

## Ecological Anatomy of Flood-Plain Ephemeral Plants of the Lower Amur Basin

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**Abstract:** The study presents results of micromorphological studies of flood-plain ephemeral species of the Amur basin in their relation to specie's ecology. The 7 species were examined: *Coleanthus subtilis* (Tratt.) Seidel (family Poaceae), *Dichostylis micheliana* (L.) Nees (family Cyperaceae), *Gratiola japonica* Miq., *Limosella aquatica* L., *Lindernia procumbens* (Krock.) Borb. (family Scrophulariaceae), *Symphyllocarpus exilis* Maxim. and *Centipeda minima* (L.) A. Br. et Aschers (family Asteraceae). All these species are mainly found at shoals throughout their habitat. A combination of typical hydromorphic and specific adaptive microscopic features is revealed. Adaptation of the species to the sandy and muddy habitats take place through histological transformations of the plant's tissues. At the same time, narrow specialization of the species does not influence the typical organization of the plant organs and does not cause the simplification of their inner structure. The following microfeatures have adaptive significance: cortex suberization and epidermis cutinization; development of aerenchyma with schizogenic air cavities, separated by live intact parenchymal cells; sclerified solid stele. The examined species exhibit more terrestrial micromorphological features (sclerification, suberization and cutinization of tissues) than hydrophytic ones. It was suggested that specific shoal complex has been mostly formed by terrestrial species.

**Key words:** Flood-plain ephemeral plants, ecological anatomy, micromorphological adaptations, lower amur, Russia's Far East

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### INTRODUCTION

Flood-plain ephemeral plants of the Amur basin have been a subject of scientific interest for a long time. Many researchers (Komarov, 1988; Nechaev, 1970; Nechaev and Gapeka, 1970; Nechaev and Nechaev, 1973; Voroshilov, 1986; Polevoy, 1989) have noted their floristic and ecological peculiarity. Species diversity, pronounced ephemeral life cycle, high endemism, presence of tropical species and non-annual vegetation have been described. The life cycle of the species is linked to short low-water periods between summer monsoon floods.

The studies of the Amur flood-plain ephemeral plants are of great current interest. It is required to perform further cataloguing of biodiversity, conduct detailed studies of the specie's ecology, structure and dynamics of local populations as well as assess the anthropogenic transformation of the river shoal's plants, including the effects of the disastrous Amur river flood of 2013. Our studies were aimed to analyze micromorphology of vegetative organs of some flood-plain ephemeral species to assess their perseverance and adaptedness to shoal environment. Our tasks were:

- To characterize the microstructure of roots, stalks and leaves of the species to assess habitual features of typical structure of the organs

- To reveal adaptive features
- To perform comparative analysis of the species' micromorphological adaptations to the environment

Autoecological studies of micromorphology of the Amur basin flood-plain ephemeral plants have not been yet performed.

### MATERIALS AND METHODS

To conduct comparative anatomical studies, we use live specimens collected at the Amur left bank area near the rail bridge, Telman and Pokrovka stations in 2012 as well as dry herbarium specimens collected in 2001 near Khabarovsk, Petropavlovskoe and Knjaze-Volkonskoe settlements and stored at the Botany Department of DVGGU. 7 species were studied: *Coleanthus subtilis* (Tratt.) Seidel (Poaceae family), *Dichostylis micheliana* (L.) Nees (Cyperaceae family), *Gratiola japonica* Miq., *Limosella aquatica* L., *Lindernia procumbens* (Krock.) Borb. (Scrophulariaceae family), *Symphyllo carpus exilis* Maxim. and *Centipeda minima* (L.) A. Br. et Aschers. (Asteraceae family).

According to previous studies (Probatova, 1985; Kozhevnikov, 1988; Barkalov, 1992; Ivanina, 1991), the subjects of our interest are highly specialized stenotopic species inhabiting the lowest narrow strand of

sandy and muddy shoals along flood-plain lakes and oxbows during low-water periods. Only few of them can be found outside of low-water shoals of freshwater bodies at the seashores and as weed plants, at the margins of rice fields, at the roadsides (*Centipeda minima*) or at recurrently flooded local depressions of flood-plains (*Limosella aquatic*). From the life form point of view, they can be described as dwarf annual plants with ephemeral vegetation. Such species as *Coleanthus subtilis*, *Limosella aquatic*, *Lindernia procumbens* and *Centipeda minima* are abundant, generally and multiregionally distributed with preeminently shoal ecology within the whole geographical range. There is a species endemic to the Amur basin among the studied ones *Symphyllocarpus exilis* included into “*locus classicus*”. There is also a species rare and threatened in Russia and the Khabarovsk Krai, *Coleanthus subtilis*.

The study was performed according to the practice generally accepted in plant anatomy (Furst, 1979). The specimens were fixed in the mixture of alcohol, glycerin and water. Cross-sections of the organs were manually prepared with the help of a razor blade. The sections were stained with safranin and analyzed with the help of the “Mikromed-2” microscope. The photographs were taken with “ScopePhoto” Software and DCM 130 camera. The measurements were performed with an ocular micrometer.

**RESULTS**

**Coleanthus subtilis (Poaceae family):** Leaf structure (Fig. 1a), cross-sectional view of the lamina reveals its

dorsoventral structure. The upper side of the lamina is more or less flat while the lower side has bulging veins. The lamina thickness is highest at the middle of the leaf, especially at the veins, being 208.0 mkm, whereas at the leaf margin it is thin and bilayered, being 41.6 mkm thick. Upper and lower epidermises are covered with thin layers of cuticle. Epidermal cells form a single layer, they are large, colourless, more or less sclerified, all of them are equally bubblelike. Leaves are amphistomatic having stomata on both upper and lower sides. Stomata are superficial or slightly embedded with small substomatal cavities. Vascular bundles are closed, collateral, having sclerenchymal sheath. Xylem contains air cavity. The leaf mesophyll is of the festucoid type. Some cells of the mesophyll near the bundles are circular, they surround the bundles forming parenchymal sheaths; within them chloroplasts are observed. Other cells of the mesophyll are rectangular, lying beneath the epidermis in 3-5 layers. The cells of upper and lower epidermises have the same shape, 4-6 sided with straight, non-thickened walls. Stomata are organized in parallel linear arrays along the vascular bundles. There are ~54 stomata per 1 mm<sup>2</sup> in the upper epidermis and ~31 in the lower epidermis. The stomatal complexes are paracytic with 2 small subsidiary cells which are parallel to the cylindrical guard cells.

Stalk structure (Fig. 1b), Stalks are covered with single layer of epidermis with thin cuticle. Stomata are superficial, guard cells are at the same level as epidermal cells, substomatal cavities are large. The outer walls of the epidermal cells are thickened, containing cellulose. The cortex consists of 2-3 layers of collenchymatous parenchymal cells that are more or less closed and contain

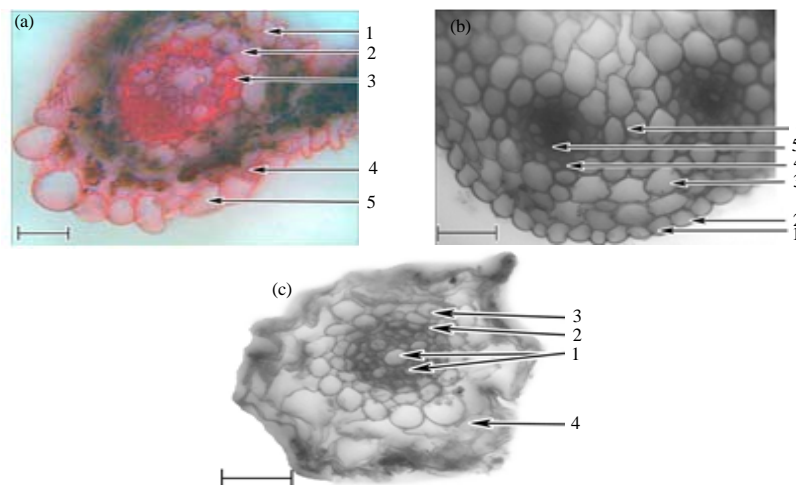


Fig. 1: *Coleanthus subtilis* structure; a) leaf, 1: upper epidermis, 2: lower epidermis, 3: stoma, 4: chlorenchyma, 5: outer parenchymal sheath of the bundle, 6: inner sclerenchymal sheath of the bundle, 7: vascular bundle; b) stalk: 1: epidermis, 2: cuticle, 3: cortex parenchyma, 4: sclerenchymal sheath of the bundle, 5: vascular bundle, 6: interbundle parenchyma; c) root: 1: xylem vessels, 2: endodermis, 3: cortex parenchyma, 4: rexigenic air cavities

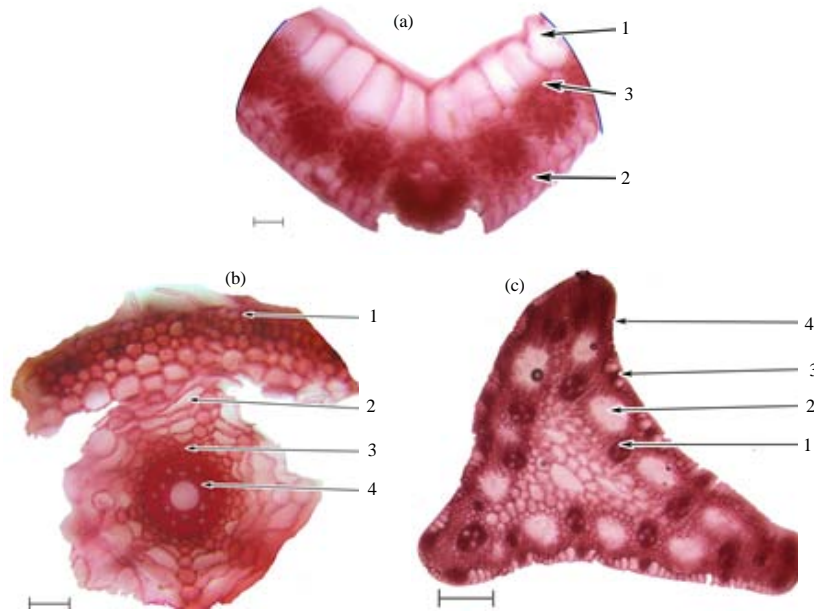


Fig. 2: *Dichostylismicheliana* structure; a) leaf: 1: upper epidermis, 2: lower epidermis, 3: sheath cells; b) stalk: 1: epidermis, 2: stoma, 3: aerenchyma, 4: vascular bundle; c) root: 1: xylem vessels, 2: endodermis, 3: cortex parenchyma, 4: epiblem

chloroplasts. Endodermis and pericycle are not prominent. Stele contain vascular bundles. The bundle's structure is common in grasses. Single circle of bundles connected by sclerified interbundle parenchyma can be observed. Pith is at first composed of large parenchymal cells, then a small air cavity of a culm is formed. Medullar bundles are absent.

Root structure (Fig. 1c), cortex contains rexicenic air cavities. Inner layers of the cortex parenchyma are suberised. Endodermis is single-layered with U-shaped lignified thickenings. Stele is totally sclerified. It contains a single broad vessel at the very central part, surrounded by 4-6 narrower vessels.

***Dichostyli smicheliana* (Cyperaceae family):** Leaf structure (Fig. 2a). Cells of upper and lower epidermises dramatically differ. The upper epidermis cells in the lamina cross-section are large, whereas the cells of lower epidermis are small, about 10 times smaller than the upper epidermis cells. Cuticle is well-marked. Flat mounts show that the upper epidermis cells are rectangular in shape, with buckled, slightly lignified walls. The lower epidermis cell walls are straight. Leaves are epistomatic, stomata are organized in rows between the bundles at the lower side of the lamina. Stomata are superficial with large substomatal cavity, of paracytic type. Stoma is surrounded by 2 small subsidiary cells which are parallel to the cylindrical guard cells. The leaf mesophyll is tightly

connected to numerous vascular bundles. Mesophyll cells semicircularly surround the bundles, forming kranz-type sheaths.

Stalk structure (Fig. 2b), cross-sectional shape of the stalk is triangular. Stalk epidermis is single-layered, formed by large colourless cells with prominent superficial stomata, consisting of small guard cells and shallow substomatal cavity. Besides stomata, there are groups of several small cells with adjacent small chlorenchymal patches among large epidermal cells. The 10 major rexicenic air cavity are observed located at periphery of the organ. Vascular bundles are collateral with sclerenchymal sheaths. Larger bundles run through the stalk edges, one at each edge and 3-4 smaller bundles are located between the edges. The stalk pith contains rexicenic air cavities. Parenchyma of the stalk is slightly lignified.

Root structure (Fig. 2c), cross-sectional shape of the root is circular. Epiblem with root hairs well-marked. Exodermis has typical structure consisting of 2-3 layers of cells. Cortex parenchyma includes 2 cell types: suberised and thin-walled. The 2-3 continuous layers of suberised cells are adjacent to the inner side of exodermis, forming single-layered radial spokes that reach endodermis. 1-2 tangentially stretched rows of thin-walled cells are located between radial rows of suberised cells. Endodermis has typical structure, formed by a single layer of cells with U-shaped thickenings. The 12 xylem vessels can be

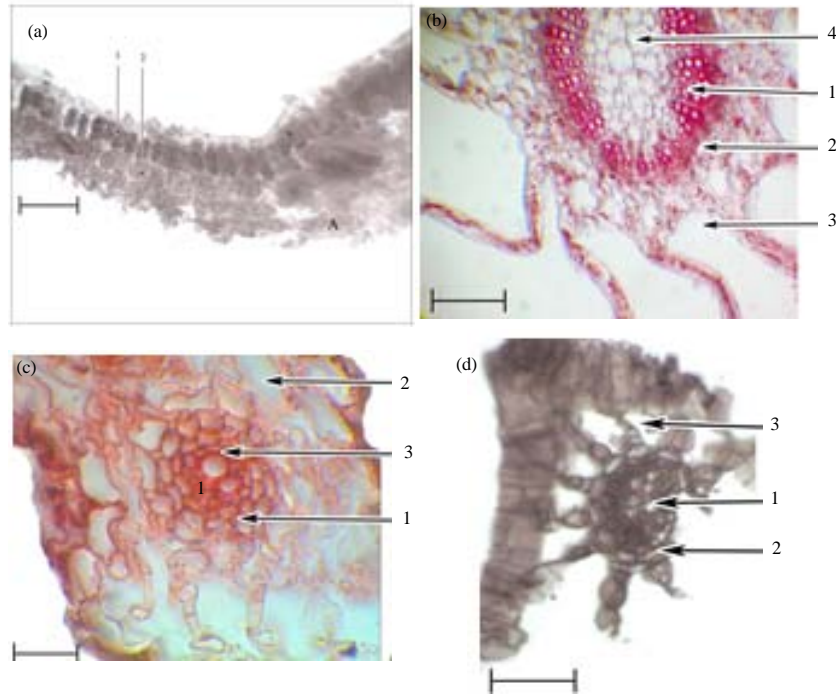


Fig. 3: Species structures: a) *Linderniaprocombens* leaf: 1: palisade mesophyll, 2: spongy mesophyll; b) *Gratiola japonica* stalk: 1: xylem vessels, 2: endodermis, 3: aerenchyma, 4: parenchyma; c) *Limosella aquatica* root: 1: endodermis, 2: aerenchyma, 3: stele; D-*Lindernia procumbens* root: 1: xylem vessels, 2: endodermis, 3: aerenchyma

observed within the stele, organized in 2 rows, each of 6 vessels. Large air cavity is located in the middle of the root.

***Gratiola japonica*, *Limosella aquatica*, *Lindernia procumbens* (Scrophulariaceae family):** Leaf structure (Fig. 3a). The examined species have single-layered palisade mesophyll, easily observed at leaf cross-sections. The palisade ratio is rather low: 35.7% in *Limosella aquatica*, 31.2% in *Lindernia procumbens*, 30.0% in *Gratiola japonica*. Mesophyll cells contain numerous chloroplasts. Upper and lower sides of lamina are equal. Leaves are amphistomatic, having stomata on both upper and lower sides of lamina. Stomata are anomocytic. The number of stomata per 1/mm<sup>2</sup> of the upper epidermis of the leaf varies between the species: 31 in *Gratiola japonica*, 34 in *Lindernia procumbens*, 58 in *Limosella aquatica* whereas for the lower epidermis this value equals 31 in *Gratiola japonica*, 44 in *Lindernia procumbens*, 68 in *Limosella aquatica*. Both sides of lamina carry ethereal oil cell complexes with four, eight and ten-cell heads.

Stalk structure (Fig. 3b), stalks of the examined species are covered with single-layered epidermis. Stomata are superficial, guard cells are at the same level as

epidermal cells, substomatal cavities are large. Cuticle is very thick, ridged and serrated. Trichomes are not prominent. Stalk cortex consists of schizogenic air cavities with assimilating parenchymal cells in between. Cell walls are slightly thickened. *Lindernia procumbens* has cords of sclereids within the stalk edges. Endodermis in form of starch sheaths can be observed only in *Limosella aquatica*, other species lack endodermis. Pericycle is undeveloped, sporadic cords of pericyclic fibres can only be observed within the stalks of *Lindernia*. Stalk stele in three examined *Scrophulariaceae* species does not have bundle structure. Secondary xylem prevails within the stele volume. Prominent single-row parenchymal rays are located between radial rows of vessels. Stalk pith of *Gratiola japonica* consists of parenchyma, whereas in *Limosella* and *Lindernia* rexicogenic air cavity is formed.

Root structure (Fig. 3c and d), cortex area of the examined species contains well-developed secondary aerenchyma with large schizogenic air cavities. Outer tangential cells of aerenchyma (1-2 layers) are slightly suberized. Aerenchyma covers larger area than the root stele. Stele is solid, filled with well-developed secondary xylem. Parenchymal rays are sclerified and hardly discernible from the xylem vessels.

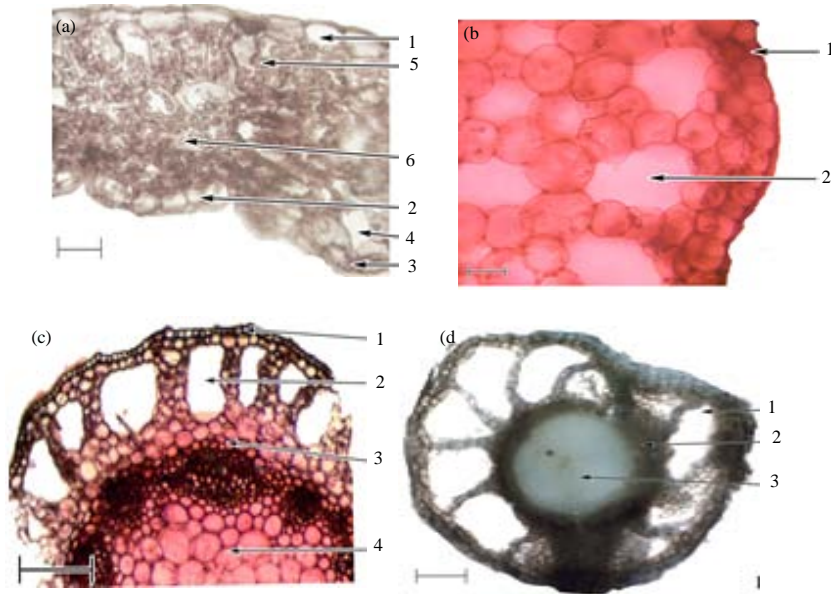


Fig. 4: Species structures: a) *Centipeda minima* leaf: 1: upper epidermis, 2: lower epidermis, 3: stoma, 4: substomatal chamber, 5: palisade mesophyll, 6-spongy mesophyll; b) *Centipeda minima* stalk: 1: epidermis, 2: aerenchyma; c) *Symphylo carpus exilis* stalk: 1: epidermis, 2: aerenchyma, 3: endodermis, 4: pith; d) *Symphylo carpus exilis* root: 1: aerenchyma, 2: cork, 3: stele

***Symphylo carpus exilis* and *Centipeda minima* (Asteraceae family):** Leaf structure (Fig. 4a) of the examined species is marked by equal upper and lower sides of the lamina. Anticlinal walls are equally twisted. Leaves are amphistomatic having stomata on both upper and lower sides. Stomata are superficial, guard cells are at the same level as main epidermal cells. Large substomatal cavities can be observed at the leaf cross-sections. Stomata pores are open. Epidermal cells contain chloroplasts. Leaf mesophyll is poorly differentiated palisade and spongy mesophyll can only be distinguished by cell orientation. Mesophyll is close-packed, almost lacking intercellular spaces, it is formed by circular parenchymal cells with large chloroplasts. Vascular and supporting tissues are poorly developed. Bundles are deeply embedded in mesophyll and surrounded by smaller sheath parenchymal cells.

Leaf species differences involve certain quantitative characteristics. Palisade ratio of *Centipeda minima* is 29.7%, it has ~32 stomata per 1/mm<sup>2</sup> of the upper epidermis of the leaf which is more than the number of stomata of the lower epidermis (~20). *Symphylo carpus exilis* has palisade ratio of 14.3%, ~22 stomata per 1/mm<sup>2</sup> of the upper epidermis and ~32 per 1/mm<sup>2</sup> of the lower epidermis.

Stalk structure (Fig. 4b and c) is marked by wide cortex area with relatively small stele. Cortex consists of aerenchyma. Small fibre “caps” above the bundles are of

pericyclic origin. Stele has bundle structure. Open collateral bundles are organized in a single circle. Interbundle cambium is absent.

Species differ in the structure of endodermis: in *Centipeda minima* it is not pronounced; on the contrary, in *Symphylo carpus exilis* endodermis is well-developed and can be easily noted due to the suberized cell walls. Root structure (Fig. 4d) combines primary and secondary tissues. The volume of cortex and aerenchyma is larger than that of stele which is formed by a solid ring of secondary vascular tissues. Several layers of immature periderm, which probably emerges from phloem are located at the interface of cortex and stele.

## DISCUSSION

Micromorphological studies of the vegetative organs of some flood-plain ephemeral plants from the Amur basin revealed that typical structure of vegetative organs, inherent in monocotyledons and dicotyledons is preserved. No deviations in general organ morphology due to ephemeral and therophytic lifestyle were detected. Moreover, no signs of microstructural simplification, linked to specific shoal environment, were observed. Anatomical differences are connected to species-specificity. However, we suppose that histological features of organs exhibit adaptive transformations.



**Leaf structure:** Leaf epidermis serves as subsidiary assimilative tissue. In *Centipeda minima* and *Symphyllo carpus exilis* epidermal cells contain chloroplasts (Fig. 4a). Ecological significance of this feature is that it provides photo synthesis during short flooding of the ecotope. Equivalence of the upper and the lower epidermis (*Amphistomatic epidermis*). Anticlinal walls are equally twisted. Leaves are amphistomatic, having stomata on both upper and lower sides. It is observed, for example, in *Gratiola japonica*, *Limosella aquatica*, *Lindernia procumbens* (Fig. 3a). Ecological purpose is to enhance transpiration and gas exchange.

**Superficial stomata:** All examined species have superficial stomata, guard cells are at the same level as main epidermal cells. Large substomatal cavities can be observed at the leaf cross-sections. Stomatal pores are open. Ecological purpose is to enhance transpiration and gas exchange.

**Shape of epidermal cells:** Epidermal cells form a single layer, they are large, colourless, more or less sclerified and bubblelike for example, in *Coleanthus subtilis* and *Dichostylis micheliana*. Ecological purpose is to enhance surface water exchange. Large colourless epidermal cells probably form a screening cover decreasing the intensity of light passing through the leaf.

**Epidermal secretion:** In *Gratiola japonica*, *Limosella aquatica*, *Lindernia procumbens* both sides of lamina bear ethereal oil cell complexes with 4-8 and 10 cell heads. Ecological purpose is to provide water-repellency of the leaves.

**Signs of kranz anatomy:** In *Dichostylis micheliana* mesophyll chlorenchymal cells semicircularly surround the vascular bundles, forming kranz-type sheaths (Fig. 2a). Such sort of connection between chlorenchyma and vascular bundles is observed in  $C_4$  photosynthetic plants. It is known that  $C_4$  species exhibit high photosynthetic activity under intense illumination and in arid environment (Polevoy, 1989). These environmental factors are constantly present at littoral shoals and we observe highly adaptive  $C_4$  photosynthesis in certain shoal plants.

#### Stalk structure

**Epidermis cutinization:** Cuticle of Scrophulariaceae species (*Gratiola japonica*, *Limosella aquatica*, *Lindernia procumbens*) is very thick and fine-ridged. Ecological purpose is to avoid drenching of inner tissues.

Cortex contains schizogenic air cavities, separated by live intact parenchymal cells. It is observed in *Gratiola japonica*, *Limosella aquatica*, *Lindernia procumbens* (Fig. 3b). Ecological purpose is to prevent water from entering the plant organs.

**Suberized endodermis:** In *Symphyllo carpus exilis* endodermis is well-developed and can be easily noted due to the suberized cell walls (Fig. 4b and c). Ecological purpose is to protect stele from water leaching.

**Air cavities within the stalk pith:** In *Limosella aquatica* and *Lindernia procumbens* rexigenic air cavities are formed there. Ecological purpose is to provide support and buoyancy in case of flooding.

#### Root structure

**Cortex suberization:** In *Dichostylis micheliana* suberized cells are organized in radial rows, forming supportive structure resembling wheel spokes (Fig. 2c); in *Coleanthus subtilis* 1-2 parenchymal layers adjacent to endodermis inner layers of cortex parenchyma are suberized (Fig. 1c); in *Lindernia procumbens* superficial cells of 1-2 layers are suberized (Fig. 3d). Ecological purpose is to prevent drenching of inner tissues and to maintain the organ structure intact under stenobiotic environmental conditions.

**Formation of aerenchyma:** Primary aerenchyma with rexigenic or schizogenic air cavities is observed in *Coleanthus subtilis* and *Dichostylis micheliana* (Fig. 1, 2); secondary aerenchyma with schizogenic air cavities is present in *Lindernia procumbens* (Fig. 3d). Ecological purpose is to assist gas exchange and to provide mechanical support.

**Stele sclerification:** In *Limosella aquatica* and *Lindernia procumbens* stele is solid, consisting of well-developed secondary xylem. Parenchymal rays are sclerified and hardly discernible from the xylem vessels (Fig. 3d). In *Coleanthus subtilis* stele is also entirely sclerified (Fig. 1c). Ecological purpose is to anchor the plant in moving alluvial substrate of littoral shoals.

#### CONCLUSION

Our studies revealed an entire complex of typical and specific adaptive microscopic features of both hygromorphic and heliomorphic nature in the examined species. Micromorphological structures are, undoubtedly, an essential part of the specie's adaptive system and promote their survival under particular conditions.

Shoak species have patient survival strategy, adjusting to the environmental conditions through special adaptations. Patient strategy was, probably, the original one, as it is preserved with the help of conservative and evolutionary stable micromorphological structures. Explerent strategy, providing adaptation for major disturbances has probably developed later as it is supported by macromorphological life form transformations. This secondary strategy does not lead to any changes in the inner structure of plants. The examined species exhibit more terrestrial micromorphological features (sclerification, suberization, cutinization of tissues, presense of secretory structures within the leaves, prominently twisted epidermal cells) than hydrophytic ones. It leads us to the conclusion that specific shoal complex has been mostly formed by terrestrial species.

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