

Morphological and anatomical observations on *Ranunculus auricomus* L. var. *biformis* L. in Vlasina Lake, Serbia

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Abstract:

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R. auricomus var. *biformis* L. (*R. binatus* Kit.) grows mainly in the suburbs of Vlasina plateau beside a variety of other species and forms one part of the peat-land association. Specimens were collected from the northeast coastal flooded area of Vlasina Lake (Latitude: 42° 41' 46 N, Longitude: 22° 22' 14 E). In this study, some morphological and anatomical characteristics were investigated. Samples for anatomical analysis were prepared according to the standard methods described by Ruzin (1999) though, slightly modified (Atanackovic et al., 2012). Morphological features such as leaf, petiole, stem, and root have been described. The anatomy presented in this work form the first of its kind available in the literature about this variety found in the Vlasina Lake. Transverse sections of ground-leaf, upper-leaf, petiole, stem and root have been examined and photographed. The observed morphological and anatomical features showed that the species grows in these flooding conditions.

Key words: Anatomy, leaf, morphology, petiole, *Ranunculus*, root, stem

Introduction

Ranunculus L. or buttercup is the largest genus of *Ranunculaceae* that contains around 600 species and described as cosmopolitan (Tamura, 1993) that mostly distributed in all continents throughout temperate, Arctic, sub-Antarctic and rarely in high elevated areas of the tropic zones excluding the Antarctic (Erst and Sukhorukov, 2011). *Ranunculus* grows in a wide range of habitats such as rivers, lakes, wet soils, damp/dry meadows and forests, and shows several morphological adaptations to different habitats (Paun et al., 2006). *Ranunculus* is well established in Central Europe where the yellow

buttercups are found in meadows, pastures, or ruderal places during spring and summer (Steinbach and Gottsberger, 1994). *R. auricomus* complex (aggregate) represents one of the larger clades within *Ranunculus* (Hörandl et al., 2005) that is distributed throughout Europe to Western Siberia, from the Arctic zone to the Mediterranean region, Greenland and Alaska (Jalas and Suominen, 1989). *R. auricomus* complex is highly diverse in morphotypes, cytotypes and ecotypes, which were the reasons to be considered as a separate section (Loos, 1997) and often subdivided into four morphological groups; *auricomus*, *cassubicus*, *fallax* and *monophyllus sensu* (Ericsson, 1992; 2001).

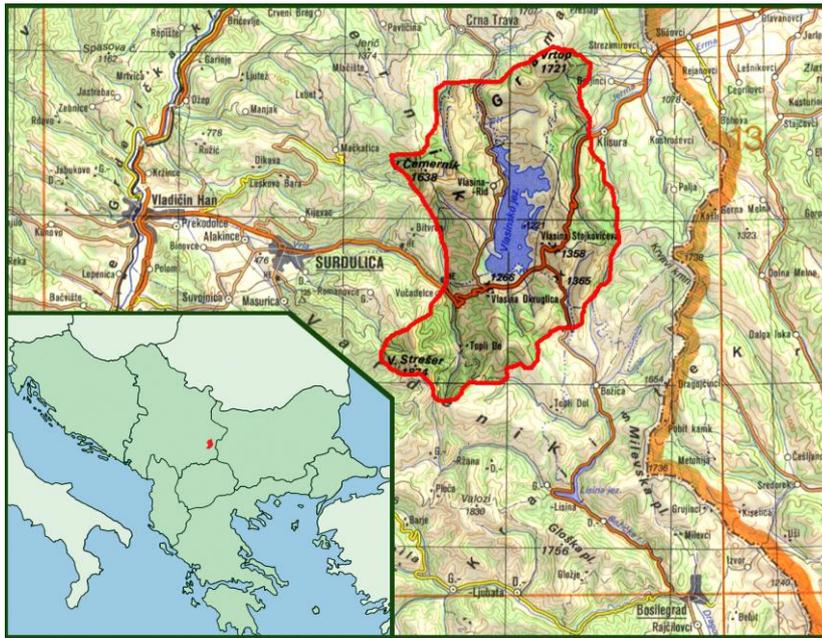


Fig. 1. Geographical position of investigated area

Hö r a n d l et al. (2005) reported that various morphological adaptations and reproductive strategies such as vegetative reproduction (stolons), self compatibility (in water-buttercups), and likely agamospermy (*R. auricomus* complex) could play important role in the *Ranunculus* ability to colonize different habitats, altitudes, and latitudes. Despite the morphological adaptations, *R. auricomus* var. *biformis* is in an extremely high risk of extinction at the regional scale and it is recognized as a critically endangered variety (R a n đ e l o v i ć et al., 2010).

Temporary or continuous flooding of soil with fresh water occurs as a result of overflowing of the lake (R a n đ e l o v i ć, Z l a t k o v i ć, 2010). The mechanisms by which flood-tolerant plants survive water-logging are complex and involve interactions of morphological, anatomical, and physiological adaptations (K o z l o w s k i, 1997; S t e v e n s et al., 2002). Generally, adverse effects of flooding include changes in plant distribution and composition as well as changes in shoot growth of many plants due to suppressed leaf formation and expansion of leaves and internodes. Soil inundation leads to increased length of the first internode and furthermore, flooding may also increases intercellular spaces in the tissues of flood-tolerant plants (A t a n a c k o v i c et al., 2012). Certain terrestrial plant species respond to poor aeration in flooded areas and produce aerenchyma mainly in the cortical tissues of the stem and root (J a c k s o n and C o l m e r, 2005; S t e v e n s et al., 2002; J u n g et al., 2008). Thus, flooded habitats may change the anatomical structure such as the cuticle layer and phloem in leaves, pith cavity area and vessel lumen

in stem (T a o et al., 2009). Different plants express different morphological or anatomical adjustments in response to environmental changes, which act as mechanisms of survival and reproduction under a wide range of conditions (B e n z et al., 2007).

We assumed that *R. auricomus* var. *biformis* adapted and survived in temporal flooded peat bog habitat due to morphological and/or anatomical adjustments. Hence, its morphological and anatomical features of leaf, petiole and stem and anatomical adaptations of root were investigated.

Material and methods

Sampling of plant material

Plant material were collected from the Northeast coastal flooded area of Vlasina Lake at latitude 42° 41' 46 N and longitude 22° 22' 14 E (Fig. 1). The variety was identified by Professor Vladimir Stevanovic. Plant samples were fixed in 50% ethanol and samples were also deposited and recorded at the Herbarium and Wet Collection of the Department of Ecology and Geography of Plants, Institute of Botany and Botanical garden “Jevremovac”, Faculty of Biology, University of Belgrade, Serbia.



Fig. 2. *R. auricomus* var. *biformis* plant growing in a peat bog habitat

Preparation of plant material for anatomical analysis

Anatomical analysis of leaf, petiole, stem and root were performed. Samples were prepared according to the standard methods described by Ruzin (1999), though slightly modified (Atanacković et al., 2012).

Morphological observations and anatomical analysis under light microscope

Certain quantitative measures were carried out for leaf, stem and root of *R. auricomus* var. *biformis* as well as description of petioles. Morphometric measures were taken for leaves that included; leaf length, leaf length from base to the widest part, leaf width, leaf perimeter, and leaf area, while those for the plant included; length of each of the first three internodes, stem length and total number of internodes. Measurements performed on the cross section of leaf included; leaf thickness, width and thickness of epidermal cells and surface, total thickness of mesophyll including thickness of spongy and palisade tissues and number of layers for each tissue. The diameter of primary cortex and central cylinder at the radius of root cross section were also measured.

Statistical analysis

To increase the accuracy of data and statistical analysis, each character was measured on 25 samples of leaf, stem and root of individuals from the plant population. All measurements of quantitative characters were performed on an "Image Analyzer" software package (QWin, Leica Microsystems Imaging Solutions Ltd, Cambridge, UK), and then the measurement results were processed by the software SPSS 15 for Windows (SPSS 15.0. 2006). For each character, basic parameters were statistically analyzed such as mean, minimum value, maximum value and standard errors.

Results and discussion

In Serbia, *R. auricomus* var. *biformis* grows in light and moist deciduous forests, bushes, thickets, damp meadows, fields from lowland regions to Alps belt, but grows mainly in the suburbs of Vlasina plateau beside a variety of other species. *R. auricomus* var. *biformis* became part of the peatland association that includes *Caricetum goodenowii* and *Caricion cannescentis-nigrae*, which belong to the class *Caricetalia fusca* and

Scheuchzerio-Caricetea fusca (Randelović et al., 2010).

R. auricomus var. *biformis* is a perennial plant that grows during spring-summer in a half-rosette and hemicryptophyte (Fig. 2). Ground leaves are rounded, kidney-shaped and partially divided into lobes. The stem is erect, in the range of 9.5 cm high and rarely covered with trichomes. The root is short and vertical with numerous hair roots.

Morphological characteristics

Leaf

R. auricomus var. *biformis* has two heterophyllous leaves here named as upper and ground leaves, which differ in shape and size. Upper leaves are sessile and cut into 4-8 linear-lanceolate lobes. These leaves are fan-like-shaped, soft, shiny, smooth and dark green, while the reverse side is light green and the length of leaf is nearly six times its width (Fig. 3a). There are two types of ground leaves namely ground leaves that are not lobed (Fig. 3b) and ground leaves that are divided into lobes (Fig. 3c). Deep ground leaves have larger lobes with rounded tips, sharply-toothed secondary parts and sometimes appear jagged with lateral lobes. Ground leaves are kidney-shaped, shallow, soft, smooth, and glossy green with long petioles. The length of leaf is slightly less than or equal to its width (Table 1).

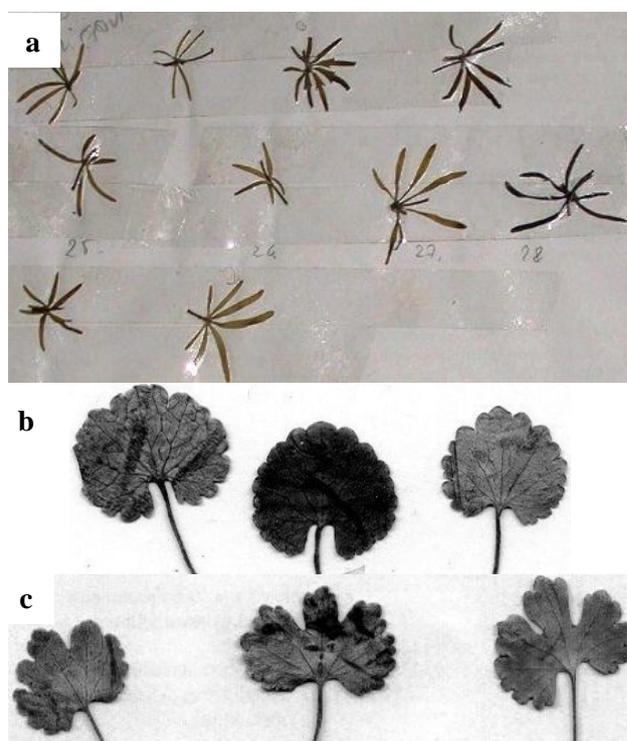


Fig. 3. Morphological features of upper (a) and ground leaves; not lobed (b) and lobed (c) from *R. auricomus* var. *biformis*.

Stem

R. auricomus var. *biformis* is an erect plant, branched and can reach 9.5 cm tall (Fig. 2). Two or three internodes of various lengths were observed and while the first internode is the longest, the second and third were slightly shorter (Table 2).

Anatomical characteristics

Upper leaf

The upper leaf of *R. auricomus* var. *biformis* is thinner compared with the ground leaf (Table 1). The adaxial layer of the epidermis consists of larger cells and a few stomata were observed (Fig. 4a). The mesophyll composed of the palisade tissue of 2-3 layers and the spongy tissue of 4 layers of cells and large intercellular spaces, but cells are smaller than in the ground leaves. The palisade tissue is slightly thicker than the spongy tissue (Fig. 4b). The vascular bundle is collateral and surrounded by sclerenchyma tissue (Fig. 4c).

Ground leaf

The two types of ground leaves are not different in structure as seen from the cross sections. The epidermis was observed as one layer of large cells on the adaxial side of leaf and the exterior walls are thicker and slightly prominent. Stomata were seen on both adaxial and abaxial surfaces or leaves are amphistomatic. Similar to the upper leaves, the mesophyll is divided into the palisade tissue that consists of 2-3 layers of extremely large cells, but the first layer of cells is slightly longer than other layers and smaller intercellular spaces were observed. The spongy tissue of 4-6 layers of cells that are irregular in shape, thick-walled and separated by large intercellular spaces (Fig. 5a). The vascular bundles are collateral, enclosed in sclerenchyma tissue (Fig. 5b) and the number of vascular bundles is between 28 and 30 (Table 1).

Petiole

The cross section of the petiole of ground leaf; lobed or not, is kidney-shaped and showed clearly distinct epidermis, primary cortex, central cylinder and pith cavity on one side towards the centre. The epidermis of the petiole consisted mostly of one layer, but two layers in some parts, covered with a well-developed cuticle and stomata were observed. The primary cortex consists of small parenchyma cells that are densely packed in groups of 6-7 layers separated by large intercellular spaces

and sometimes there might be one or two large voids. Five vascular bundles were seen and the central bundle is the largest, while the marginal were the least developed. All bundles are partially enveloped in sclerenchyma that was observed on both sides of the phloem and xylem and the cambium was visible in the central bundle. The pith cavity is irregular in shape (saddle-shaped) and broken in some areas and remains of the pith could be seen (Fig. 6).

Stem

The cross section of stem clearly showed distinct epidermis, primary cortex, vascular tissue and pith cavity located at the centre. The epidermis of stem consists of mostly two layers covered with a well-developed cuticle and stomata were also observable in some areas (Fig. 7a). The primary cortex consists of slightly thicker-walled small parenchyma cells and densely packed but in some areas large intercellular spaces were visible. The chloroplasts and starch reserves were observable in parenchyma cells (Fig. 7b). The vascular tissue consists of 9-10 vascular bundles partially enveloped in sclerenchyma tissue, which is found only on the phloem side of the vascular bundle. In addition to the basic parenchyma tissue, the vascular tissue contains xylem vessels and phloem in a collateral arrangement and remains of the cambium could be seen (Fig. 7c). The central cavity forms the pith cavity and remains of the pith were observed in some areas (Fig. 7a). The thickness of stem cortex is $\frac{3}{4}$ of the radius of pith cavity (Table 2).

Root

The cross section of root showed the primary cortex and central cylinder (Fig. 8a). The parenchyma of primary cortex consists of thin-walled, densely packed cells filled with reserve materials and intercellular spaces between cells scarcely observable. The endodermis forms the last layer of the primary cortex. The vascular tissue consists of the pericycle of 1 or 2 layers of cells, the xylem and phloem that were arranged in radials (Fig. 8b). The xylem consists of 3-4 bundles each contains 4-10 vessels and typical to the normal root with a central stele. The plates of phloem are radial in the vascular bundles. The protophloem mesh tubes were seen larger and located on the periphery, while the metaphloem is near the centre (Fig. 8c). The primary cortex is five times larger than the central cylinder (Table 2).

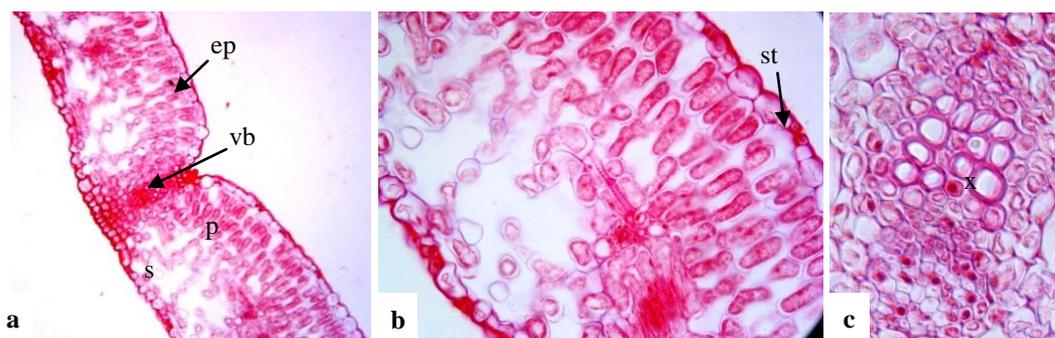


Fig. 4. A cross section through the upper leaf of *R. auricomus* var. *biformis* (a) shows the upper and lower epidermis, mesophyll tissue; palisade and spongy tissue, vascular bundle of mid-rip, and stomata (b) and close up of the vascular bundle of mid-rip (c). ep; epidermis, p; palisade tissue, s; spongy tissue, vb; vascular bundle of mid-rip, st; stoma, x; xylem vessel.

Table 1. Morphological and anatomical features of the upper and ground leaf from *R. auricomus* var. *biformis*

	Upper leaf	Ground leaf (not lobed) ¹	Ground leaf (lobed)
Morphology			
Leaf length (mm)	06.20 ± 0.20	/	/
Length from the base to widest part (mm)	03.07 ± 0.10	05.8 ± 0.30	05.5 ± 0.2
Leaf width (mm)	01.40 ± 0.40	15.6 ± 0.60	18.2 ± 0.3
Leaf perimeter (mm)	71.20 ± 0.20	51.7 ± 0.30	31.4 ± 0.1
Leaf blade area (cm ²)	09.80 ± 0.01	14.3 ± 0.01	03.1 ± 0.1
Anatomy			
Leaf thickness (µm)	106.813 ± 0.4	120.80 ± 0.2	114.0 ± 0.2
Width of upper epidermis (µm)	014.300 ± 0.6	012.40 ± 0.4	012.4 ± 0.3
Thickness of upper epidermis (µm)	010.900 ± 0.5	010.40 ± 0.3	010.6 ± 0.4
Mesophyll tissue thickness (µm)	086.700 ± 0.4	099.90 ± 0.2	098.9 ± 0.3
Palisade tissue thickness (µm)	056.800 ± 0.2	056.50 ± 0.2	045.7 ± 0.2
Spongy tissue thickness (µm)	046.900 ± 0.3	055.00 ± 0.1	055.1 ± 0.1
The number of layers of palisade tissue	002.000 ± 0.0	003.00 ± 0.0	003.0 ± 0.0
The number of layers of spongy tissue	004.000 ± 0.0	006.00 ± 0.2	005.0 ± 0.2
Width of lower epidermis (µm)	010.900 ± 0.5	009.20 ± 0.3	008.8 ± 0.3
Thickness of lower epidermis (µm)	009.200 ± 0.4	008.92 ± 0.3	008.5 ± 0.2

¹ Mean ± Standard Error

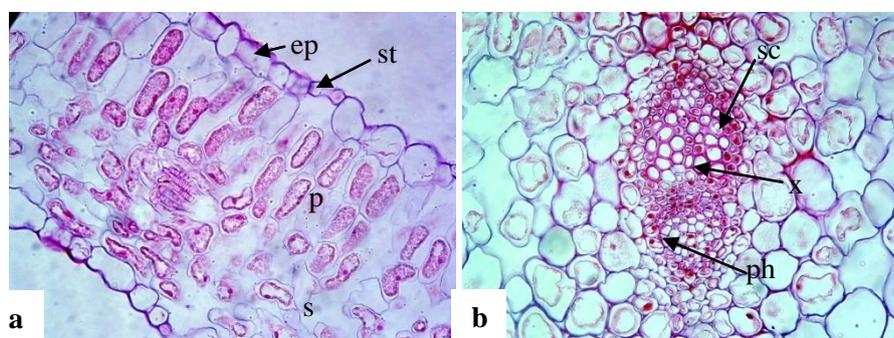


Fig. 5. A cross section through a ground leaf of *R. auricomus* var. *biformis* (a) shows a vascular bundle (b). ep; epidermis, st; stoma, p; palisade tissue, s; spongy tissue, x; xylem vessel, ph; phloem, sc; sclerenchyma.

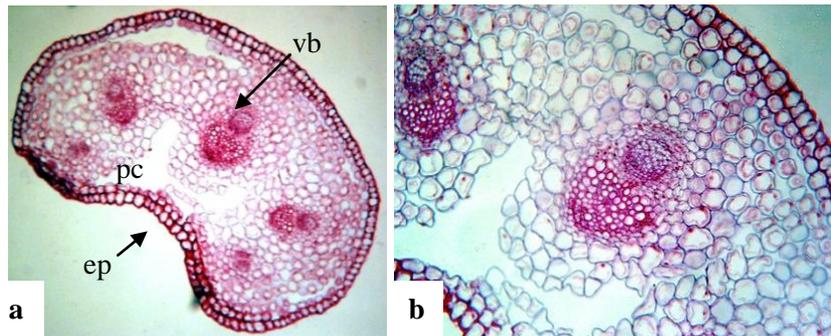


Fig. 6. A cross section of the leaf petiole of *R. auricomus* var. *biformis*. ep; epidermis, vb; central vascular bundle, pc; pith cavity.

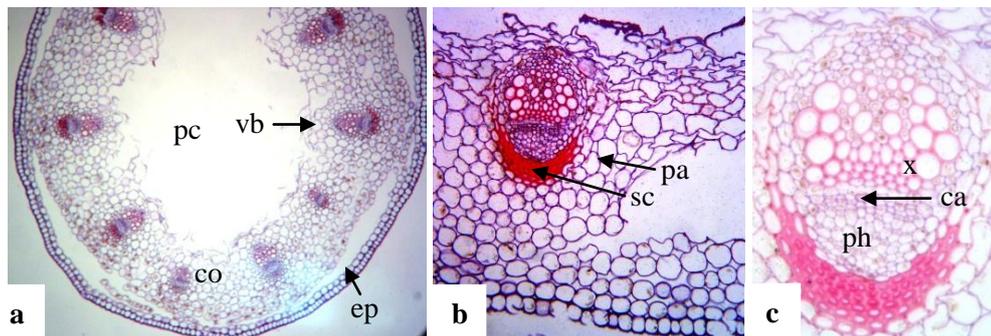


Fig. 7. A cross section through the stem of *R. auricomus* var. *biformis* shows a distinct epidermis, primary cortex, vascular tissue and pith cavity located at the centre (a) a vascular bundle in a parenchyma tissue (b) and details of a vascular bundle (c). ep; epidermis, co; cortex, vp; vascular bundle, pc; pith cavity, sc; sclerenchyma, pa; parenchyma, x; xylem vessel, ca; cambium residues, ph; phloem.

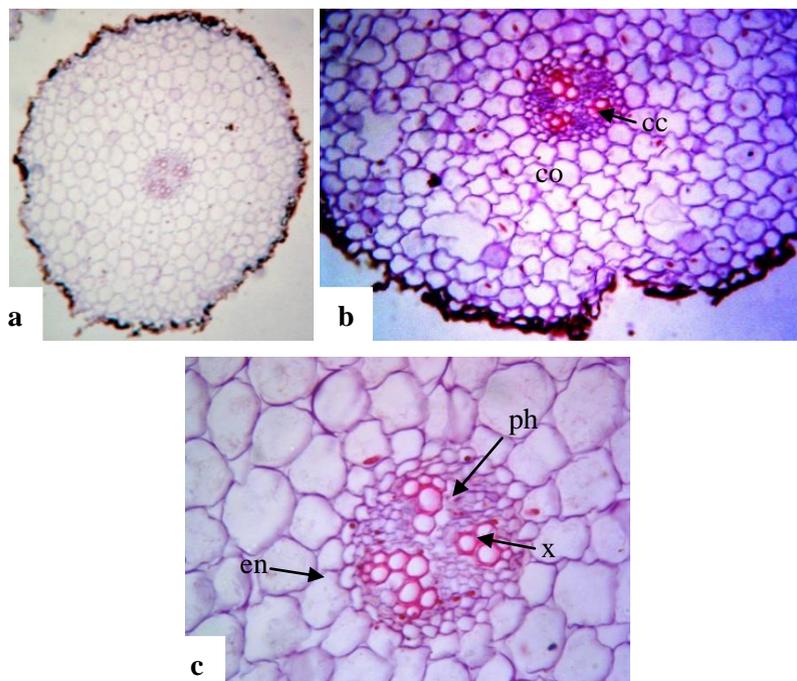


Fig. 8. A cross section through the root of *R. auricomus* var. *biformis* (a) shows the central cylinder and cortex (b) and details of the central cylinder (c). co; cortex, cc; central cylinder, en; endodermis, x; xylem vessel, ph; phloem.

Table 2. Morphological and anatomical features of the stem and root of *R. auricomus* var. *biformis*

Feature	Mean ± SE ¹
Stem morphology	
Stem length (cm)	9.40 ± 0.005
Length of first internode 1(cm)	5.72 ± 0.400
Length of second internode (cm)	1.96 ± 0.400
Length of third internode 3(cm)	3.07 ± 0.400
Total number of internodes	2.30 ± 0.900
Stem anatomy	
Stem cortex thickness (µm)	303.1 ± 0.1
Radius of pith cavity of stem (µm)	399.9 ± 0.9
Root anatomy	
Cortex diameter of root (µm)	527.9 ± 0.1
Radius of central cylinder (µm)	126.5 ± 0.5

¹ Mean ± Standard Error

Discussion

R. auricomus var. *biformis* is characterized by a terrestrial life form. Despite this terrestrial mode of life, *R. auricomus* var. *biformis* managed to adapt and survive during periods of drought in mostly wet meadows. The durability of this modified form of life varies and depends on microclimatic changes in a given habitat (Personal observation during this study). Morpho-anatomical analysis indicated that *R. auricomus* var. *biformis* considers specific structure and typical terrestrial/aquatic forms.

R. auricomus var. *biformis* belongs to the ecological group of hygromesophytes and grows as a terrestrial plant in temperate and moist areas on the slopes around Vlasina Lake that is characterized by slight changes of humidity. On this terrestrial type and according to the position on plant, two types of ground kidney-shaped leaves are formed. These leaves are characterized by one layer of epidermis on the adaxial surface, large cells, elongated palisade tissue cells and large intercellular spaces, which can be correlated with significant surface moisture due the wet habitat. The plant is characterized by a small diameter of the primary cortex of stem.

Morphological characteristics of leaves and stem

Two kinds of heterophyllous leaves were observed, namely; upper linear-lanceolated-lobed and sessile leaves and ground leaves that were lobed or not. The length of the internodes varied and the first internode was the longest that could be a consequence of the wet habitat (Table 2), which is in contrary to the shortest first internode of *R. lingua* from the same area (Atanackovic et al., 2012).

Anatomical characteristics of leaf, petiole, stem and root

The upper leaf of *R. auricomus* var. *biformis* was thinner compared with the ground leaf (Table 1). The three types of leaves contained one layer of epidermis and the stomata were rarely observed, which could be an adaptation to the habitat. Smaller intercellular spaces were seen in the mesophyll of the upper leaves compared with the ground leaves. Large intercellular spaces between cells in the spongy tissue and long palisade cells are characteristics influenced by water availability on leaves.

The plant stem showed structural features of the genus *Ranunculus* that consists of primary cortex, vascular tissue and pith cavity located at the centre (Fig. 7). One layer of epidermal cells contains only few stomata, primary cortex composed of small cells that are separated by large intercellular spaces, which could be a characteristic anatomical feature of a plant living in a wet habitat. The central cylinder consists of 9-10 vascular bundles. The petiole of ground leaves showed similar structure as the stem, but less number of vascular bundles, i.e. around 5.

The root consisted of primary cortex and central cylinder, though the structure was not different from other *Ranunculus* species.

Conclusion

The goal of this study was to observe the morphological characteristics of leaves and stem and anatomical characteristics of leaves, petiole, stem and root of *R. auricomus* var. *biformis* in peat bog habitat, in SE Serbia. The wet habitat partially influenced the anatomical structure of leaf, stem and root, while the morphological characteristics did not show any influence by the habitat except for the length of first internode of the stem. Nevertheless, the constraints in a term of temporal flooding could not affect the species due the stable structure of plant organs.

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