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RED BOOK SPECIES OF SPARGANIUM GRAMINEUM IN THE IVANOVO REGION

The genus Sparganium L. belongs to one of the large genera of the *Typhaceae* family, in which there are from 7 to 27 species. The presence of interspecific hybrids greatly complicates their definition; therefore, I use modern molecular methods (PCR, sequencing) to define them, on special laboratory equipment (sequencer, amplifier).

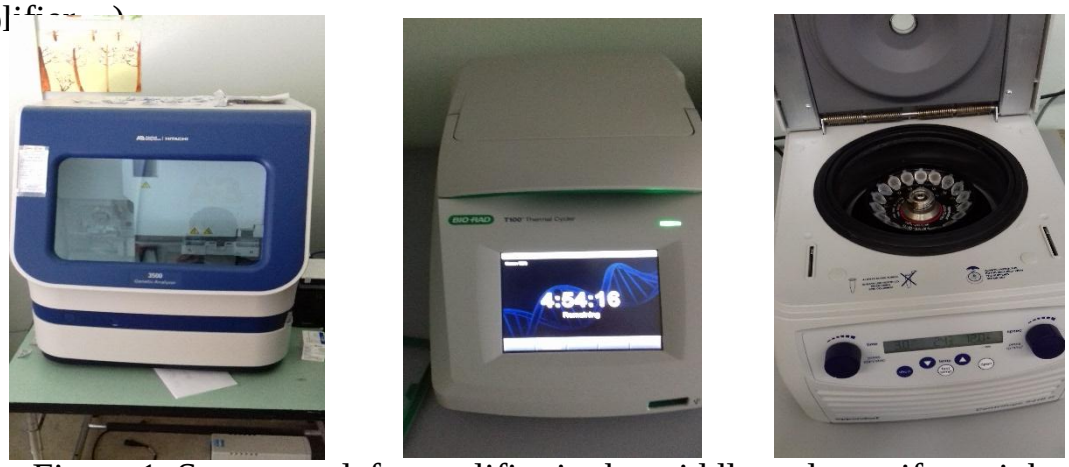

Figure 1. Sequencer left, amplifier in the middle and centrifuge right.

In the Ivanovo region, there are 4 know species: *S. emersum* Rehm, S. *microcarpum* (Neum.) Raunk. *S. gramineum* Georgi. S. natans L. *S. gramineum*, is very rare, is included in the regional Red Book (2010) with a rare status category 2. This species forms hybrids, mainly with *S. emersum*, known as *S. × longifolium*. Therefore, the aim of this work was to identify hybridization in this species in the Ivanovo region using molecular and morphological comparisons.

To achieve this goal, the following tasks were set:

1) Molecularly examine samples of S. Gramineum from 3 lakes in the Ivanovo region (Valdayskoe, Vysokovskoe and Spasskoe)
2) Morphologically compare species
3) Investigate pollen from collected species

In the period from 2017 to 2019, 3 lakes were examined in the Ivanovo Region, in which a rare species S. gramineum was indicated. These are the lakes Valdaiskoe, Spaskoe (Losevskoe) and Vysokovskoe. During the research period herbarium samples were collected, which are now transferred to the herbarium of the Institute of Inland Water Biology RAS (IBIW). For molecular studies, green, healthy parts of the leaf were collected in bags “zip-lock” filled with silica gel.

Figure 2. Thickets *S.* × *longifolium* on lake Valdayskoe (left) and Vysokovskoe (right)

Collected samples were examined by morphological and molecular methods to identify and confirm hybridization. As a result of molecular studies, when comparing nuclear DNA sequences using the ITS marker, up to 13 polymorphic positions were identified that are different from the parent species, which indicates a clear hybridization of the species in these lakes. When studying chloroplast DNA by the matk marker, it was revealed that the maternal species is *S. gramineum*, since chloroplast DNA is inherited by the maternal line.

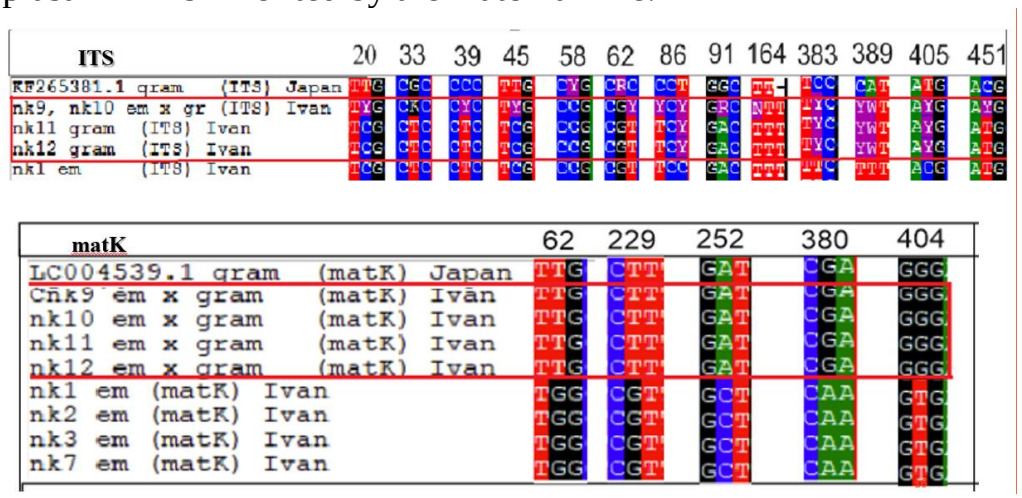

Figure 3. DNA sequences of the nuclear marker ITS and chloroplast matk.

From the maternal species *S. gramineum*, which was shown by chloroplast markers, the hybrid inherited long and thin leaves, also a branched inflorescence, in contrast to the rather short, rigid leaves and simple inflorescences in *S. emersum*, and from the paternal *S. emersum* - keeled leaves , while *S. gramineum* leaves are flat. Also, the leaf width of the hybrid is closer to the values of *S. emersum* than to *S. gramineum*. Fruits of the hybrid have a hooked styloid like the maternal species. Some of the samples from Lake Valdaiskoe have deformed inflorescences and no pistil heads.

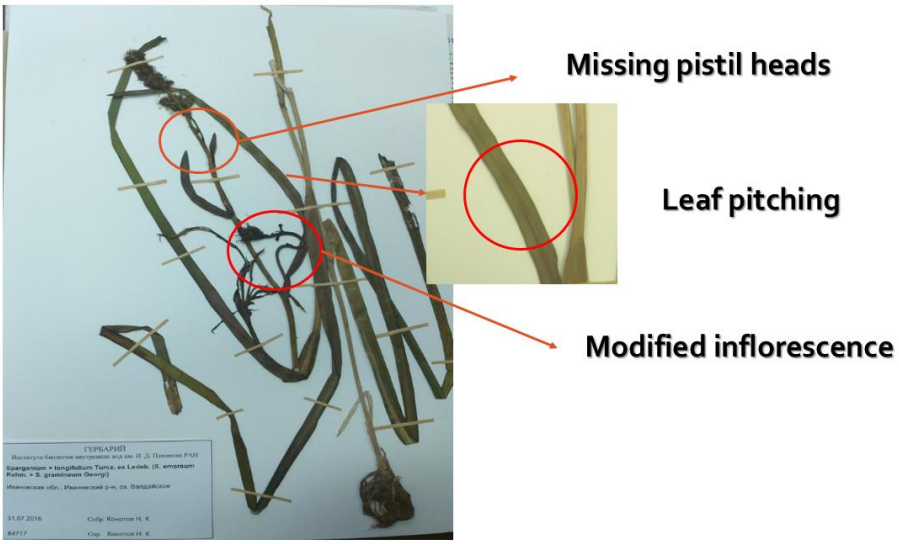

Figure 4. Morphological features *S. × longifolium*.

When painting and examining the pollen of a hybrid under a microscope, it was found that up to 80% turned out to be fertile, while in the male heads it is quite small. This feature indicates that the hybrid can be sexually crossed, because species of the genus sparganium have the same number of chromosomes 2n = 30. These phenomena indicate late or cross hybridization of *S. gramineum* on explored lakes.

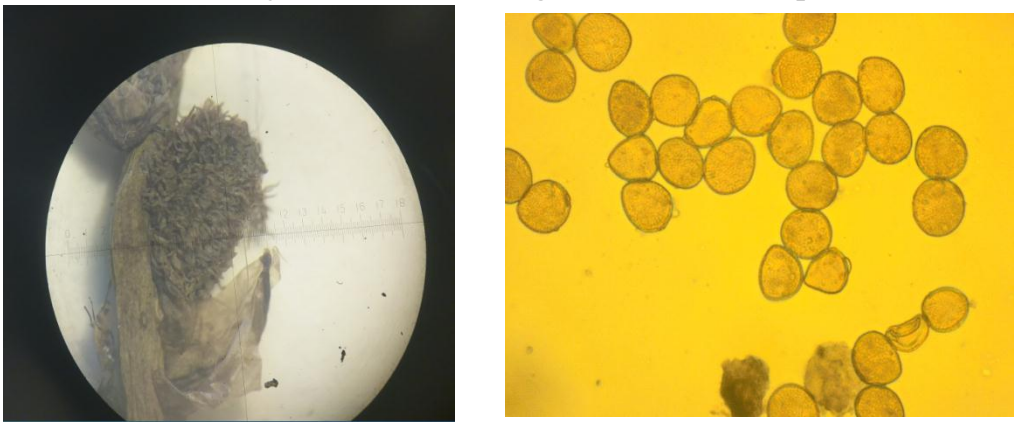

Figure 5. Stamen head and fertile pollen *S. × longifolium*.

Based on the results of this work, we can draw the following conclusions:

1) Found up to 13 polymorphic positions in nuclear DNA.
2) According to the chloroplast DNA of the hybrid, it was revealed that the maternal species is *S. gramineum*.
3) The hybrid has intermediate morphological characteristics: keeled leaf, branched inflorescence, long, but much wide leaves.
4) The hybrid has fertile pollen, which indicates the possibility of interbreeding sexually.
5) The red-book species *Sparganium gramineum* should be assigned status 1, a species that is under threat of extinction.

The work was carried out based on the laboratory of the Institute of Biology of Inland Waters, Russian Academy of Sciences, Borok, as part of the institute's planned topic.

Special thanks to my supervisors: Dr. Biol. sciences, prof. E. A. Borisova, Ph.D. biol. sciences. A.A. Bobrov.