**Euryale ferox** Salisb. and *Nuphar japonica* DC. (Nymphaeaceae) micromorphology study from the Amur region

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**Abstract.** The article presents the results of a micromorphological study of two species of nymphaeans, *Euryale ferox* Salisb. and *Nuphar japonica* DC., fam. Nymphaeaceae Salisb. Detailed descriptions of the leaf blade and petiole are given. The main histological and topographic complexes and their proportions, and the histochemical features have been determined.

Common microstructural features in the structure of both species are dorsoventrality of floating leaves, anomocytic stomatal apparatus, the presence of astrosclereids, epidermal and epidermal-subepidermal outgrowths (covering and capitate trichomes, hydropods, emergencies), compact (rather than diffuse) subepidermal collenchyma in the petiole, atactostelia, and the absence of cambium. It is shown that aerenchymatization and weak sclerification of parenchymal tissues, and reduction of conductive elements are effective adaptations to the conditions of the aquatic environment. The taxon-specific trait is the type of the conducting bundle. Paired concentric bundles with a common air cavity were found in *E. ferox*, and collateral bundles were detected in *N. japonica*.

1. **Introduction**

The objects of our research were two species, which are *Euryale ferox* Salisb. and *Nuphar japonica* DC. fam. Nymphaeaceae Salisb. Both species are endangered in the northern and northeastern limits of distribution and are included in the list of protected plants [1].

*Euryale ferox* is a South Asian subtropical species with a relict range in the countries of eastern Asia of the temperate zone (China, the Korean Peninsula, and Japan), covering the south of the Russian Far East [2]. In the Russian Far East, the natural locations of the species are noted mainly in the Ussuri River valley [3]. They are also found in the vicinity of the city of Birobidzhan in the Jewish Autonomous Region [4].

*Nuphar japonica* is the Amur-Korean-Japanese species [5, 6]. In the Far East of Russia, the species is found only in the lower reaches of the Kiya River, a tributary of the Ussuri River [7]. Narrow local Far Eastern populations of this species represent the northernmost continental part of the range of predominantly island Japanese species.
Analysis of the literature showed that, despite a significant interest in species as relict representatives of the regional flora, the issues of morphology, anatomy, biology and phylogeny of species are still poorly studied.

The aim of this work is to generalize the results of micromorphological studies of the vegetative organs of *E. ferox* and *N. japonica* based on the material from the Amur region.

2. Materials and methods

The samples of *E. ferox* were collected in the vicinity of Birobidzhan, in the Jewish Autonomous Region in September 2020. The coordinates were N 48°81’44.92” and E 132°90’16.61”. The samples of *N. japonica* were acquired over the Kiya River, in the village of Pereyaslavka in the Lazo District of the Khabarovsk Territory in August 2014. The coordinates were N 47°57’22.35” and E 135°03’39.58”.

The studies were carried out according to the generally accepted method of microstructural analysis [8]. The finished preparations were examined using the Biolam-LOMO microscope and the Altami BIO 8 digital microcomplex. When determining the cell size and the proportion of histological zones, we used the standard eyepiece micrometer M-LOMO and the object micrometer OMO. Visualization of the slides was carried out with a digital camera for the Toupecam microscope using the Toupecview software.

3. Results and discussion

3.1 Constitutional features

The anatomical features of the leaf in the species studied correlate with the degree of exposure to their air environment. The mesophyll of floating and emergent leaves has a dorsoventral structure. The palisade parenchyma is photosynthetic, relatively compact and multilayered with longitudinally elongated narrow cells. The spongy parenchyma is represented mainly by diffuse intercellular spaces with a diameter of 120–500 µm. The leaf is reinforced with astrosclerids with the smallest rhombic crystals. Numerous and multi-branched astrosclerids are developed in *N. japonica*. In *E. ferox*, they are few in number and slightly branched (Figures 1a and 1e).

Leaf blade of leaves floating on the water surface in *E. ferox* is of the epistomatic type. The leaves of *N. japonica* towering above the water are of the hypostomatic type. The stomata are of normal structure. The stomata are large (~160 µm long and ~130 µm wide), the guard cells are reniform-bean-shaped, and the stomatal slits are wide open. In the species studied, the stomatal apparatus is of the same type – anomocytic (Figure 1d). Various outgrowths of the epidermal origin were found including hydropots, coverts, and capitate trichomes in *E. ferox* (Figure 1c), and essential oil glands in *N. japonica*. *E. ferox* has numerous long-pointed emergences (spines) of the epidermal-subepidermal origin.

The petiole of the species investigated is characterized by the primary microstructure of the axial organ with typical histological and topographic complexes. The petiole epidermis of the species studied is single-layered and performs a secreting function. The zone of the primary cortex is differentiated into the external and internal areas. The outer cortex in the species examined is equally occupied by 5–6–7 layers of angular collenchyma (Figure 1c).

Compact (rather than diffuse) localization of multilayered subepidermal angular collenchyma is atypical for macrohydrophytes [9]. The inner cortex is structured in different ways. It is performed by diffuse aerenchyma in *E. ferox*, whereas in *N. japonica*, on the contrary, by openwork aerenchyma with a regular network of air cavities (Figures 1b and 1e).

In the species studied, an atactostella is found in the petioles. The differences between the species are manifested in the type of conducting bundles. In *E. ferox*, peculiar paired concentric bundles with a common air cavity were found (Figure 1b), while in *N. japonica* collateral bundles were detected (Figure 1e). The vascular elements of the xylem are partially or completely replaced by one or two air cavities. Bundles are without covers. Cambium is absent.
Figure 1. Micrographs of the examined species: a – cross-section of the leaf blade (*Euryale ferox*), b,c – cross-section of the petiole (*Euryale ferox*), d – adaxial epidermis (*Nuphar japonica*), e – cross-section of the petiole (*Nuphar japonica*). Designations: it – intercellular space, co – collenchymas, pp – palisade parenchyma, ph – phloem elements, ps – air cavity, sc – astrosclereids, sp – spongy parenchyma, str – secretory trichome, x – xylem elements.
3.2 Adaptive features
In the species examined, the leaves exhibit a spectrum of micromorphological adaptations characteristic of the hydromorphic evolution of plants. General aerenchymatization, reduction of conductive elements, weak tissue sclerization are adaptive features of the species. Assimilating, normally developed leaves are characterized by typical traits of organs found in two habitats. A common feature in their structure is the dorsoventrality of the mesophyll and the difference between the upper and lower sides of the leaf blade. Secretory formations of the epidermal origin provide waterproofing protection to plant organs in the aquatic environment.

3.3 Taxon-specific features
Taxonomic characters of the Nymphaeaceae family include the presence of atactostela in the axial organs and astrosclereids in the vegetative organs [10]. The atactostela and astrosclereids found in the examined representatives give grounds to consider them as part of the Nymphaeaceae family. The study also confirms their belonging to different subfamilies by the micromorphological signs. The type of the conducting bundle can be considered as a sign of the subfamily rank. *E. ferox* is close to *Brasenia schreberi* J. F. Gmel in the presence of paired concentric bundles from the monotypic family Cabombaceae A. Rich. [11].

4. Conclusion
To summarize, the microstructure of the leaf and petiole was studied in two species of the nymphaean family *E. ferox* and *N. japonica*. The factual data obtained in the course of the study complement the general characteristics of the species and provide some grounds for clarifying their taxonomic position in the system of the Nymphaeaceae family. Further research will be related to the microstructural analysis of the root system and the reproductive organs of the species.

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