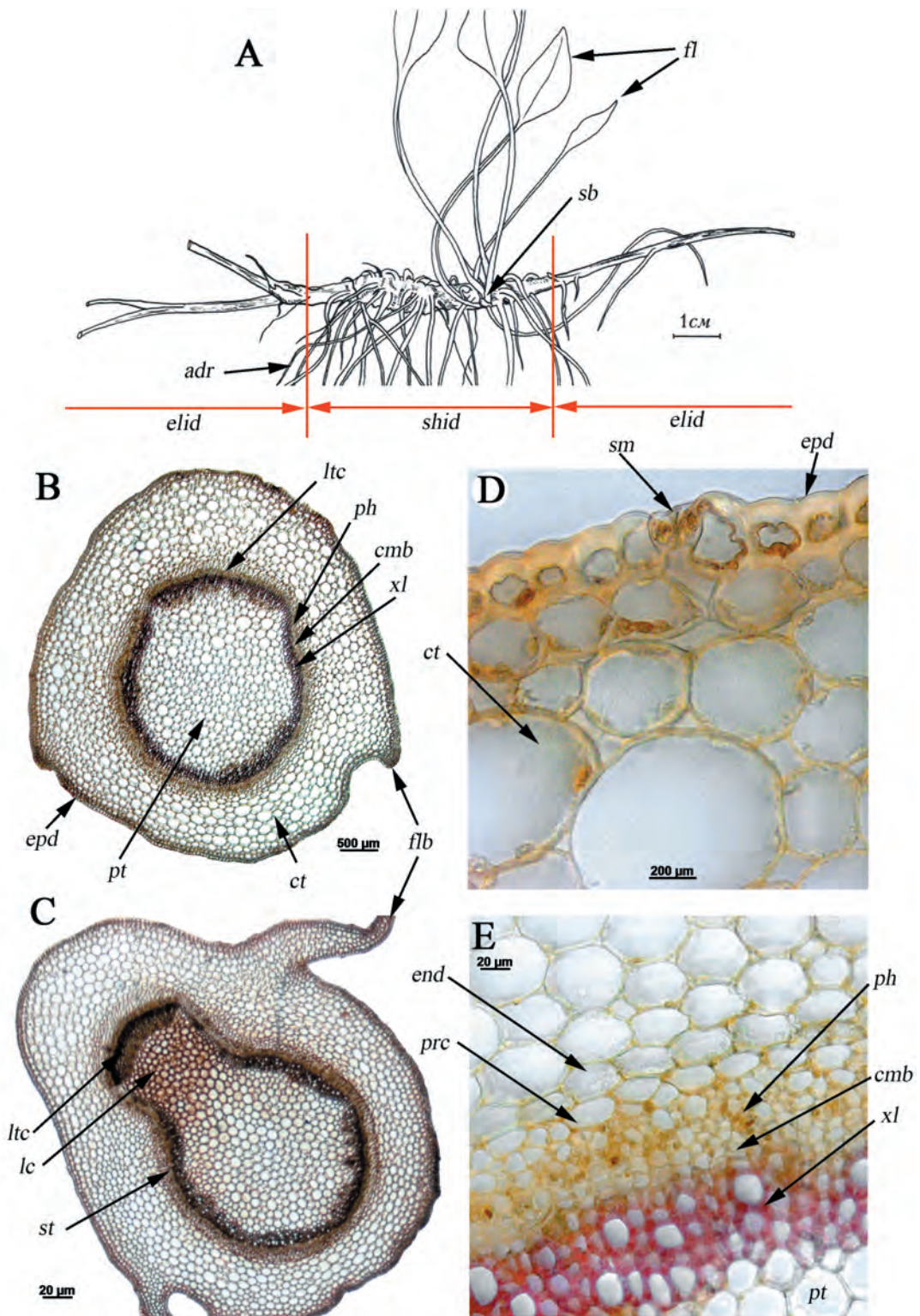


Figure 6. *Omphalodes verna*. A – part of aerial epigeogenous rhizome with emerging buds in the zone of shortened internodes; B–E – cross sections of an elongated, horizontal, annual rhizome through an internode (B) and a node (C); a peripheral fragment of an elongated aerial rhizome with a stoma and the epidermis (D); a part of stele adherent to the endodermis (E).

cell layers contains small starch grains. The endodermal cells are small, more rounded and closely situated. The cambium activity appears to be poor, as only a few xylem elements (5–7 in a radial cord) are present. Cork is missing.

In *B. sibirica*, the pith and 8–9-layered cortex of the vertical (upright) epigeogenous rhizome contain a lot of compound starch grains (with 2–3 hila). The epidermis and 2 subepidermal layers are tanniferous; tannins are also present in the endodermis and in phloem parenchyma cells. Vascular bundles are rounded to oval in outline, with a limited number of tracheary elements, located in a circle solitarily or in small tangential groups of 2–4.

The vertical, 1-year-old epigeogenous rhizome of *Brachybotrys paridiformis* (Fig. 5B, C) has leaves of two shapes, scalariform ones at base, further replaced by foliage leaves. Contrary to other species, it is found to have epidermal cells with relatively thin walls (Fig. 5D). It may be connected with the fact that the species grows in low light conditions under the canopies in forests and sometimes also in increased humidity, in river valleys. The cortex consists of 8–9 cell layers. Small endodermal cells possess narrow Casparian stripes (Fig. 5E). The pericycle is one-layered. Vascular bundles, located far apart and having a small number of tracheary and sieve elements and vascular cambium, alternate with minute, sometimes incomplete ones, which differentiate for a long time in the meristematic interfascicular zone. Starch-containing pith appears to take up a significant part of the volume. Cataphyll-bearing nodes are unilacunar, with 3–4 vascular bundles; those with foliage leaves are also unilacunar, but their leaf traces have a greater number (up to 7) of closely located vascular bundles (Fig. 5F). The structure of the horizontal hypogeogenous rhizome is generally similar, differing only in the higher activity of the vascular cambium (Fig. 5G).

The aerial, anisotropic branched, sympodial, green-leaved rhizome of *Omphalodes verna* has a noteworthy structure. Each branch (Fig. 6A), including up to 3 annual growth rings, is differentiated into relatively thin horizontal zones formed by elongated internodes and zones with nodes brought closely together, bearing leaves, axillary buds and adventitious roots. The fact that the rhizome has developed above ground and its external differentiation into the two zones is expressed in its microstructure, too. In both zones (those of elongated and shortened internodes, near foliage leaves bases, Fig. 6B, C), a one-year-old aerial rhizome of *O. verna* has a well-presented smooth cuticle. The epidermis, and at some sites also the subepidermal layer, is composed of thick-walled cells; solitary stomata can be seen (Fig. 6D). The cortex of 10–11 cell layers; the endodermis consists of light, tightly connected cells with Casparian stripes and solitary starch grains. One-layered pericycle adheres to the endodermis from inside (Fig. 6E). Vascular tissues form an almost continuous ring around the abundantly developed pith. The primary medullary rays are narrow, 3–4-seriate, with slightly lignified cell walls near the xylem. Nodes are unilacunar, with large leaf traces; due to this circumstance, the internode cross section outlines at different zones of the rhizome vary (Fig. 7A, B). Late in the vegetation season, the endodermal cell cavities fill up with tannins. Due to nutrient outflow from the clustered leaves, the activity of the vascular cambium and root formation increases. The adventitious roots serving as support and storage are pentarch (Fig. 7D). In 2–3-year-old rhizomes, the storage pith volume is much greater (3.5–4 times); the cortex dies off; outer phloem layers obliterate. Layers of dead cortex cells, persisting at the surface of the rhizome, as well as bases of semi-amplexicaul leaves serve to protect the organ.

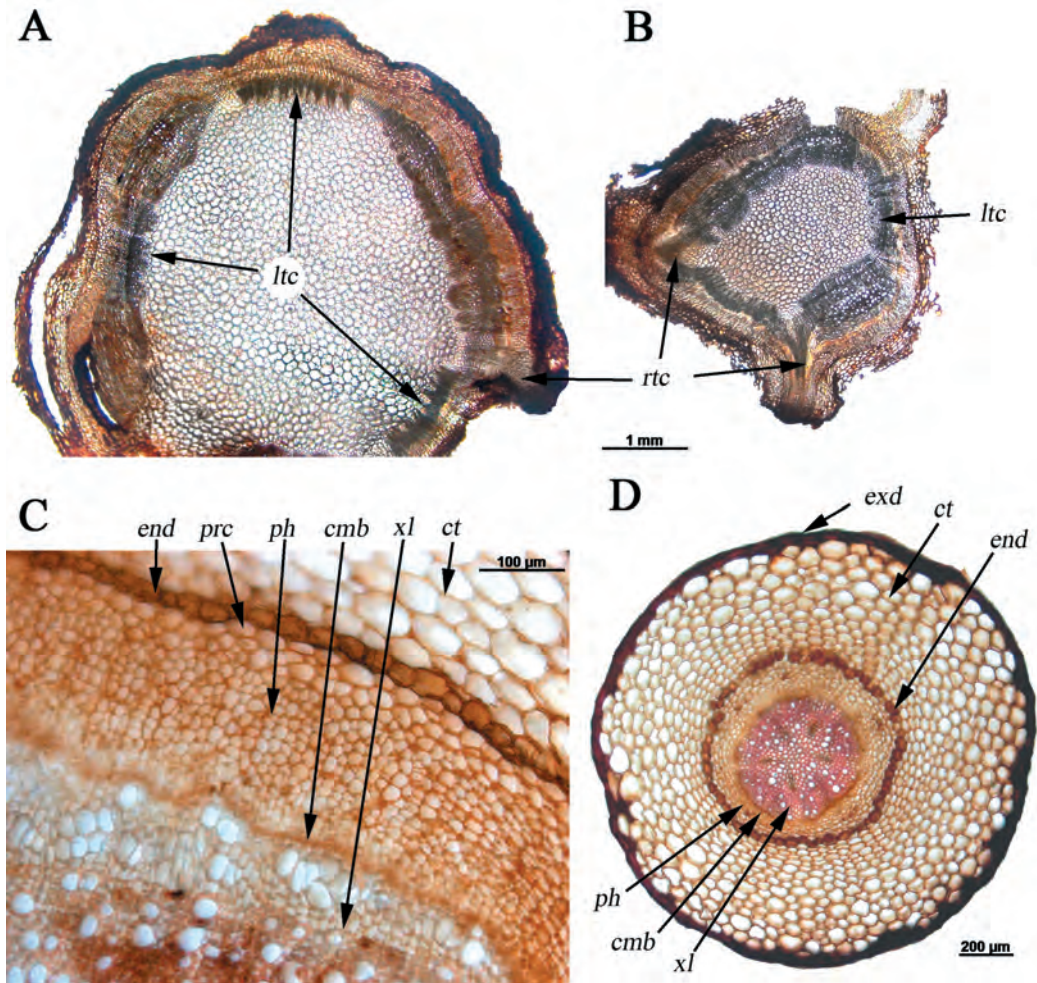


Figure 7. *Omphalodes verna*. A, B – rhizome cross sections at the level of shortened internodes in its wide and narrow parts; C – fragment of a cross section of a 2–3-year-old epigeogenous rhizome with its starch sheath and pericycle, naturally stained brown; D – 2-year-old pentarch adventitious root.

Mertensia maritima appears to be very close to *Omphalodes verna* in terms of morphogenesis and the structure of decumbent (lodging) shoots transformed into sympodial few-year-living rhizomes. At the same time, this plant is known to have a number of biomorphological peculiarities. This halophyte with a short tap root system can be found at the seashores of Europe, Asia and North America, where it grows on gravels and sandy substrates in considerable salinity (SHIBNEVA 2008). Brief information regarding the habit of *M. maritima*, the structure of its vegetative and reproductive organs can be found in papers of LID & LID (2005), AIKEN et al. (2007) and SKARPAAS (2008). The plant's shoot system (Fig. 8A, B) is represented by numerous branched shoots of 2 types: vertically oriented, elongated, monocyclic, reproductive ones and procumbent, anisotropic, semi-rosette, usually dicyclic shoots, developing from the buds on basal metameres of the parent shoot. Each procumbent (lodging) branch begins as a plagiotropic runner of 1–2 internodes; only after a while, its apical bud curves upward, producing a rosette-like part with compact nodes, bearing 2 leaf formations (scalariform and foliage) as well as the terminal and lateral buds. During the first year, the procumbent dicyclic shoot remains vegetative. At the

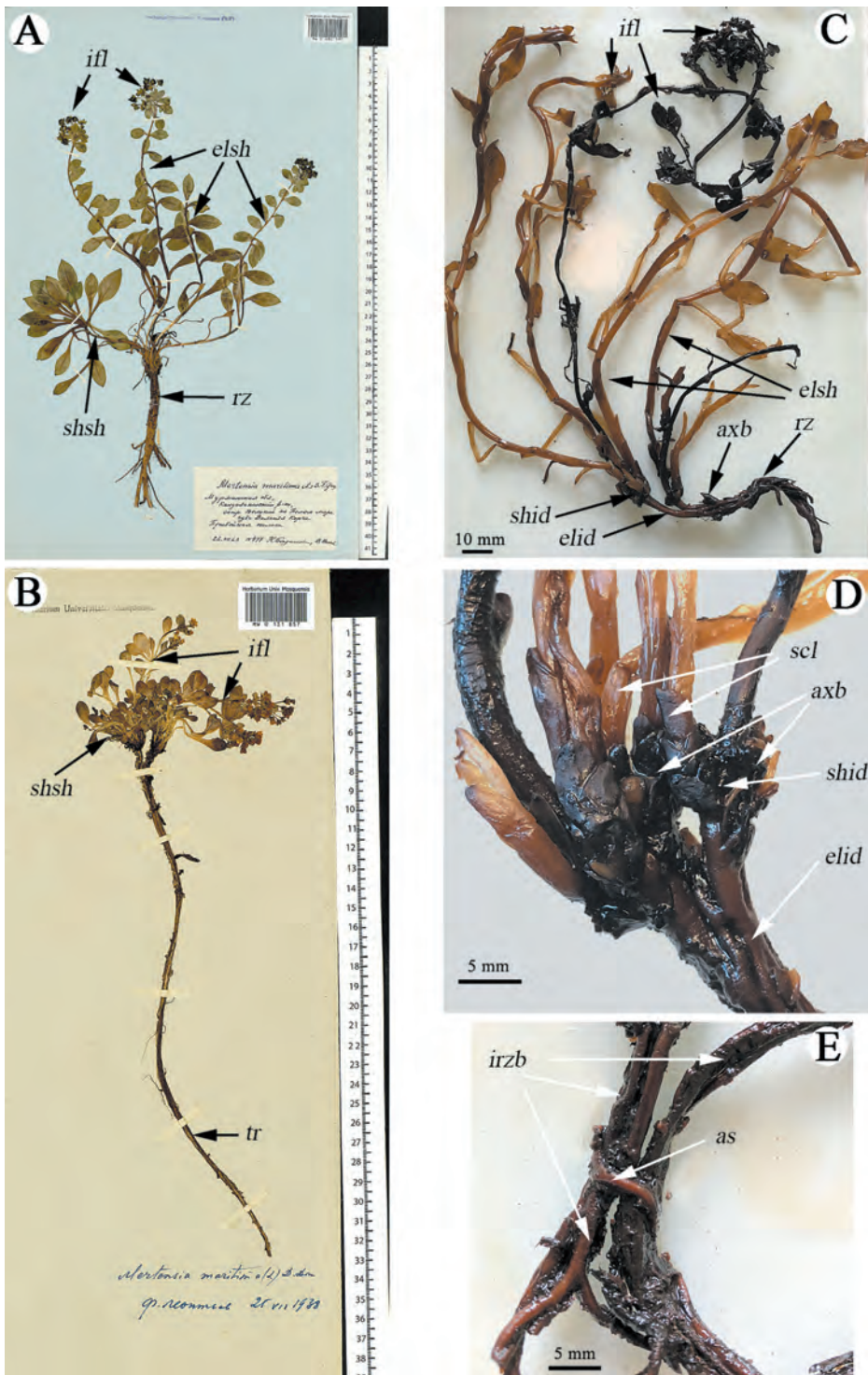


Figure 8. *Mertensia maritima*. A, B – herbarium specimens [MW] of a mature reproductive plant, collected in the surf (reference) zone at Velykyi island (Kandalaksha district, Murmansk region, the White sea); C – part of the shoot system of a perennial plant with annual flowering shoots and biennial rhizomes; both include sites with shortened and elongated internodes (alcohol-preserved material); D – closely located nodes of vertical shoots with emerging axillary buds; E – 2–3 parallel-growing, spirally twisted branches (stems) of a 2-year-old rhizome, connected by an anastomosing strip.

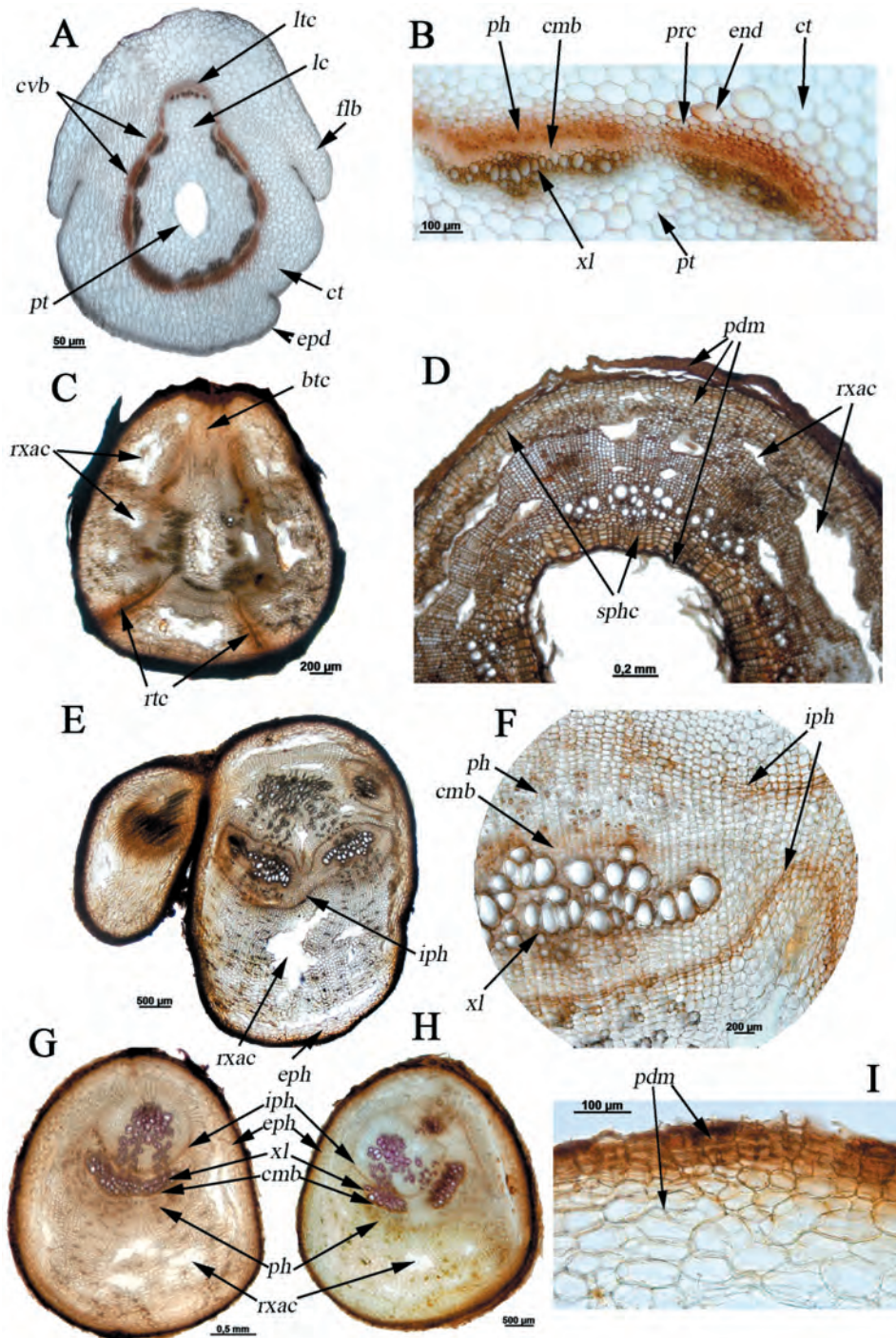


Figure 9. *Mertensia maritima*. A – an annual photophilic shoot near a foliage leaf-bearing node: node unilacunar, with many bundles; B – a fragment of the vascular system in a vertical annual shoot; C – cross section through a shortened node of a rhizome with traces of two adventitious roots and a lateral bud; D – segment of an elongated internode of creeping, anisotropic, biennial rhizome: at the periphery of the stele and from the direction of the crushed pith, a phellem ring of cells with suberized walls is visible; E – cross section of an elongated, plagiotropic, biennial rhizome: next to the 3-bundled branch, a thinner single-bundled one is formed after the fusion; F – inner phellem initiation around the secondary xylem and phloem elements of the isolating bundle; an early stage of inner phellem formation in the single-bundled (G) and three-bundled (H) branches; I – site of outer periderm on the surface of a biennial rhizome.

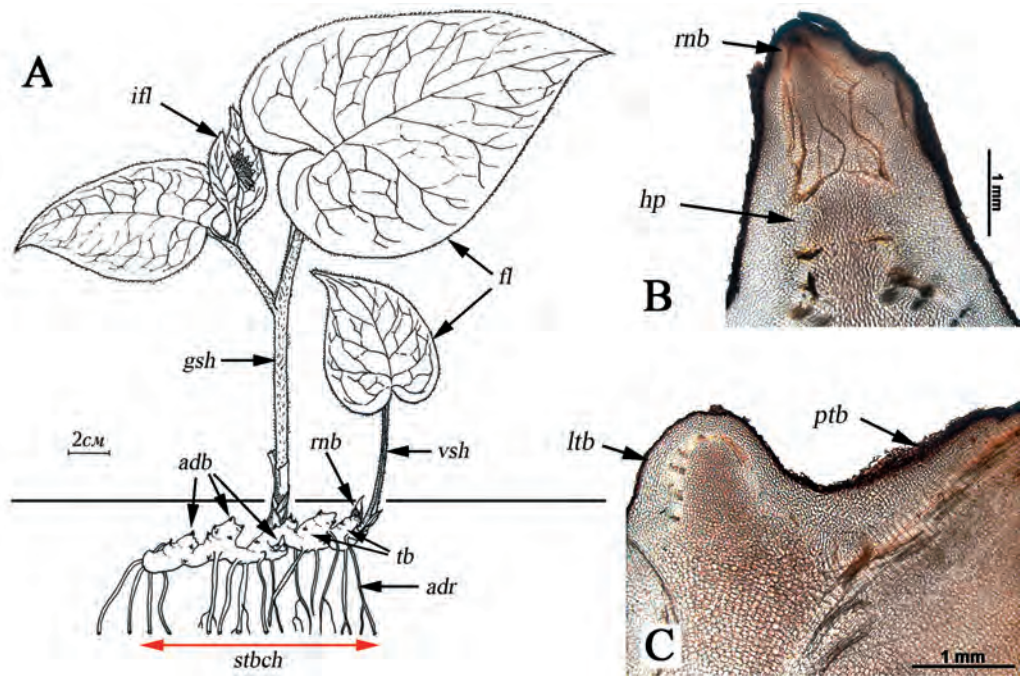


Figure 10. *Symphytum cordatum*. A – mature reproductive plant; B – early stage of a growing renewal bud formation on the short stolon due to intercalary elongation of the hypopodium; C – development of a new tuber from the scalariform leaf's axillary bud, located near the functioning parent stem tip.

end of the vegetative season, cork development takes place on its surface, whereas the ground parenchyma of the stem accumulates starch, resulting in the formation of a thin bi- to triennial dimorphic rhizome. The zone with closely located nodes, bearing buds and being capable of producing roots, submerges into the moist substrate and subsequently, when the perishable runner-like part disintegrates, it becomes separated and grows independently as a daughter individual.

In the course of annual spring sympodial renewal, 2–3 closely located lateral buds of the procumbent runner's shortened zone often start growing simultaneously (Fig. 8C, D). Shoots emerging from these buds grow parallel. It was noticed that they twist spirally (like in a rope) being regularly exposed to the mechanical impact of moving sandy and rocky substrate, and sometimes their stems fuse at points of contact for 2–3 mm (Fig. 8E). Then, they diverge and develop autonomously. Natural self-graftings are quite widespread in plants (KRENKE 1950). The shoot transformations in *M. maritima* mentioned above are reflected in the microstructure, too (Fig. 9A–I). However, changes in the bark and stele structure that take place in case of fusion and the details of 'particle' formation can be discussed only after thorough microscopic studies. Here, we just state the fact of the longitudinal stem fusion, subsequent active development of separating particles, each of them usually having a different number of open vascular bundles, isolated from the ground parenchyma by the cork tissue.

Among Boraginaceae, tuberous forms are present. They belong to a few representatives of the genus *Symphytum* from *Tuberosum* section. Tubers are believed to be phylogenetically relatively young shoots specialized in vegetative propagation. Tuberous life forms are the most adapted ones for living in alpine habitats, on mobile rankers or rocky substrates. Among the tuberous species

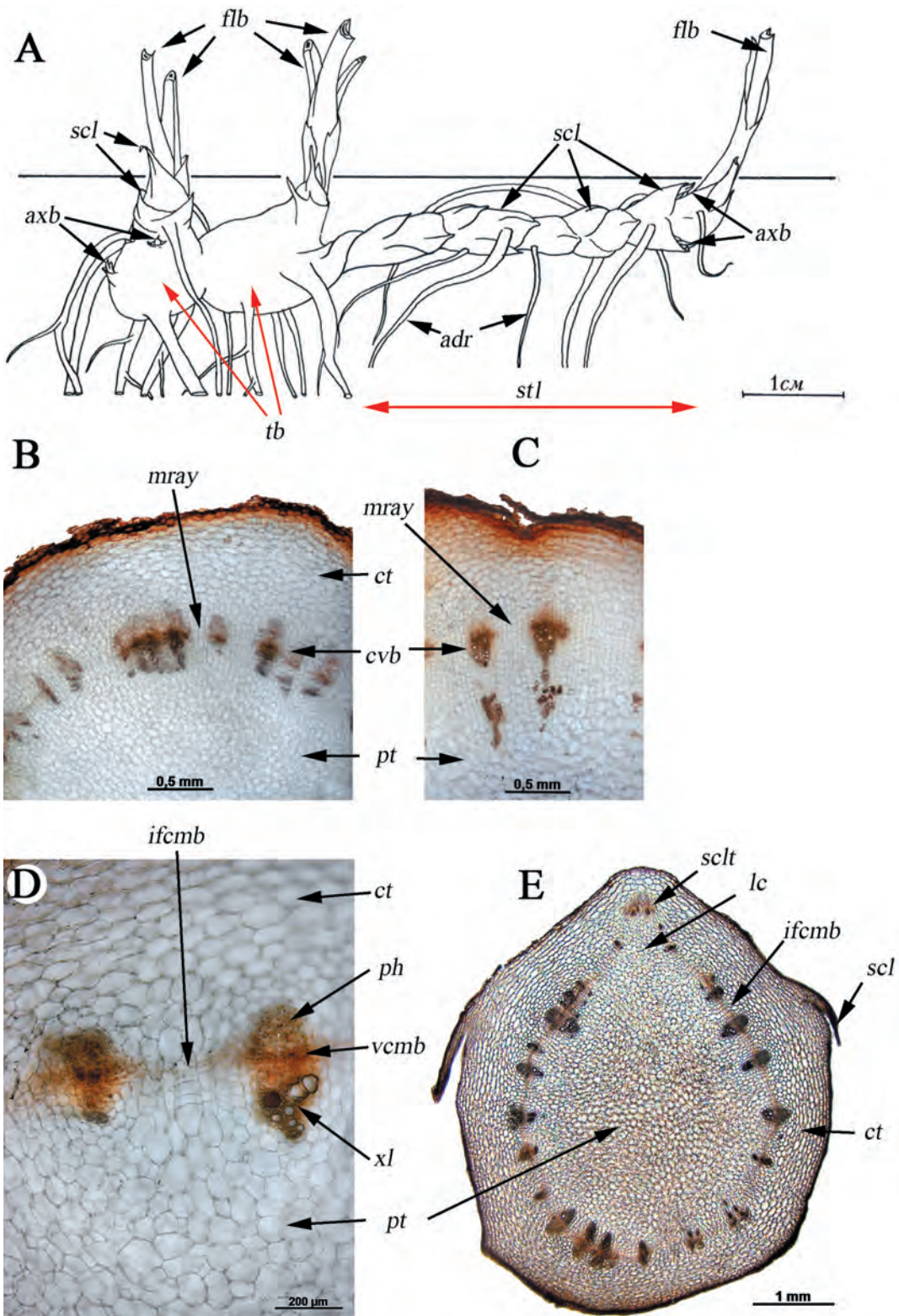


Figure 11. *Symphytum tuberosum*. A – stolon-tuberous rhizome, habit view; B–E – cross sections of annual (B) and biennial (C) tubers; anatomical structure of a fleshy stolon, bearing scalariform leaves at the cortex-stele border (D); near the node (E), wide-spaced open vascular bundles and interfascicular cambium are visible.

studied, there are *S. cordatum* and *S. tuberosum*. In these species, the tuber formation occurs prior to the aerial shoot development, no matter if vegetative or flower-bearing (BARYKINA & ALYONKIN 2008) (Fig. 10A). In 3–4-year-old juvenile plants of both species, the parent tuber of hypocotyl origin continues to grow as new metameres of the main rosette shoot submerged into the soil. One or less often 2–3 lateral buds begin to sprout on the parent tuber, so the apex, due to stolon-like elongation of the hypopodium, keeps moving away from the parent tuber (Fig. 10B). The daughter tuber formed due to the bud axis's increase in thickness (Fig. 10C) is found to bear renewal buds and adventitious roots in addition to the scales and 2–3 assimilating leaves in the second year of life. 5–6-year-old plants grown from seeds are shown to possess several lateral tubers of ascending order, which compose a rather compact sympodial chain. Later on, in mature vegetative and young plants in the reproductive stage, the tuber formation is preceded by a marked thickening of both the hypopodium and a few metameres, bearing scalariform leaf primordia. As a result, a thick anisotropic underground stolon of 5 cm or more, similar to a hypogeogenous rhizome in thickness, is formed (Fig. 11A). Growing monopodially, its terminal bud produces a shortened, tuber-like thickened, orthotropic rooted shoot, located near the soil surface at the end of the vegetation period. In the spring of the following year, it will continue to develop as a photophilous reproductive shoot.

Out of the axillary buds on the overwintering shoot, usually one or, less often, two become the renewal buds; the rest normally do not sprout. In *S. tuberosum*, tubers, which are the first stage in the morphogenesis of a dicyclic, monocarpic shoot (Fig. 11B, C), anatomically differ greatly from both the underground, horizontal stolon-like part (Fig. 11D) and the aerial, leaf-bearing, reproductive part. Both demonstrate pronounced growth of the cortical and the pith starch-containing parenchyma. The stele has around 30 far apart, open collateral vascular bundles, located in 2 tangential groups and including quite a small amount of tracheary elements. No supporting tissues are found. Closely located traces of adventitious roots and scale-like leaves (cataphylls) are well seen. Nodes, bearing the latter, are unilacunar, with 3 bundles. 2–3 peripheral cortical layers with metacutinized tanniferous cell walls serve as protective cover. Tubers of *S. cordatum* appear to have similar structure. In *S. tuberosum*, the fleshy stolon is rounded to oval in cross section (Fig. 11E). The cortex consists of up to 20 layers of thin-walled cells; the two outer layers, being naturally coloured brown due to tannins, perform the protective function. 25–30 vascular bundles, pear-shaped and radially elongated, possess a limited number of vascular elements and are separated from each other by wide (up to 15 cell layers) medullary rays. The bundle sheath consists of small-celled parenchyma. In the rays, the derivatives of the interfascicular cambium are well seen. The major part of the stolon is composed of the well-developed pith of 40 or more cells in diameter. The pith as well as other living cells of the cortex and the stele is filled with storage starch and aleurone grains.

Thus, in the morphogenesis of a monocarpic, dicyclic shoot in stoloniferous and tuberous species of the genus *Symphytum* (e.g. *S. tuberosum* and *S. cordatum*) and species with long, structurally dimorphic rhizomes (e.g. *Brachybotrys paridiformis*, *Omphalodes verna*, *Mertensia maritima*, *Brunnera sibirica*) share many traits in terms of the vegetative dispersal strategy, quickness of capture and retention of the habitat. Long, aerial, stolon-like rhizomes and underground hypogeogenous ones serve for quick and efficient population of new territories, while vertically oriented tubers developing at their tips as well as shortened epigeogenous rhizomes with bundles of adventitious roots and renewal buds on them ensure high intensity of vegetative propagation.

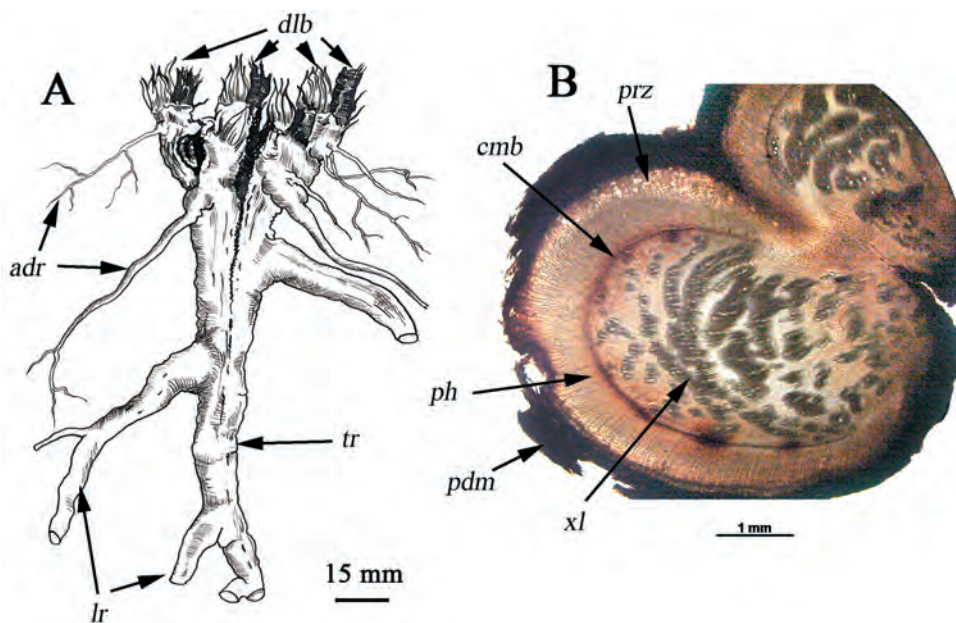


Figure 12. Senile particulation. A – a perennial rhizome of *Symphytum x uplandicum*; B – fragment of the cross section of a particulating rhizome in *Anchusa pseudochoroleuca*.

Particulation as a peculiar form of optional vegetative propagation is characterized by longitudinal splitting of an epigeogenous rhizome or a caudex and the tap root into separate particles capable of independent living appears to be extremely rare among Boraginaceae. Here, we are not discussing the senile (*Symphytum x uplandicum*, *Anchusa pseudochoroleuca*) particulation (Fig. 12), but the one connected with anomalous secondary increase in thickness of axial organs which can be traced in the virginile ontogenetic stage or in young reproductive individuals (BARYKINA 2009). Some indications to hidden particulation can be observed in the Central Asian tap rooted petrophyte *Rindera tetraspis* Pall., possessing a multicipital caudex. This species was studied by BUTNIK et al. (2009) in detail. As inferred from the figures and descriptions of reproductive shoot bases and their macro- and microstructure, one can see the transition from radial symmetry of their stele to polycentric structure, i.e. the presence of groups of differently oriented vascular strands. Regrettably, further development of several autonomous loci of the secondary increase in thickness was not particularized by these authors.

Conclusions

The revealed diversity of vegetative propagation variations within the family Boraginaceae demonstrated not only the adaptations of species to their habitats, but also various strategies of capturing and populating new living spaces ('anchoring' and 'creeping' forms), the speed of movement and the renewal degree in the vegetative offspring. Similar specialized reproductive organs in different taxa are the result of the convergent evolution. A certain correlation of the propagation mode and the plant's life form, shoot structure and root system type (allorhiz and homorhiz) can be observed. For tap-rooted wood biormorphs, sobole formation is a common trait. Modes of vegetative propagation are more diverse in herbaceous plants, both short tap-rooted and homorhiz with fringe-like fibrous root system, as they are more evolutionary adaptable and flexible. They are found to show various types of sarmentation, conditioned by

the morphology of modified axial organs, their development peculiarities and structure. Together with sobole formation, these could be: individualization of separate rooting sites, epigeogenous, hypogeogenous or mixed (epigeogenous-hypogeogenous) underground and stolon-like aerial rhizomes, specialized recumbent biennial shoots as well as tubers. Vegetative mobility and high regeneration activity of Boraginaceae are largely due to the microstructure of the axial organs aimed at vegetative propagation, in particular, profound parenchyma development and extensive supply of ergastic substances (starch, aleurone), poor lignification, lack of specialized supporting tissues or cork (except for *Ehretia* and *Mertensia maritima*). The protective function is usually performed by the peripheral cortex layers, which are often tanniferous, having metacutinized cell walls and wide, persisting leaf bases, remaining on the surface for a long time. Specialized organs of vegetative propagation are characterized by accelerated differentiation and development, short-term activity of vascular cambium (with the average of 2–4 years), quick renewal, extended ability to form adventitious buds and roots.

The revealed diversity of vegetative propagation and dispersal modes together with other biological and morphological peculiarities allowed Boraginaceae to populate various ecological niches and ensured their thriving in the vegetative cover of subtropical and non-tropical areas in the present time.

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