Ecologo-anatomic characteristics of the rare species *Cladium mariscus* (L.) Pohl.

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**Abstract.** The anatomic structure of the vegetative organs of the rare and protected species *Cladium mariscus* (L.) Pohl is studied in order to expose its adaptation and resistance to the growth conditions. For the first time the microstructural organization of the root, rhizome and leaf was described. The vegetative organs of *C. mariscus* have a common plan of the anatomic structure similar to other species of the Cyperaceae family; along with this substantial differences were exposed in the structural organization of the lamina. There was exposed the combination of typical hygromorphic and specific xeromorphic adaptive indicators in the structure of the vegetative organs, caused by the peculiarities of the ecotope’s hydrological conditions.

1. **Introduction**

Nowadays the problem of preserving the biological diversity has become of paramount importance in the world and is discussed at different levels, along with this in the Crimea many species of vascular plants, first and foremost stenotopic ones, are under the threat of the extinction [1-3].

The coastal-water species with the disjunctive natural habitat *Cladium mariscus* (L.) Pohl. has a very low ecological amplitude and competitiveness, that is why it is protected by the law of many countries [4-7], including the Russian Federation – at the federal and regional levels [3, 8-11]. Despite the nature conservation status, many populations, including the ones in the region of Sevastopol, are under the threat of the extinction, and in Bashkortostan, Tatarstan, in the Northern Caucasus this species is considered to have been disappeared [3, 8, 11].

On the Crimean peninsula *C. mariscus* is a rare representative of the flora with the only location on the territory of Sevastopol, where it grows on the strip of the coast (about 2 km) to the west of Lermontov cape. The saw-grass grows here in the non-typical conditions: on the steep, open and well-insolated seaside slopes, in the places of the underwater output, and along the riverbeds of the mineralized sources, in the conditions of the dry climate of the Heracles peninsula [3, 12].

Despite the high nature preservation status, the data on morphology and anatomic structure of *C. mariscus* are practically absent. Besides, such complex researches, as it is known, help to understand the ecological chronotope, supplement the botanical characteristics, and also evaluate the plasticity, potential and functional possibilities of the species in the conditions of the changing hydrological
regime of the ecotope [13, 14]. The study of the vegetative organs is topical as in many populations the renewal of the species is only vegetative [15]. In connection with this the aim of the research is the study of the micromorphological structure of the vegetative organs of *C. mariscus* for exposing the adaptations and stability of the species to the growth conditions in the Crimea.

2. Materials and methods
The research was done in the period of 2017-2020. The native material was taken in the phase of blossoming in the natural cenopopulation *C. mariscus* near the cape Lermontov (Sevastopol) in 2017 and preserved (spirit: glycerine : water in the ratio 1 : 1 : 1). The anatomic study was done according to the generally accepted methodology on the preparations obtained with the help the microtome Rotmic 2-P [16-24]. For the comparative-morphological analysis of the epidermal structures of *C. mariscus* leaf temporary preparations from the fresh leaves were prepared according to the standard methods [25, 26]. The quantitative determination of separate morphological elements (stomata, the main epidermis cells) on the micropreparations was done in the 30-multiple repeatability. The processing of the data was done by the standard methods of the mathematical statistics [27]. The research of temporary and constant preparations was done with the use of the microscope Olympus CX31RTSF. The objects were photographed by the digital camera Olympus (Industrial Digital Camera TOUPCAM™ U3CMOS10000KPA).

3. Research results

3.1 Root
The main root of *C. mariscus* dies off very early and is substituted by the system of supplementary roots in the bushing out zone. From the outside the root is covered with epiblem. The zone of the initial cortex and central cylinder is well-exposed in the anatomic structure. The outer layer of the initial cortex is presented by the exoderm, consisting of small cells with thickened cell walls, located in 1-2 rows. The mesoderm is presented by big thin-walled parynchematous cells with well-developed blind pits. The inner layer of the mesoderm, adjacent to the endoderm, is sclerified. The endoderm is single-row, consisting of tightly close cells, oblong towards the radial direction. The characteristic feature of the initial cortex of *C. mariscus* is the presence of weakly-expressed radial rays in the mesoderm, between which a bit larger cells of the parenchyma are located (it is typical of the family Carex). At the last stage of the root initial structure formation small rexygen cavities are formed between the radial bands. The central cylinder is a radial polyarchy conducting bunch with the alternating on the radius conducting elements of the initial xylem and phloem. Large vessels of the metaxytem elements have well-sclerified walls. Parynchematous and conducting xylem elements of the central cylinder are also sclerified.

3.2 Rhizome
As a result of the anatomic structure study of the saw-grass rhizome it was found out that it has a fascicular structure and is covered with a single-layer lignified epidermis (Figure 1). The initial cortex is well-developed. The sub-epidermal parenchyma of the initial cortex consists of thin-walled cells with small blind pits, it contains starch grains. In the parenchyma of the initial cortex there are singular conducting bundles, surrounded by the sclerenchymatous covering. Endoderm is poorly expressed. The central axial cylinder is surrounded by the sclerenchymatous pericyclic ring. The conducting bundles of the central cylinder are concentric, amphivasal, are concluded into the boot of the sclerenchyma, located absenty. The space between the bundles is filled with the medullarly parenchyma. Both the initial cortex and the medulla contain idioblasts with dark content (presumably tannins).
Figure 1. The cross-section of the C. mariscus rhizome. 
Designations: 1 – epidermis; 2 – sclerified parenchyma of the initial cortex; 3 – the main parenchyma of the initial cortex; 4 – endoderm; 5 – pericycle; 6 – conducting bundles of the central cylinder; 7 – conducting bundles of the initial cortex; 8 – idioblasts.

3.3 Leaf
The leaf of C. mariscus is linear, on the edges and along the central tendon from the abaxial side it is rugged, leathery and has small unicellular protuberances, in the cross-section it is folded along the central tendon.

The research of the anatomic structure of the lamina showed that for C. mariscus equiphacial type is characteristic. From the outside the leaf is covered with a single-layer epidermis with a developed cuticle. The results of the research of the epidermal structures showed that the leaf of C. mariscus has an amphistomous and tetracid type of the stoma apparatus.

On the abaxial and adaxial sides of the leaf there are the main cells of two morphological epidermis types: of the split shape, located above the areas with the assimilated tissues, and of the rectangular shape, located above the areas with mechanical tissue (Figure 2). Both cell types are grouped in three, five-cell rows along the lamina.

Figure 2. Types of the epidermal cells of C. mariscus. 
Designations: 1 – the main epidermal cell of the rectangular shape; 2 – the main epidermal cells of the split shape; 3 – stomata.

Epidermal cells of the abaxial side of the leaf, which are located above the areas of the assimilative tissue, are of oblong or split contours with flexuous anticlinal walls, obtuse or acute angles on the adjacent boundaries. The size of the epidermal cells varies from 9.81 to 41.11 μm in length and from 10.09 to 21.12 μm in width. The amount of the cells on 1 mm² on average is 2940±46.12 units. The epidermal cells of the abaxial side of the leaf, located above the areas of the mechanical tissue, have a rectangular shape with the wavy anticlinal walls, right angles on the adjacent boundaries. The size of the epidermal cells varies from 19.8 to 47.75 μm in length and from 11.3 to 17.9 μm in width. The amount of the cells on 1 mm² on average is 1733±31.24 units.
The epidermal cells of the adaxial side, located above the areas of the assimilative tissue, as well as of the abaxial side, are of oblong or split contours with flexuous anticlinal walls, obtuse or acute angles on the adjacent boundaries. They are distinguished by a bit larger size: from 24.31 to 46.35 μm in length and from 9.12 to 24.31 μm in width. The amount of the cells on 1 mm² on average is 2400±39.26 units. The epidermal cells of the adaxial side of the leaf, located above the mechanical tissue, also have a rectangular shape with the wavy anticlinal walls, right angles on the adjacent boundaries. The size of the epidermal cells varies from 19.33 to 66.59 μm in length and from 9.92 to 18.94 μm in width. The amount of the cells on 1 mm² on average is 1629±32.71 units.

The stomata are surface, with weakly noticeable understoma cavity, oblong-rounded shape with four nearstoma cells. For C. mariscus a group arrangement of stomata is characteristic: they are located only in the areas of the epidermis along the assimilative tissue. This is conditioned by the functional and physiological peculiarities of the underlying tissues. The amount of the stomata from the lower side of the leaf was on average 1067±15.76 units/mm², on the upper side – 633±13.87 units/mm².

The lamina of C. mariscus is filled with the homogeneous, non-differentiated small-cellular mesophyll with numerous chloroplasts and underdeveloped blind pits (Figure 3). The average size of the mesophyll cells is 16±2.01 μm. The cells of the mesophyll are of a rounded or a bit oblong shape in the tangential direction. In the mesophyll’s parenchyma there are idioblasts with cellular inclusions. In the central part of the young leave’s lamina there are formed large thin-walled cells, which, while growing and developing the anatomical leaf’s structure, explode and in their place rexygen air cavities, occupying the most part of the mature leaf volume, are formed. The ratio of the mesophyll volume and the cavity is 1:1.5. Such air cavities guarantee the intensive interchange of gases in the C. mariscus leaves.

In general the conductive system of the C. mariscus leaf deserves more attention, as it has some peculiarities. The leaf’s mesophyll is pierced by the conducting bundles. The central tendon stretches along the whole lamina and is presented by the closed collateral bunch with a well-developed multilayer sclerenchymatous covering and a single-layer parynchematous covering. The conducting bundles, located along the edge of the lamina, and the central tendon have a single location. Fibrovascular bundles of the most width part have two-row location. The conducting bundles of larger sizes alternate with the less ones. The large conducting bundles of the upper and lower sides are connected by the sclerenchymatous band, dividing the air cavities of the leaf into separate chambers. The parynchematous covering of the conducting bundle of the mature leaf continues along the sclerenchymatous band, restricting it from the air cavity (Figure 3 B). The sclerenchymatous band between the bundles provides the strength of the leaf and prevents from the rupture along the lamina. On the whole, the leaf is significantly sclerofied: the mechanical bands accompany small side tendons; they are located between the conducting bundles on the both sides of the lamina, on the edge of the leaf.

4. Discussion of the results
The comparative analysis of the anatomic structure of the C. mariscus vegetative organs with the data of other species of the family Cyperaceae demonstrated their similarity [28, 29]. The set of the histological zones and the root and rhizome tissues, constituting them, in general are typical, however, there is a range of peculiarities: less development of the air cavities and more sclerofication of the tissues. More significant differences have been found out in the structural organization of the lamina. The leaf C. mariscus is equiphacial; the conducting bundles of the lamina are located in pairs in two rows and are oriented by the xylem to each other. Between the large conducting bundles, located one above another, there is a sclerenchymatous band, preventing the rupture on the air cavity along the lamina. Quite a strong sclerofication of the lamina is found out.
Figure 3. The anatomic structure of the lamina *C. mariscus*.

A – the general view of the lamina in the cross-section; B – the middle part of the mature lamina; C – the edge of the lamina of the mature leave. Designations: 1 – the conducting bundle; 2 – the air cavity; 3 – epidermis; 4 – xylem; 5 – phloem; 6 – sclerenchyma; 7 – the parynchmatous facing of the conducting bunch; 8 – mesophyll; 9 – idioblasts.

*C. mariscus* are ascribed to hygrophytes or mesoxerophytes. It is a typical coastal-water species, being able to exist in the conditions of constant water level fluctuation [7,30;31]. As a rule, saw-grass prefers hygrophilous meadows, bogs, and shallow waters of the basins, often with a turf layer, in the seaside zone it grows in the areas, becoming waterlogged, in the relief lowering. For every macrovegetation the water level fluctuation determines the structural changeability of the species: a high level of the surface impoundment positively influences the growth and the development of the plant. The plants reach the maximum sizes in the basins with the water thickness of 1-1.5 m [7]. The ecotope of the studied population by us is characterized by the absence of the surface impoundment and constant change of the hydrological regime in the environment (depending on being away from source riverbed) and in the time (in different seasons and years). Besides, in humid ecotopes *C. mariscus* forms thick high (more than 2 m) brushwood with a huge amount of generative sprouts, and in drier ecotopes it occurs in scattered small groups, formed by low (up to 1 m) plants. On the whole, it is evident, that the population is in the optimum ecotope conditions, and the threat is mainly presented by the irregularity of watering slopes and the strengthening collapse-landslide processes of the anthropogenic genesis.

The conducted microstructure analysis of the vegetative organs of the Crimean population *C. mariscus* showed that along with the hygromorphic indications of the researched species the xeromorphic traits of the structural organization were exposed, mainly, in the structure of the leaf. As it is known, during the change of the plants’ ecotope first of all the qualitative changes in the anatomic
structure of the leaf occur, and the parallelism between the modified and structural adaptations occur, providing the adaptation of the plant to the changing conditions of the environment [32, 33].

Thus, the presence of well-developed air cavities in the lamina and thinned parenchyma of the initial root cortex, a thin cuticle and non-submerged stomata belong to the hygromorphic adaptive peculiarities of the vegetative organs of C. mariscus. A well-developed system of blind pits and air cavities in the plant provide intensive interchange of gases during the excess of the soil moisture. The surface location of the stomata and a relatively thin cuticle provide the intensive water cycle.

On the other hand, in the structure of the C. mariscus leaf xeromorphic features of the organization prevail: small cellularity of the epidermis, the amphistomatic leaf, a large amount of stomata on the unit of the area and the equiphacial leaf. Besides, the inner tissues of the leaf are also distinguished by the small cellularity and strong sclerification. So far as C. mariscus inhabits in the well-illuminated areas, in the leaf structure it is possible to state the presence of heliomorphous indications. Such a leaf microstructure provides the regulation of the transpiration in the conditions of heightened insolation and water deficit. Thus, in the anatomic structure of the leaf C. mariscus xeromorphic characteristics prevail, they allow adapting to the arid conditions of existence.

Probably, according to the evolutionary formed set of the histological zones and the tissues, constituting their microstructure organization of the vegetative organs, C. mariscus is the most adapted to the well-moistened ecotopes, the presence of hygromorphic indicators evidences that. However, due to the peculiarities of the ecotope the Crimean population of saw-grass suffers from the moisture deficit, this, probably, causes the xerophytization of the plant, and first and foremost is reflected in the leaf structure. However, for the complex understanding of the dynamics of the adaptive species’ transformations and exposing its structural transformations the further comparative researches of the biology and ecology of C. mariscus in different ecotopes are necessary.

5. Conclusion
Therefore, for C. mariscus the microstructure of the vegetative organs is similar to other species of the family Cyperaceae. The substantial differences are revealed in the structural organization of the lamina: less development of the air cavities, more sclerification of the tissues, conducting bundles are located in pairs in two rows and are oriented by xylem to each other, large conducting bundles of the upper and lower sides of the leaf are connected by the sclerenchymatous bands.

There were exposed the peculiarities of the C. mariscus vegetative organs’ microstructure, conditioned by the adaptation of the hydrological regime of the ecotope: combination of typical hygromorphic indications for the species (presence of the air cavities in the leaf, open parenchyma of the initial root’s cortex, thin cuticle, surface location of the stomata) and specific xeromorphic adaptive indications (small-cellularity of the epidermis and internal tissues of the leaf, amphistomatic leaf, high density of the stomata, equiphacial leaf, sclerification of the vegetative organs).

For the complex understanding of the species’ adaptive transformation and exposing its structural transformations the further researches of biology and ecology of C. mariscus in different ecotopes are necessary.

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