

Syncotyly in seedlings and sprouts of some Boraginaceae: genesis, structure and function of the cotyledon tube

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Summary: Eleven herbaceous species of Boraginaceae were used to demonstrate the phenomenon of syncotyly in seedlings and sprouts expressed to various degrees. It can be displayed in a slight tendency towards the formation of the cotyledon tube or, as in most representatives, in the formation of a distinctive relatively short tube containing the plumule. Isolated cases of syncotyly are reported as early as at the embryo stage. Along with sheathing cotyledon bases, their petioles can be involved in the tube formation as well. The tube's functions are to protect the plumule and axillary buds of the cotyledons (if present) from physical damage, dehydration and temperature changes. In some species, it also plays the role of the hypocotyl in elevating and removing the cotyledon blades from the seed coat and other covers. Comparative anatomical studies of the cotyledon tube (at the node level and above) in annual, biennial and polycarpic perennial species of various life forms revealed a number of common traits, such as: median veins of 2–3 vascular bundles, presence of oil drops and aleurone in the ground parenchyma of the wall, pubescence mainly present on the adaxial surface facing the tube's lumen (inner surface) and unilacunar cotyledon node with several discrete strands.

No marked differences in the microstructure of the cotyledon tube in different species of a genus were found. Some revealed qualitative differences are apparently connected with specific habitual conditions and do not bear any unambiguous taxonomic value.

Keywords: Boraginaceae, seedling, cotyledons, syngensis, syncotyly, cotyledon tube, genesis, functions, anatomy

Many extant dicotyledons are known for a phenomenon of so-called syncotyly i.e. fusion of cotyledons observed at early embryogenetic stages. The congenial fusion can be observed, when the entire margins of cotyledons are united (amphicotyly) resulting in the formation of a cup-like structure, or it can be partial, when only some parts of the cotyledons fuse, primarily their adjoining sides (hamocotyly). As a result of syncotyly, a cotyledon tube (a closed hollow chamber containing the plumule) is formed. The cotyledon tube is “simple in structure but complicated in origin” (YANISHEVSKYI & PERVUKHINA 1948: 272), as it can be formed with the contribution of the sheathing bases as well as of basal parts of cotyledons' petioles grown together.

Up to now, the cotyledon tube is an object of complex multifaceted studies. Its genesis, structure, functions are being investigated along with its biological, ecological, adaptive, taxonomic and phylogenetic value (LEWIS 1904; YANISHEVSKYI 1910; VASILCHENKO 1937, 1941; YANISHEVSKYI & PERVUKHINA 1948; HACCIIUS 1952, 1953; BARYKINA & GULANJAN 1978; ZOZ 1962; BARYKINA 1971, 1999; ILYINA 1976; BARYKINA & POTAPOVA 1994; TSUTSUPA et al. 2001; and many others).

Some researchers consider the phenomenon of syncotyly a possible way how a monocotyledonous embryo could have emerged. Long ago, SARGANT (1904) hypothesized the syncotylous origin of monocots from dicots, their derivative nature due to the fusion of two cotyledons into one as an adaptation to living in harsh climatic conditions (geophily). However, numerous records of cotyledon fusion in dicots and, as VASILCHENKO (1936: 10) pointed out, “their phylogenetic

significance cannot be considered sufficiently covered in literature”. Cotyledon fusion, even partial, has been observed in various taxonomic groups and could be a manifestation of convergent evolution.

The degree to which syncotyly is expressed can vary, but the latter is always connected with the epigeous cotyledonary, less often hypocotylous and cotyledonary seedling emergence pattern. The fusion is accompanied by the formation of a long or a short cotyledon tube, hypocotyl reduction and plumule lowering towards the ground. The cotyledon tube is primarily characteristic of herbaceous polycarpic plants, rarely of annuals and shrubs, e.g. *Paeonia lutea* (BARYKINA & GULANJAN 1978).

The biological significance of the cotyledon tube is quite diverse. Its presence, in some species at the embryo stage, indicates the early manifestation of geophily attesting the high level of its structural and functional specialization (VASILCHENKO 1936). The role of the cotyledon tube is frequently limited to the protection of poorly developed plumule and axillary buds primordia from physical damage and dehydration until well-differentiated leaves are developed (ILYINA 1976; TSUTSUPA et al. 2001). In some Apiaceae, the cotyledon tube serves as an adaptation to moisture deficit and huge temperature swings (YANISHEVSKYI & PERVUKHINA 1948; ZOZ 1962). The connection of syncotyly with habitat conditions is quite obvious at the example of the genus *Rheum*: in species populating plains, the cotyledon tube is a lot longer than in those growing in mountainous areas (YANISHEVSKYI 1910). The most diverse role played by the cotyledon tube is found in pseudomonocots (Ranunculaceae, Berberidaceae, Apiaceae, etc.), in which the monocotyledonous type of embryo results from underdevelopment (abortion) of a cotyledon or from syncotyly. In the former case, e.g. in *Anemone caucasica*, the cylindrical cotyledon tube is formed by the elongated sheathing base of the only cotyledon (BARYKINA & POTAPOVA 1994).

In some geophytes (*Podophyllum*, *Anemone*, some Apiaceae, etc.), a long (2 cm and longer) cotyledon tube bears a function unusual for a leaf-like organ – it serves as root (lower part with positive geotropism) and hypocotyl (upper part); this developmental deviation affected not only its morphogenesis but also its tissue development (HACCIUS 1953; BARYKINA 1971, 1999). This temporal and spatial heterogeneity of the cotyledons apparently allows us to make an assertion about the unified phylogenetic origin of axial and appendicular organs in angiosperms from the standpoint of ontogenetic morphogenesis.

According to literature data, the phenomenon of syncotyly is widespread among dicotyledonous plants. Boraginaceae are rather poorly investigated in this relation. A noteworthy paper titled “Ecological anatomy of desert plants in Central Asia” was published by BUTNIK et al. (2009); it provides a more or less detailed description of seedling microstructure of only one Boraginaceae species, *Rindera tetrapsis* Pall., an ephemeroïd plant growing in the rock crevices, possessing a long (1–1.5 cm) cotyledon tube.

The aim of the present study is to elucidate and cover the newly obtained data on syncotyly, its manifestation rate, the nature and structural details of the cotyledon tube, its adaptive significance for the seedlings and sprouts in some Boraginaceae of various life forms.

Materials and methods

The objects of our study were 11 herbaceous (annual, biennial and perennial) species from the family Boraginaceae Juss.: *Brachybotrys paridiformis* Maxim. ex Oliv., *Omphalodes verna* Moench,

O. linifolia (L.) Moench, *Echium vulgare* L., *Anchusa pseudochroleuca* Des.-Shost., *Lindelofia stylosa* (Kar. & Kir.) Brand, *L. macrostyla* (Bunge) Popov, *Macrotomia euchroma* (Royle) Paulsen, *Symphytum × uplandicum* Nyman, *Trichodesma incanum* (Bunge) A. DC., *Hackelia uncinata* (Benth.) C.E.C. Fisch. The material for morphological and anatomical research was collected in the nature (Russian Federation, Moscow and Oryol regions; former USSR republics, Moldova, Tadjikistan and Uzbekistan) as well as in cultivation (Botanical garden of the Lomonosov Moscow State University). The investigation combined comparative morphological and anatomical as well as ontogenetic research techniques. To clarify the biomorphological characteristics of certain species, herbarium samples from D.P. Syreishchikov's herbarium [MW] were studied. The embryo structure traits were analyzed after removing the seed coat; the presence and type of storage substances (starch, aleurone, etc.) were identified according to the reference book on microscopic techniques (BARYKINA et al. 2004). Seedling and sprout microstructure was observed in serial cross sections using light microscope Micromed 3. Sections were photographed using the light microscope Axioplan-2 and AxioCam MRc camera. Obtained photographs were edited using Adobe Photoshop CS6.

Results

Brachybotrys paridiformis Maxim. ex Oliv. is a perennial hemicryptophyte with a short horizontal rhizome. The species occurs in shady broadleaf forests of Russian Far East and China, mostly on light loamy soils (GÜRKE 1893; POPOV 1953).

The embryo bears large cotyledons with pronounced procambial strands. The cotyledons are 2.4 mm long which is 3 to 3.2 times longer than the axial part of the embryo, the latter being 0.74 mm. A longitudinal section of the embryo clearly shows the cotyledon tube (a hollow chamber) containing the undifferentiated plumule (Fig. 1A).

Seedling emergence pattern is cotyledonary or hypocotylous and cotyledonary (Table 1, Fig. 1B, C). The seedlings have two pubescent, fleshy rounded to ovate cotyledons with long petioles. Cotyledon bases are partially fused forming a continuous tube of 2–5 mm in length. In some individuals, adjoining sides of cotyledons' petioles are also fused (hamocotyly). In this case, the cotyledon tube may reach 1–1.2 cm in length. The first assimilating leaf of the seedling emerges through the upper opening of the cotyledon tube. In the nodal area, the tube is shaped as a continuous cylinder. In cross section, it is rounded-ovate with its wall made of 5–6 cell layers (Fig. 1E, F). The epidermal cells on the tube's abaxial surface are larger than those on the adaxial side. No stomata or pubescence were observed. The storage parenchyma is presented by 8–9 layers of relatively large rounded cells. The vascular bundles are 6–8 in number, collateral, with no cambium present; the bundles or median veins are markedly clustered. Just before entering the hypocotyl stele, both median and lateral vascular bundles merge into two opposite strands of vascular tissue. The cotyledonary node is one trace unilacunar.

Omphalodes verna Moench, a perennial stoloniferous polycarpic plant with a short rhizome, is known to grow in moist deciduous forests of the lower montane belt, on rocky slopes of gorges, under trees, along river valleys among shrubs on rich, loose, slightly alkaline soils. The plant occurs in the southeastern Alps and the Romanian Carpathians (POPOV 1953; HEGE 1972).

Its fully developed embryo is found to possess two large cotyledons (2 mm), which exceed the embryo's axial part (0.56 mm) 3.5 times (Fig. 2A). A poorly differentiated plumule is located

Table 1. Anatomical and morphometrical parameters of syncoryly in the studied Boraginaceae species with different seedling emergence patterns, life forms, ecology and habitats.

Species	Life form	Seedling emergence pattern	Embryo's axis (hypocotyl and root) length (A) to coryledons length (S) ratio (A/S)	Coryledon shape	Number of lacunae and number of vascular bundles in the coryledon trace	Coryledon tube			Geographical range	Habitat
						Length (mm)	Thickness (number of cell layers)	Number of vascular bundles		
<i>Brachybotrys paridiformis</i>	perennial hemicryptophyte with a short horizontal rhizome	coryledonary or hypocotylous and coryledonary	A<S 1/3–3.2	petiolate, rounded to ovate	unilacunar, one-trace	5–12	8–9	6–8	Russian Far East (Ussurisk area, Sikhote-Alin), China (Manchuria), Korean peninsula	Shady broadleaf forests, in river valleys. Light soils, loams.
<i>Omphalodes verna</i>	perennial hemicryptophyte with a short rhizome	coryledonary or hypocotylous and coryledonary	A<S 1/3.5	petiolate, broad elliptic	unilacunar, one- or two-trace	10–12	5–6	6–8	Southeastern Alps and the Carpathians	In moist deciduous forests of the lower montane belt up to 1300 m. Along river valleys among shrubs and herbs on rich, loose, slightly alkaline soils.
<i>Omphalodes linifolia</i>	taprooted therophyte	hypocotylous and coryledonary	A<S 1/2	petiolate, rounded to ovate	unilacunar, two-trace	1.5–2	5	6–8	Southwestern Europe (Spain, France, Portugal) and northern Africa (Algeria)	Dry and semi-dry sunny habitats. Habitats with loose, well-drained soils.
<i>Echium vulgare</i>	biennial taprooted hemicryptophyte	hypocotylous and coryledonary	A<S 1/1.2	petiolate (petioles short), broad elliptic	unilacunar, one- or two-trace	3–4	5–6	8–9	Throughout Europe, western Siberia and west of Central Asia	Boreal and steppe zone on dry slopes, in ravines and gulleys, on wastelands. A weed, growing on pastures, in crop plantations, along the roads.
<i>Anchusa pseudochroleuca</i>	perennial hemicryptophyte with a short taproot and polycephalous caudex	hypocotylous and coryledonary	A<S 1/2.5	sessile, acuminate, elliptical	unilacunar, two-trace	3–4	10–11	8	Moldova, Middle-Dnieper and Greater Black Sea areas	Calcareous steppe slopes, dry meadows, landslides, along the roads, less often at clearings in dry oak forests.

<i>Lindelofia stylosa</i>	perennial taprooted hemicryptophyte with a polycephalous caudex	cotyledonary	A<S 1/3.5	with short petioles, elliptical or broad-lanceolate	unilacunar, two- to four-trace	12–15	14	10	Central Asia (Tien Shan, Pamir-Alay), Himalayas, Tibet	Rocky slopes, at temporarily moist habitats, in alpine deserts. In the middle and upper (subalpine) montane belt at 1700–4500 m.
<i>Lindelofia macrostyla</i>	perennial taprooted hemicryptophyte with a polycephalous caudex	hypocotylous and cotyledonary	A<S 1/2.5	with short petioles, rounded	unilacunar, two-, three- and four-trace	3–4	11	7–8	Central Asia (Tien Shan, Pamir-Alay), Iran, Afghanistan, Pakistan	Semi-savanna habitats, subalpine forests, sagebrush deserts. On rocky and rubbly slopes, gliders. In the lower and middle montane belt at 700–3400 m.
<i>Macrotomia euchroma</i>	perennial hemicryptophyte with a thick taproot and polycephalous caudex	cotyledonary	A<S 1/3	with short petioles, rounded or oblong to elliptical	unilacunar, two-trace	15	22	18	Central Asia (Tien Shan, Pamir-Alay), Iran, Afghanistan, Pakistan, Himalayas, western Tibet	In the subalpine meadow belt, forb and grassland steppes, alpine deserts. On rocky and rubbly slopes, gliders, and rocks, along river valleys. In the upper montane belt at 2800–4400 m.
<i>Symphlytum x uplandicum</i>	perennial fibrous-rooted hemicryptophyte or geophyte with a short rhizome	cotyledonary	A<S 1/3	petiolate, oval to elliptical	unilacunar, two-trace	2–3	6–7	8	Europe, North America, Central Russia (Moscow city, Moscow region)	Plains at wet meadows, marshy areas, along rivers and streams, in ruderal or dwelling sites.
<i>Trichodesma incanum</i>	perennial hemicryptophyte with a strong branching rhizome	hypocotylous	A<S 1/3.5	petiolate, rounded to spatulate	unilacunar, two-trace	0–1	14–15	7–9	Central Asia (western Tien Shan, Pamir-Alay, Kopet Dagh), Iran, Iraq, Afghanistan, Pakistan,	In the semi-savanna and desert belt on rocky or rubbly slopes. In the lower and middle montane belt at 600–2900 m.
<i>Hackelia uncinata</i>	perennial hemicryptophyte with a short rhizome	cotyledonary	A<S 1/3	petiolate, rounded to ovate	unilacunar, two-trace	2–3	6–7	4	Asia (from Kashmir to Nepal, western China)	In montane forests at 2400–3500 m, on moist soils.

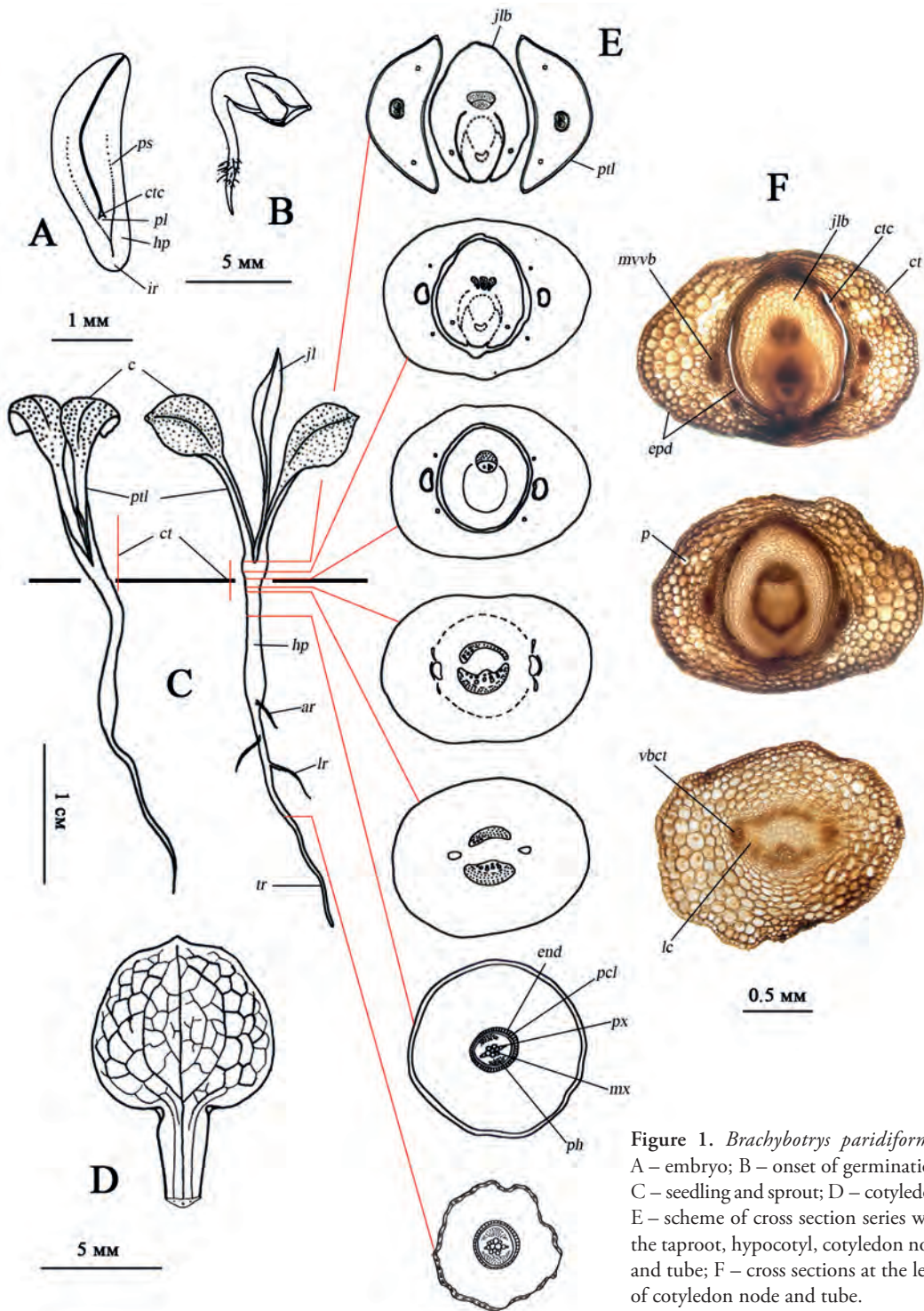


Figure 1. *Brachybotrys paridiformis*. A – embryo; B – onset of germination; C – seedling and sprout; D – cotyledon; E – scheme of cross section series with the taproot, hypocotyl, cotyledon node and tube; F – cross sections at the level of cotyledon node and tube.

ar – adventitious root; *c* – cotyledons; *cb* – cotyledon base; *cpb* – cotyledons' petiole base; *ct* – cotyledon tube; *ctc* – cotyledon tube cavity (chamber); *end* – endodermis; *epd* – epidermis; *h* – hair; *hp* – hypocotyl; *ir* – incipient root, radicle; *jl* – juvenile leaf; *jlb* – juvenile leaf base; *lc* – lacuna; *lr* – lateral root; *lbp* – lateral buds primordia; *lvb* – lateral vascular bundles; *mvvb* – median vein's vascular bundles; *mx* – metaxylem; *p* – parenchyma; *pl* – plumule; *ph* – phloem; *pcl* – pericycle; *ps* – procambial strands; *ptl* – petiole; *px* – protoxylem; *rac* – rexigenous air cavity; *rh* – root hairs; *s* – stomata; *sc* – seedcoat; *tr* – taproot; *vbct* – vascular bundles of the cotyledonary trace.

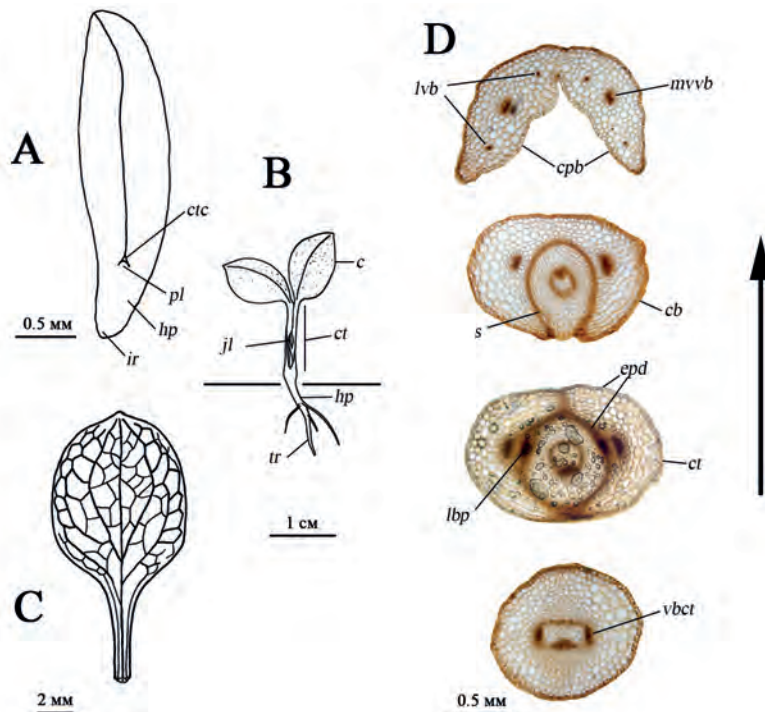


Figure 2. *Omphalodes verna*. A – embryo; B – seedling; C – cotyledon; D – a series of cross sections at the level of cotyledon tube base and upper. For abbreviations see Fig. 1.

inside a small chamber, the cotyledon tube. Parenchymal cells of the cotyledons and the embryo's axial part contain numerous oil drops and aleurone grains. The seedlings emerge according to the cotyledonary or hypocotylous and cotyledonary pattern (Table 1, Fig. 2B). The cotyledons' blades are broad elliptic, clearly distinct from their long petioles. The cotyledon tube is 1–1.2 mm long, its nature being complex: in its lower part (in the cross section), it is shaped as a continuous ring and formed by the bases of the fused cotyledons, while in the upper part it is triangular, which appears to result from one-sided fusion of the petioles throughout their entire length. The tube's epidermal cells are minute; its inner surface is found to have some stomata, however scarce; no trichomes are found. The ground parenchyma of 5–6 cell layers is penetrated by 6–8 collateral vascular bundles. The cotyledonary node is unilacunar, one- or two-traced. The basal (continuous) part of the cotyledon tube bears pronounced meristematic primordia of the cotyledons' axillary bundles (Fig. 2D).

Omphalodes linifolia (L.) Moench, contrary to the previously described species, is a taprooted annual (Table 1) species. Its geographical range includes southwestern Europe and northern Africa; the plant predominantly populates dry habitats with well-drained soils.

Many morphological traits of this species resemble those of *O. verna*: a large embryo (Fig. 3A), cotyledons being two times longer than its axial part; poorly differentiated plumule situated in a small chamber, formed through the syngensis of the cotyledons' bases; storage substances aleurone and lipid drops; hypocotylous and cotyledonary emergence pattern (Fig. 3B–E). The cotyledons are born on long petioles; their blades are rounded-ovate. Different from *O. verna*, the cotyledon tube in *O. linifolia* is very short (1.5–2 mm) and is formed solely by the fused cotyledon

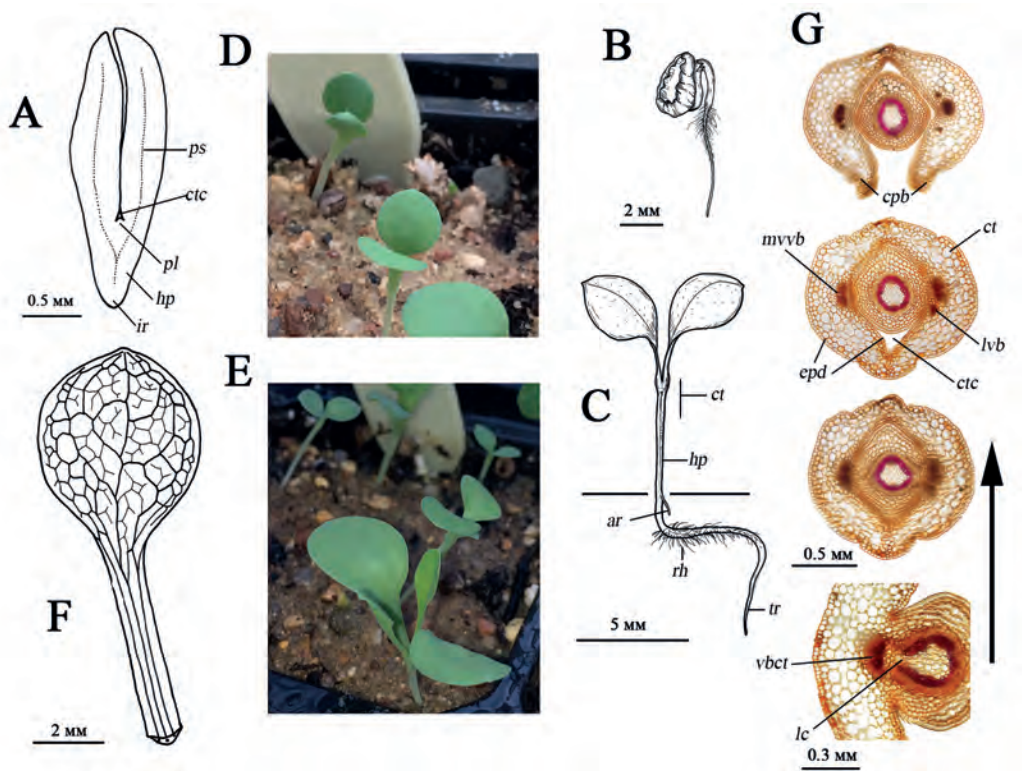


Figure 3. *Omphalodes linifolia*. A – embryo; B – onset of germination; C, D – seedlings; E – sprouts, stands; F – cotyledon; G – a series of cross sections at the level of cotyledon tube base and upper. For abbreviations see Fig. 1.

bases and only in solitary cases, slight one-sided fusion was also extended to the petiolar area. The key parameters of cotyledon tube anatomy are identical to those of perennial *O. verna*, except the former lacks the stomata in the tube's epidermal tissue and no primordia of the cotyledons' axillary buds are found (Fig. 3G).

Echium vulgare L. – a taprooted biennial hemicryptophyte, widespread throughout Europe, western Siberia and Central Asia. *E. vulgare* is a weed, abundant on pastures and in crop plantations, along the roads (POPOV 1953; HEGE 1972).

In its large (2.3–2.56 mm long) straight embryo, the cotyledons are almost equal to the axial part in length (Table 1, Fig. 4A). The plumule is represented by a group of meristematic cells, no leaf primordia are reported. No chamber is found in the embryo around the plumule. All parts of the embryo contain abundant lipid drops and aleurone.

The seedling emergence pattern is hypocotylous and cotyledonary (Fig. 4B, C). In the seedlings, the cotyledons are broad-elliptic, with short petioles; together with the hypocotyl, they are densely covered by long unicellular trichomes. The short cotyledon tube (3–4 mm) is formed by the enlarged cotyledon bases and is shaped as a continuous ring in cross section (Fig. 4D). Its adaxial and abaxial surfaces are covered by multiple long one-celled hairs, no stomata are found. The tube's wall is composed of a small-celled epidermal layer, 5–6 layers of large-celled parenchyma and 6–8 vascular bundles. At the base, the number of vascular bundles reduces to 4–6. The cotyledonary node is unilacunar, one- or two-traced.

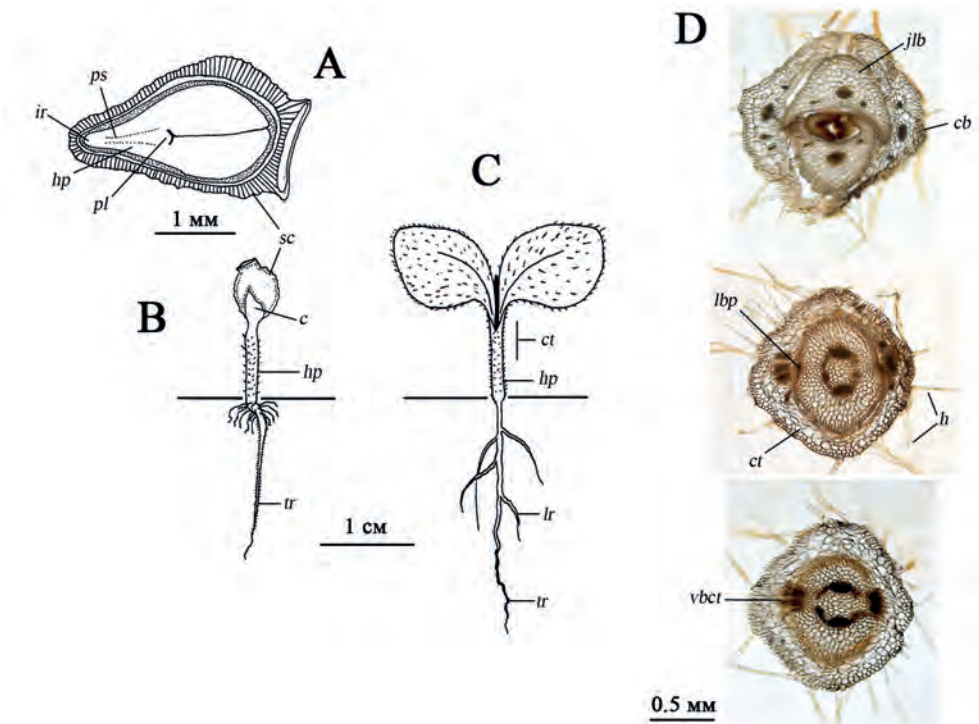


Figure 4. *Echium vulgare*. A – seed in longitudinal section; B – onset of germination; C – seedling; D – a series of cross sections at different levels of the cotyledon tube. For abbreviations see Fig. 1.

Anchusa pseudochroleuca Des.-Shost. is a perennial hemicryptophyte with a short taproot and a polycephalous caudex. The plant occurs on the calcareous steppe slopes of Moldova, less commonly in Middle-Dnieper and Greater Black Sea Area (POPOV 1953; HEIDEMAN 1975).

In its developed embryo (Table 1, Fig. 5A), the cotyledons exceed the axial part almost 2.5 times, being 2.1 mm and 0.89 mm respectively. Seedling emergence pattern is hypocotylous and cotyledonary (Fig. 5B). The seedling's cotyledons are acuminate, elliptical, broad-ovate (12 mm long, 8.3 mm wide), born on short petioles or sessile (Fig. 5C). A short (3–4 mm) cotyledon tube is characterized by peculiar genesis (Fig. 5D), not observed in other studied species. With the exception of the basal part, where amphicotyly takes place, the major part of the seedling displays incomplete fusion of the cotyledons' bases by their adjoining parts (hamocotyly). The cotyledon tube is covered by the small-celled epidermis, with solitary stomata present on the adaxial side. Short one-celled living trichomes are found both inside and outside the tube, being especially abundant near where its parts split and diverge. The ground parenchyma is large-celled, consisting of 10–11 cell layers, penetrated by 8 collateral vascular bundles with no cambium inside. Opposite median veins are represented by paired vascular bundles. The cotyledonary node is unilacunar, two-traced.

Lindelofia stylosa (Kar. & Kir.) Brand is a taprooted polycarpic plant with a polycephalous caudex. It is widespread in the belt of microthermic steppes, alpine deserts of Central Asia, on rocky slopes, at temporarily moist habitats (ZAKIROV 1961; CHUKAVINA 1984).

The plant is characterized by the cotyledonary seed emergence pattern (Table 1, Fig. 6B–D). In seedlings, the cotyledon blades are elliptical or broad-lanceolate, tapering into a short petiole,

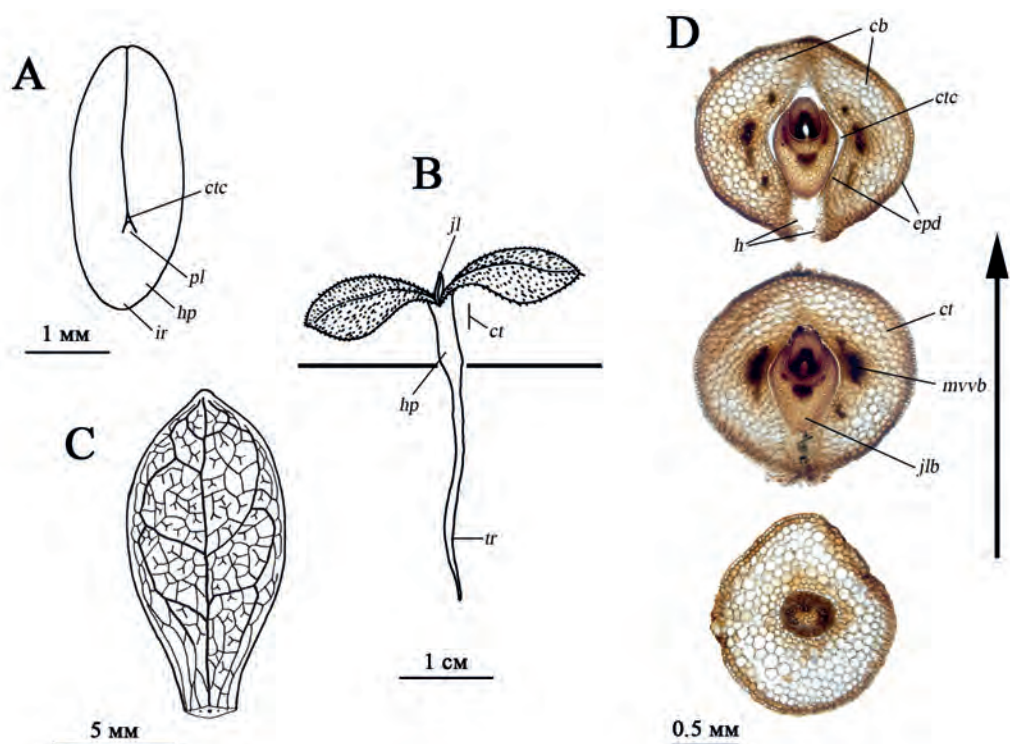


Figure 5. *Anchusa pseudochroleuca*. A – embryo; B – seedling; C – cotyledon; D – cross sections through hypocotyl and cotyledon tube. For abbreviations see Fig. 1.

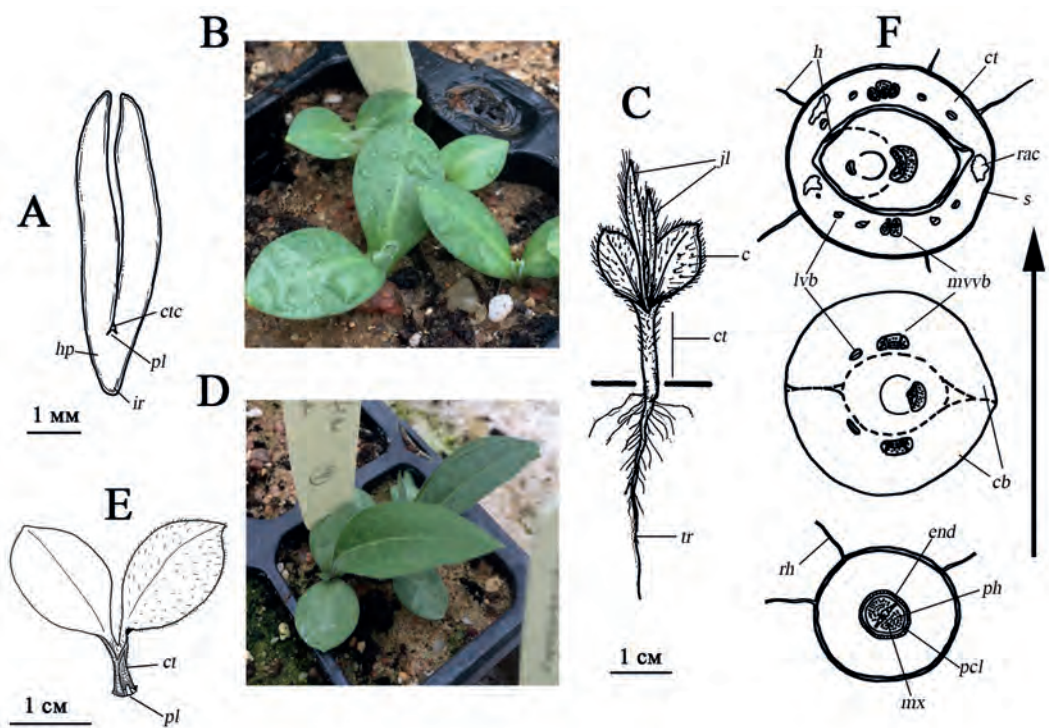


Figure 6. *Lindelofia stylosa*. A – embryo; B – seedling; C, D – sprouts, stands; E – cotyledons, fused into a tube by their bases; F – schemes of cross sections of the root, cotyledon node and tube. For abbreviations see Fig. 1.

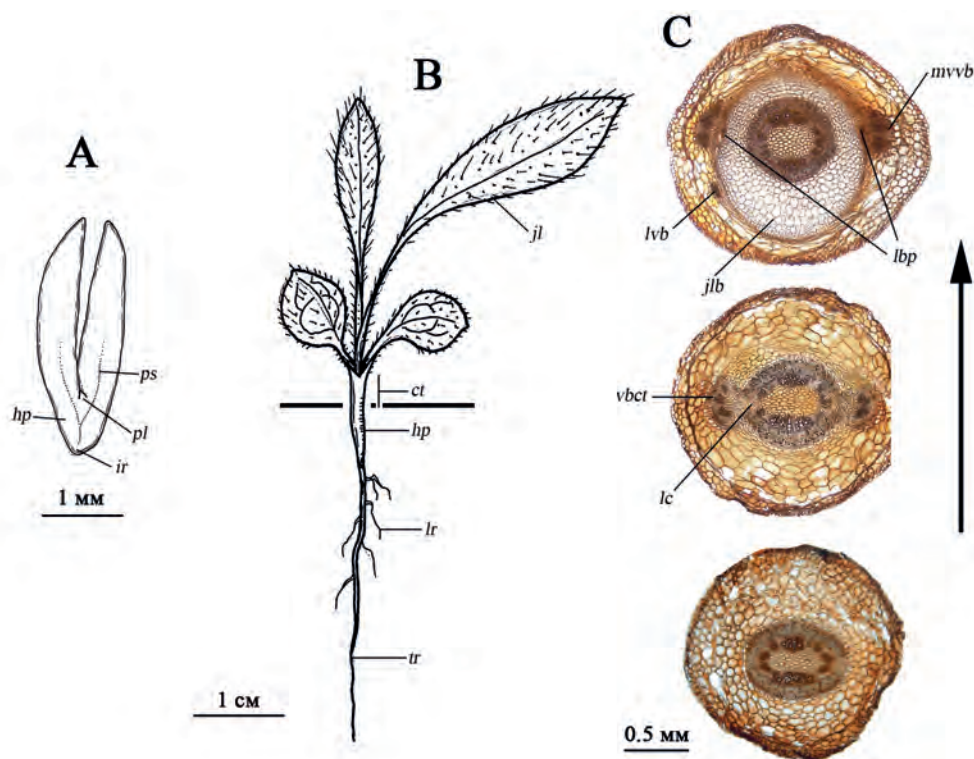


Figure 7. *Lindelofia macrostyla*. A – embryo; B – juvenile plant; C – a series of cross sections at the level of hypocotyl, cotyledon node and tube. For abbreviations see Fig. 1.

fleshy, with fused bases. The latter undergo intercalary growth forming a long (1.2–1.5 cm) continuous tube, embracing the epicotyl together with the two leaf primordia. Only slight traces of syncotily can be noticed in the embryo; large cotyledons exceed the axial part 3.5 times (Fig. 6A). Small-celled epidermis of the tube's abaxial and adaxial sides is strigose near the blade; trichomes subulate, one-celled, living; scarce stomata can be seen. The ground parenchyma of 14 cell layers comprises small air cavities and is penetrated by 10 collateral bundles, out of which 2–3 are part of opposite median veins and the others are lateral. At the level of the cotyledonary node, the bundles are brought together reducing in number (2–4). They enter the hypocotyl stele as a single strand; the node is unilacunar.

Lindelofia macrostyla (Bunge) Popov resembles *L. stylosa* in its life form. The species is known to grow on rocky slopes in the middle and upper (subalpine) montane belt of Central Asia (ZAKIROV 1961; CHUKAVINA 1984). Seedling emergence pattern is hypocotylous and cotyledonary (Table 1, Fig. 7A, B). The cotyledons possess long petioles; the cotyledon tube is short (3–4 mm), basal parts of the petioles may contribute to its formation (Fig. 7C). The tube wall includes up to 11 parenchymal cell layers and up to 7–8 vascular bundles. The cotyledonary node is unilacunar, two- to four-traced. Some individuals are found to have small meristematic primordia of future lateral buds located in the cotyledons' axils.

Macrotomia euchroma (Royle) Paulsen is a perennial hemicryptophyte with a thick (up to 2 cm) staining taproot and polycephalous caudex. It occurs on rocky slopes in the middle and upper (subalpine) montane belt (ZAKIROV 1961; CHUKAVINA 1984). The plant is characterized by

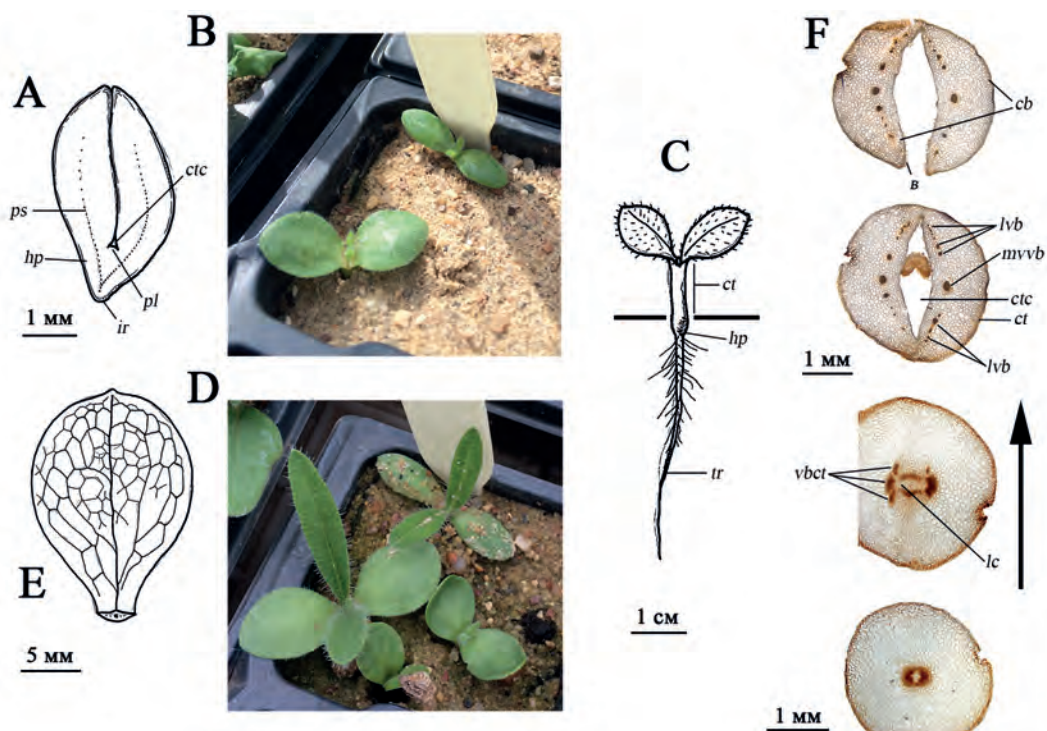


Figure 8. *Macrotomia euchroma*. A – embryo; B–D – seedlings and sprouts; E – cotyledon; F – a series of cross sections through hypocotyl, cotyledon node and leaf sheath. For abbreviations see Fig. 1.

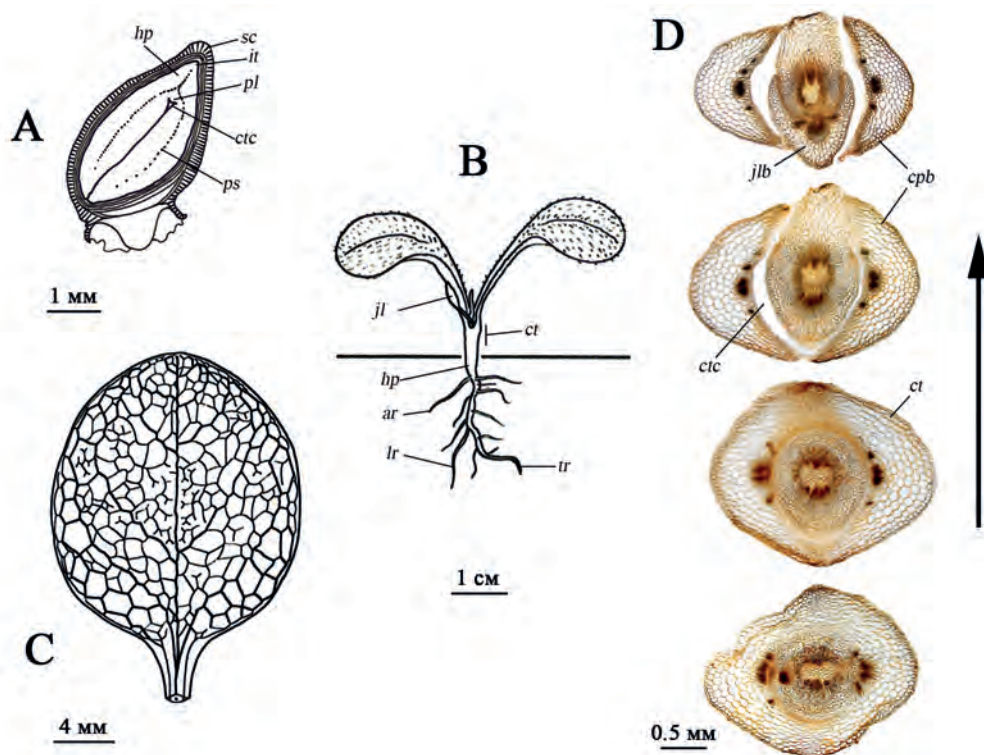


Figure 9. *Symphytum x uplandicum*. A – seed in longitudinal section; B – seedling; C – cotyledon; D – a series of cross sections at different levels of the cotyledon tube. For abbreviations see Fig. 1.

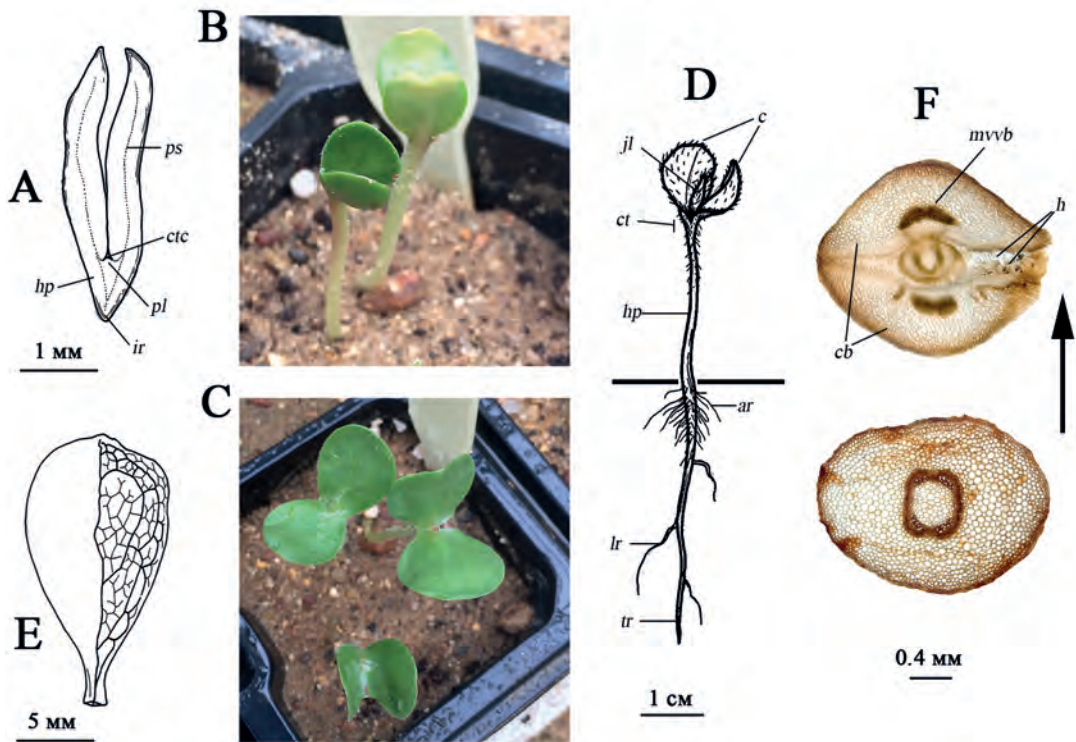


Figure 10. *Trichodesma incanum*. A – embryo; B–D – seedlings; E – cotyledon; F – hypocotyl cross section near the cotyledon node and cotyledon tube base. For abbreviations see Fig. 1.

the cotyledonary seed emergence pattern (Table 1, Fig. 8B–D). Densely pubescent seedling cotyledons are rounded or oblong to elliptical, born on short petioles (Table 1, Fig. 8E). The cotyledon tube is pronounced, thick (3 mm), long (up to 1.5 cm), pink in colour. It is quite abruptly delimited from the relatively thin hypocotyl, which possesses short adventitious roots. The tube formation in *M. euchroma* results from the fusion of base margins of both cotyledons and can be seen as early as at the embryo stage (Fig. 8A); subsequently, they undergo intercalary elongation. The tube wall (Fig. 8F) is found to have the highest (among the studied species) number of cell layers (22) of storage parenchyma throughout its almost entire length and the highest vascular bundle count (18). Close to the cotyledonary node, median and lateral bundles draw together and unite in two opposite synthetic strands. The node is unilacunar, two-traced.

Symphytum × uplandicum Nyman is a perennial fibrous-rooted hemicryptophyte or geophyte with a short rhizome, quite widespread in Europe and North America; only very few localities of this species in Central Russia are known (Moscow city and Moscow region). The plant grows in wet meadows, marshy areas, along rivers and streams (PAWŁOWSKI 1972; HEGE 1972; TIKHOMIROV et al. 1999).

In its large (2.3–2.5 mm long, 1.8–2 mm wide) embryo, the axial part is 3 times shorter than the cotyledons (0.65 mm and 1.9 mm respectively) (Fig. 9A). The seedling emergence pattern is cotyledonary (Table 1, Fig. 9B). Cotyledon blades are oval to elliptical, born on long petioles. The cotyledon tube is short (2–3 mm), formed by the fused basal parts of the petioles (Fig. 9D). The tube wall is covered by small-celled epidermis lacking trichomes or stomata. Large-celled

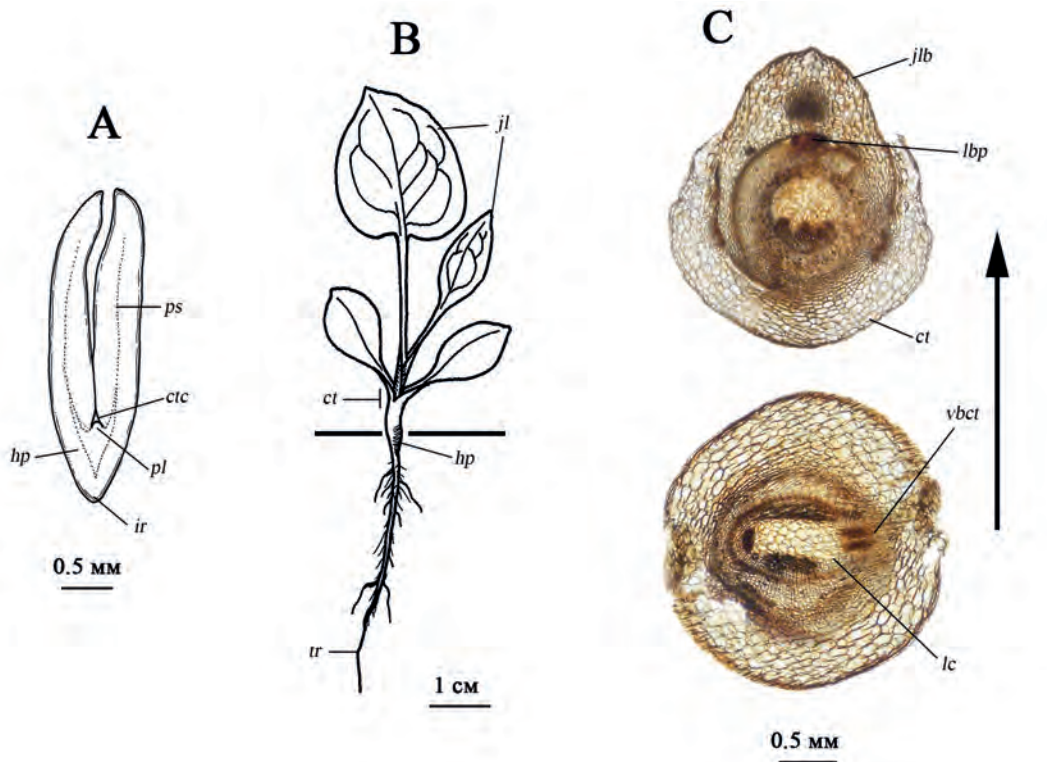


Figure 11. *Hackelia uncinata*. A – embryo; B – juvenile plant; C – cross section of the cotyledon tube. For abbreviations see Fig. 1..

parenchyma (6–7 layers) comprises up to 8 vascular bundles, out of them paired bundles are median veins and singular ones are lateral veins. When entering the hypocotyl stele, the lateral bundles unite with the median ones. The node is unilacunar, two-traced.

Trichodesma incanum (Bunge) A. DC. is a perennial hemicryptophyte with a strong branching rhizome. The species is spread in Central Asia, growing on rocky slopes in the lower and middle alpine belt at the altitude of 600–2900 m (ZAKIROV 1962; CHUKAVINA 1984). The seedling emergence pattern is cotyledonary (Table 1, Fig. 10A–D). Rounded to spatulate in shape, born on short petioles, the fleshy cotyledons of the species' seedlings fuse at their margins by some very small spots without forming a pronounced cotyledon tube (Fig. 10). At the node level, multiple long, living trichomes on the adaxial surface can be seen. Solitary stomata are located between them. Cotyledons fully embrace the plumule along with foliar primordia providing solid protection and sufficient hydration to the latter (Fig. 10F). Vascular bundles 7–9, drawn together into 2 massive median strands; the node is unilacunar, two-traced.

Hackelia uncinata (Benth.) C.E.C. Fisch. is a perennial hemicryptophyte with a short rhizome. The plant occurs in Asian montane forests at 2400–3500 m. Moist soils are preferred by this species. The seedling emergence pattern is cotyledonary (Table 1, Fig. 11A, B). Rounded to ovate cotyledons possess long petioles. The latter are slightly displaced from each other; they elevate cotyledon blades to the surface having undergone intercalary elongation. Cotyledon bases form a short (2–3 mm) continuous tube, surrounding the rapidly developing plumule (Fig. 11C). In the seedling, primordia of axillary buds are situated in the axils of cotyledons and the first leaf.

The tube's relatively thin wall comprises 6–7 layers of thin-walled parenchymal cells and 4 paired vascular bundles. The cotyledonary node is unilacunar, two-traced.

Discussion

All representatives of Boraginaceae covered by the present study are found to possess protein-free seeds with large embryos. Their own nutrient supply (fatty oils, aleurone) is stored predominantly in the cotyledons which are much greater in length than the embryo's axial part (Table 1). The seedling emergence pattern is epigeous: cotyledonary or hypocotylous and cotyledonary, less frequently hypocotylous. In the first two cases, syncotyly is reported. Its traces can sometimes be noticed as early as at the final stage of embryogenesis, indicating the early manifestation of geophily (*Brachybotrys paridiformis*, *Omphalodes verna*, *O. linifolia*, *Anchusa pseudochroleuca*, *Lindelofia stylosa*, *Macrotomia euchroma*, *Symphytum* × *uplandicum*, *Trichodesma incanum*, *Hackelia uncinata*). It is, however, much more pronounced in the seedling and sprouts. Syncotyly results in the formation of a hollow cotyledon tube relatively small in length (2–5 mm). The longest cotyledon tube (around 1.5 cm) was reported in *Lindelofia stylosa* (Fig. 6), a polycarpic plant growing on rocky slopes in alpine deserts of Central Asia. According to BUTENKO et al. (2009), the cotyledon tube of similar length is found in *Rindera tetraspis* Pall., a Central Asian species growing in rock crevices.

Two ways of cotyledon tube formation can be hypothesized. In species with sessile cotyledons or those with short petioles, the tube is mostly composed by the fused margins of their enlarged bases (*Anchusa pseudochroleuca*, *Echium vulgare*, *Lindelofia stylosa*, *L. macrostyla*, *Macrotomia euchroma*, *Trichodesma incanum*, *Hackelia uncinata*), whereas in species with cotyledons born on long petioles, the syngeneses is primarily limited to the petioles (*O. linifolia*, *Symphytum* × *uplandicum*). Nonetheless, the fusion can be restricted to their basal parts only or they can be grown together throughout their entire length, but near the blades it is usually unilateral (*Brachybotrys paridiformis*, *Omphalodes verna*).

The comparative anatomical analysis of the cotyledon tubes in the studied species of Boraginaceae has revealed, along with a great deal of structural resemblance, a number of differences. They are related to the tube wall thickness, the number of collateral vascular bundles, the presence of stomata and pubescence. The most massive tube wall was found in *Macrotomia euchroma* (Table 1, Fig. 8F). It comprises up to 22 layers of ground parenchyma together with the small-celled epidermis. 16–18 vascular bundles are embedded into it; the central ones draw together in groups, forming opposite veins of two to three bundles, the other ones are lateral. The number of bundles decreases towards the tube base, reaching 6–8 in the nodal area. Just like in other species, they enter the stele of the hypocotyl on both sides as united synthetic median strands of three to four bundles in each. The cotyledonary nodes are unilacunar, two-traced. The number of vascular bundles in the middle of the tube wall varies widely, ranging from 4 (*Hackelia uncinata*) to 18 (*Macrotomia euchroma*), which is likely due to the cotyledon sizes and the vascular supply pattern in their parts (blades, petioles, bases), contributing to the tube formation.

The pubescence and stomata are evident only in the upper part of the tube in *Anchusa pseudochroleuca* and *Lindelofia stylosa*; the most pronounced pubescence is characteristic of *Echium vulgare* (Fig. 4D). The living trichomes as well as the stomata, can occur on both sides of

the tube wall, but prevail on the adaxial side. A small number of stomata is reported in *Lindelofia stylosa* (amphistomatic pattern), *Anchusa pseudochroleuca* (epistomatic pattern) and *Omphalodes verna* (hypostomatic pattern).

Summing up the above stated data on seedlings and sprouts in Boraginaceae, we point out that syncotyly is a significant adaptive feature shared by all investigated species. The formation of the closed cotyledon tube, serving primarily to protect the poorly differentiated plumule, is common in plants of various habitats (Table 1). However, in extreme conditions, e.g. alpine deserts of Central Asia, the tube genesis, its length, morphology and anatomy can differ markedly in species of the same genus. Thus, in *Lindelofia stylosa*, growing at relatively high altitudes, the cotyledon tube is much longer and better developed than in *L. macrostyla*, a plant with a similar life form but growing in habitats at lower altitudes (lower and middle montane belt) (Table 1, Figs 6–7). These data are in diametric opposition to YANISHEVSKYI (1910) regarding the cotyledon tube length in *Rheum* species growing in mountains and plains.

No direct correlation between syncotyly and plant life form was revealed in the studied Boraginaceae (Table 1). Nevertheless, plumule development into the primary shoot is faster in perennials than in annual plants (*Omphalodes*) (Figs 2–3). A relatively long (1–1.2 cm) cotyledon tube in perennial *Omphalodes verna* provides good, solid protection not only for the poorly differentiated plumule, but also for the emerging metamerous of the leaf-bearing shoot as well as for the primordia of the cotyledons' axillary buds, appearing rather early at the seedling stage. Highly nutritive substances stored in larger cotyledons of *O. verna* promote and facilitate faster seedling growth and development in this species than in the annual *O. linifolia*.

All in all, the question of the interrelation between the phenomenon of syncotyly and the plant life form as well as the question of the cotyledon tube's taxonomic value within Boraginaceae remains unresolved due to the limited number of species studied.

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