

# Liliopsida anatomical traits in the *Trollius* species (Ranunculaceae)

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**Abstract.** Floral shoot anatomy in *Trollius asiaticus* and seedling morphology in *T. altaicus*, *T. apertus*, *T. asiaticus*, *T. europaeus*, *T. ledebourii* and *T. sajanensis* were studied in early ontogeny stages. In four species, there occurs syncotyly, its distribution among the species of the genus being probably a valuable characteristic to understand the genus phylogeny. In *T. asiaticus*, vascular bundles are closed, stems are unable to any secondary increase in thickness; sclerenchyma is not located radially but forms massive strands externally to the vascular bundles.

## Introduction

Despite the fact that phylogeny issues are paid particular attention, evolutionary relationships between the flowering plant taxa are still a continuous matter of dispute limited by the lack of information. Heterobathmic taxa in which the characters of evolutionary advancement are combined with primitive ones present the particular difficulty. According to A.L. Takhtajan [1], the ancestor of the taxa with heterobathmic characters is the taxon in which both types of traits are at the primitive stage of development. So exhibiting the characters of monocots or dicots is a characteristic of basal taxa forming the foundation of the phylogenetic tree. Thus far, it can be said with certainty that no anatomic-morphological attributes strictly define one or another clade. Nonetheless, some characters are typical for the vast majority of the taxa representing one clade and relatively rare in the other clade. For example, the adventitious root system mainly occurs in *Liliopsida* and also, in some very few representatives of *Magnoliopsida*.

In *Magnoliopsida* clade, presence of developed endosperm as a storage tissue of seeds is a well-studied character to prove the basal position of the taxa, while this is typical for *Liliopsida*. As primitivism or advancement criteria such characters as structure of vessels, cambial elements, pith rays and stoma types [1].

However, phylogenetic significance of some characters has not been recognized yet. They are syncotyly or adnation of cotyledons and pseudomonocotyly or false monocotyly when either cotyledon petioles form cotyledonary tubes or underdevelopment of one cotyledon is not revealed. These morphological peculiarities are being all-round studied; there are investigations on their anatomic-morphological structure and new taxa with such characters are found [2-6]. In *Ranunculaceae*, syncotyly occurs in *Anemone blanda*, *A. bucharica*, *A. caucasica*, *Clematis serratifolia*, *Pulsatilla alba*; pseudomonocoledony was established in

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*Anemone* genus (*A. blanda*, *A. caucasica*, *A. flaccida*, *A. multifida*, *A. ranunculoides*), *Thalictrum* (*T. sultanabadense*), *Eranthis*, *Ficaria* [7, 8].

*Trollius* (*Ranunculaceae*) representatives are polycarpic grasses with fibrous root system and dicyclic semi-rosette shoots [9-11]. The adventitious root system analogous to most *Liliopsida* species can very often be seen in *Ranunculaceae* family. Apart from morphological structure, typical for monocots anatomic characters are also exhibited. For instance, in *Trollius europaeus*, root structure stays primitive life-long, as roots are unable to secondary increase in thickness [12]. The stem comprises angular collenchyma, vascular bundles are collateral with hardly notable cambium and lignified pith rays [12]. The author concludes that the *Ranunculaceae* plants with more primitive flower design have more typical for *Liliopsida* characters.

If characters peculiar for monocots or any other characters that prove the phyletic belonging of plants are established in *Magnoliopsida*, it contributes to better understanding of differentiation in the course of phylogenesis and also to revealing both development patterns and evolutionary position of the taxa.

More wide-spread in *Liliopsida* characters occur in *Trollius* representatives at different levels of their structure organisation. Microsporangium examination allowed to reveal the centripetal differentiation of the anther wall layers in *T. ledebourii* [13]. Such differentiation results from the fact that the development of microsporangium layers follows the monocotylous mode.

By studying the morphology of *Trollius* seedling it was found out that cotyledon petioles are adnate in *T. asiaticus* and not adnate in *T. chinensis*, *T. pumilus*, *T. europaeus* [14]. The author offers the degree of cotyledon adnation as a diagnostic character, although no discussion on its phyletic importance is given. Nevertheless, this character also occurs in representatives of other genera. It can determine the genus in whole, but more often being the character of an intrageneric group [4, 15, 16]. In its turn, syncotyly is not inherent in *Liliopsida* since the plants are monocots, but this character should be paid attention as it is rather rare and thus scarcely studied in *Magnoliopsida*.

It is acute to search for anatomic characters of *Trollius* species which either are more spread in *Liliopsida* or have less phyletic significance. In that regard, we have studied the anatomy of plants at the early ontogenetic stages and of elongated shoots at their generative ages of some *Trollius* species.

## Materials and methods

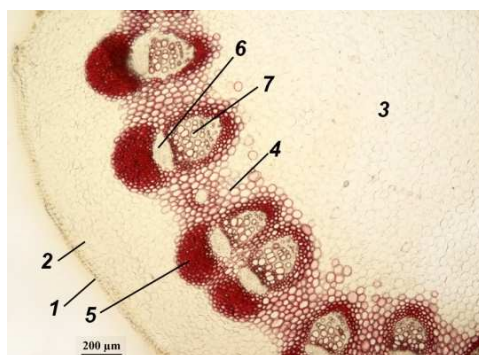
Early ontogenetic stages were studied in the following *Trollius* species: *T. altaicus* C.A. Meyer, *T. apertus* Perf. ex Igoschina of section *Trollius* Doroz.; *T. asiaticus* L., *T. ledebourii* Reichenb. of section *Longipetala* Doroz.; *T. sajanensis* (Malychev) Sipl. Seedling anatomy was investigated only in *T. asiaticus*, *T. ledebourii*.

Swelled seeds and seedlings were fixed in FAA (formalin, acetic acid, ethanol), then longitudinal and transversal slices were prepared by standard technique on the freezing sliding microtome MS-2 with a thermoelectric cold plate TCP-II [17]. The material was dyed with phloroglucinol or alcian blue [18] and examined under the light microscope Carl Zeiss Axio Scope A1.

## Results and discussion

The floral shoot stems in both *Trollius* species under the study are upright. Vascular bundles are located in one row as in most *Magnoliopsida*. The stem structure is similar to the one of *Liliopsida* plants; vascular bundles are closed with no interbundler or bundular

cambium, thus secondary increase in stem thickness cannot take place (Fig. 1). Sclerenchyma is not located radially but forms massive strands externally to the vascular bundles. That provides it good support function, but makes the stem as break-brittle as a culm.



**Fig. 1.** Transversal slice of the *Trollius asiaticus* L floral shoot dyed with phloroglucinol. 1. Epidermis, 2. Chlorenchyma, 3. Medullar parenchyma, 4. Interbundular parenchyma, 5. Sclerenchyma, 6. Phloem, 7. Xylem; Bar 0.2 mm

In the literature, vascular bundles of *Trollius europaeus* are described as open ones [12]. However, the author does not provide the figures of stem vascular bundles. Analysing her pictures, we failed to find the cambial layer there. It is neither observed in the vascular bundles of elongated shoots in *T. asiaticus*. The medullar parenchyma is loose; it early withers away and the stem turns hollow.

Adnate cotyledon petioles have been observed in *T. asiaticus*, in the hybrid species *T. apertus*, and in introgressive natural forms *T. altaicus* (the Seminski range, the Altai Republic, Russia) and *T. sajanensis* (Hamar-Daban, Irkutsk Oblast, Russia). Introgressive forms are determined by morphological criteria. Despite presented a group of diagnostic characters defining their species membership, these forms differ from the typical specimens by insignificant deviation towards *T. asiaticus*, as they have longer and more numerous petals. In *T. europaeus*, syncotyly was not revealed, however the seedlings of our artificial hybrid *T. asiaticus* × *T. europaeus* exhibit syncotyly in all the specimens of the F<sub>2</sub> generation. That makes us consider syncotyly as an inheritable character which is steadily passed by hybridisation.

In syncotyloous species and forms, initial stages of germination are the same. Embryos in fully developed seeds are immature, usually, development stages from the early heart to the early torpedo ones. Position of the two symmetric cotyledon germs is normal, but the cotyledonary tube, hypocotyl and primary root are not differentiated yet.

Embryo development and its germination take place in the course of stratification. The vegetative cone of the shoot appears in the center of the embryo axial part as a group of meristematic initials. Then the layer of axial cells straight above the meristematic apical zone undergoes lysis. The cell layers located higher and aside from the apex form the cotyledonary tube. Germination (growing from the seed) begins after the part of the embryo under the epical meristem differentiates into a hypocotyl and primary root.

In *Trollius*, seed germination is cotyledonous. According to Barykina and Chubatova [19], this term should be used for the seeds whose seed coat is brought above the soil surface by cotyledons (in this case, by the cotyledonary tube), while the plumule stays at the ground level. Cotyledonary *Trollius* species have hardly expressed hypocotyl with no growth potential; its diameter is close to the diameter of the cotyledonary tube and just slightly more than the one of the root. A young shoot apex is presented by a weakly convex vegetative cone. During the further development, symmetry of the cotyledonary tube shifts

to the side position. The slit which appears due to the lysis process over the vegetative cone is filled with leaf primordia. With their growth, the first simple leaf emerges by breaking through the epidermis of the cotyledonary tube (Fig. 2) which surrounds the vegetative cone and primordia of true leaves at the height of 1–1.5 mm.



**Fig. 2.** *Trollius altaicus* seedlings (Seminski range, the Altai Republic, Russia) with a cotyledonary tube (in the right) and a first true leaf (in the center); bar 10 mm.

The scholars, who study syncotylous plants, mention positive geotropic growth of the lower part of the cotyledonary tube. Thus the plumule is pulled to some depth into the soil and protected from negative environmental conditions. In the process, both hypocotyl and the lower part of the cotyledonary tube can perform absorption and have hairs, as for example in some representatives of *Trifolium* and *Podofillum* [4, 15]. In *Trollius* plants, the hypocotyl is clearly separated from the root and does not produce root hairs. Differentiation of hypocotyl sub-epidermal layers is photo-dependent. Initially, they differentiate as chlorenchymous cells, but soon lose chlorophyll and develop as parenchymous ones.

Unlike the *Podofillum* genus [4], in *T. asiaticus* seedlings, the cotyledonary tube is filled with intercalary meristem cells at the early stages and there are no root hairs in its base, while later the chlorenchyma differentiates sub-epidermally so as to give rise to axially elongated cells of parenchyma located closer to the centre. Primary vascular system emerges as common in the root and hypocotyl, discontinues at the apex area and then can be seen as two strands in the cotyledonary tube. On further development, the tear of the vascular system disappears.

Accordingly, the cotyledonary tube of *T. asiaticus* should not be considered as the expression of pseudomonocotily, because for the entire duration of embryo development there can be clearly seen two cotyledons. As a term, “adnation of cotyledons” describes the process of cotyledonary tube forming not clearly. It initiates in the axial part of an embryo and has not any sign of division into two petioles. In *Trollius*, syncotily is an inborn inherited character and we suppose the phenomenon not to be linked with hypomorphosis. The cotyledonary tube of *Trollius* species performs protection of the vegetative cone and bringing first assimilative organs of the seedling to the surface.

Adnate cotyledons are typical for evolutionary basal taxa as *Trollius* and *Podophyllum*, and also for rather advances as *Amyema*, *Barathranthus*, *Helicanthes*, *Lysiana*, *Mocuiniella*, etc. [4, 16]. The matter of interest is the fact that in the vast majority of genera, adnate cotyledonary tubes characterize not the whole taxon but a group of genetically close species, hence this character should be analysed from the position of intra-generic differentiation. In some species of *Trollius Longipetala*, syncotily is not observed. These are *T. ledebourii* by our data and *T. chinensis* by literature [14]. However, the phenomenon occurs in the other two species of the section: *T. apertus* and *T. altaicus*. This character does not correspond to the interpretation of the section subdivision by [20]. Taking into

account the genetic stability of the character, we recommend to include it to the next sectional revision of the genus. Also, its phylogenetic significance should be evaluated.

Analysis of literature and results of our investigations makes us conclude that close to *Liliopsida* characters are registered in the anatomic-morphological structure of root system, anatomy of vascular bundles, presence of endosperm as a storage tissue of seeds, and differentiation of microsporangium walls. Stem vascular bundles located in one layer and syncotyly in some species should be referred to specific traits. Such a combination creates an individual cluster of characters typical for all the *Trollius* representatives.

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