

How do you find it? Dualistic approach reveals novel *Paramecium* species

M. Melekhin¹, L. Nikitashina¹, N. Lebedeva², G. Petroni³, O. Lanzoni³, I. Nekrasova¹, S.I. Fokin^{1,3} and A. Potekhin¹

1. Faculty of Biology, Saint Petersburg State University, Saint Petersburg, Russia

2. Centre of Core Facilities “Culture Collections of Microorganisms”, Saint Petersburg State University, Saint Petersburg, Russia

3. Department of Biology, University of Pisa, Pisa, Italy

Paramecium (Ciliophora) is divided into five subgenera. *Cypriostomum* subgenus is the less studied group, and traditionally includes four morphospecies difficult to discriminate, namely *P.calkinsi*, *P.nephridiatum*, *P.polycaryum*, and *P.woodruffi*. We analyzed more than 100 strains belonging to this group from all over Eurasia. Firstly, we performed complete morphological analysis focusing on micronuclear and contractile vacuole characteristics. Such features allowed to distinguish five well-recognizable morphological groups. Two strains could not be attributed to any of them. Morphological analysis was confirmed by three-loci molecular characterization, proving presence of seven *Paramecium* morphospecies in *Cypriostomum*. However, only *P.nephridiatum* and *P.polycaryum* were fitting to the original descriptions, while *P.woodruffi* and *P.calkinsi* were redescribed, and three novel species were documented.

The application of the inverted approach, when molecular data suggested to search for morphological peculiarities, also appeared to be efficient. Phylogenetic analysis using different molecular markers showed that *P.multimicronucleatum* includes two branches, possibly corresponding to cryptic species (Tarcz et al., 2012). We isolated a strain from Siberia, which by 18S rDNA phylogeny appeared to belong to the less abundant cluster within *P.multimicronucleatum*. Surprisingly, this strain had a single endosomal-like micronucleus, completely different from numerous vesicular micronuclei typical to *P.multimicronucleatum*. These results together attribute this strain to a novel morphospecies. The molecular characterization of two “aurelia” type strains isolated in St Petersburg region (Russia) revealed that they were very similar to “*Eucandidatus Paramecium germanicum*” (Krenek et al., 2015) by 18S rDNA and COI gene sequences. Microscope observations showed unique organization of their micronuclei, thus allowing to consider “*Eucandidatus Paramecium germanicum*” as the valid morphospecies.

Herein, five new morphospecies were discovered in *Paramecium* genus, *Cypriostomum* and *Paramecium* subgenera were revised, and several species were redescribed. Finally, our data confirm once again, that the combination of morphological and molecular approaches is required for ciliate diversity investigations.

Supported by RSF 16-14-10157.