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Morphology and Phylogeny of a New *Frontonia* Ciliate, *F. paramagna* spec. nov. (Ciliophora, Peniculida) from Harbin, Northeast China

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Abstract

This paper describes a new *Frontonia* ciliate, *F. paramagna* spec. nov., sampled from freshwater in Harbin, northeast China, based on its morphology, infraciliature, ultrastructure and small subunit ribosomal RNA gene information. The new species is defined by the following features: large sized freshwater form, 400–610 × 110–160 μm *in vivo*, about 179–201 somatic kineties, three peniculi, each with four kineties, three vestibular and six or seven postoral kineties, one elongated-elliptical macronucleus, centrally-located, a single contractile vacuole, without canals, located right-dorsally in the posterior half of the body. Sequence alignment and phylogenetic analyses based on the small subunit ribosomal RNA (SSU rRNA) gene indicated that the new species has characters distinct from its known congeners. The ultrastructure of the trichocyst and other extrusomes, and the subpellicular fibre system, were observed by both scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Much of the ultrastructure is here given for the first time by SEM, and these features provide complementary data for taxonomic purposes.

Key words: Ciliates, *Frontonia*, new species, phylogeny, ultrastructure, taxonomy

Introduction

Frontonia ciliates are frequently found in marine, brackish and freshwater habitats (Al-Rasheid 1999; Carey 1992; Fan *et al.* 2011a, 2012; Long *et al.* 2008) and many species have been described using live observation and silver impregnation methods (Borror 1963; Burkovsky 1970; Bullington 1939; Foissner 1987; Foissner 1994; Gil & Perez-Silva 1964a, b, c; Kahl 1931; Pan *et al.* 2013; Petz *et al.* 1995; Roque 1961a, b, c; Roque & de Puytorac 1972; Song & Wilbert 1989). These species have typically been distinguished from each other by the combination of the following characteristics: their body shape and size, the number and location of their contractile vacuoles, the morphology of their oral apparatus, and their general somatic ciliary pattern (Corliss 1979; Dragesco 1960; Dragesco & Dragesco-Kernéis 1986; Foissner 1994; Roque & de Puytorac 1972). Only some species have been well-described based upon both live observations and silver impregnation, however, while the description of ultrastructure has been especially rare. Many Oligohymenophorea species are inadequately investigated in respect to current taxonomic criteria; that is, they are poorly defined and described and, often, lack a statement of the type material (Burkovsky 1970; Fan *et al.* 2011b, 2011c; Long *et al.* 2005; Pan *et al.* 2011, 2013a, 2013b, 2013c; Petz *et al.* 1995; Roque & Puytorac 1972).

These organisms should ideally be identified morphologically by a combination of features coming from both live and silver impregnation, like the buccal and somatic ciliature, position and character of the contractile vacuoles, size and shape of the body and some ultrastructure features coming from SEM and TEM observations, as well as their habitat (Foissner 1987; Foissner *et al.* 1994). Moreover, with the application of molecular techniques

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First, *F. leucas* is more slender in size, at 120–360 × 110–120 µm *in vivo*, while *F. pallida* (150–160 × 60–66 µm) and *F. terricola* (70–110 × 60–73 µm) are much smaller than *F. paramagna* spec. nov. (400–610 × 110–160 µm).

Second, the last two species have different kinety rows in peniculi 1–3. *F. pallida*'s peniculi 1–3 contain four, three and two kinety rows respectively, *F. terricola*'s peniculi 1 and 2 have four kinety rows, while peniculus 3 has three kinety rows (comparedto four rows in peniculi 1–3 in *F. paramagna* spec. nov.).

Third, *F. paramagna* differs from the other species in the number of vestibular and postoral kineties. The number of postoral kineties in *F. leucas* is four or five, in *F. pallida*, five, and *F. terricola,* six (compared to six or seven in *F. paramagna* spec. nov.). Meanwhile, the number of vestibular kineties in *F. pallida* is four (compared to three in *F. paramagna* spec. nov. and the other species). Hence, *F. paramagna* spec. nov. can be clearly distinguished from *F. leucas*, *F. pallida* and *F. terricola*.

In addition, SSU rRNA analysis results revealed that *F. paramagna* spec. nov. is close to *F. vernalis* Bullington, 1939, however, there are clear differences between them in morphology. *F. vernalis* is smaller (197 × 108 µm compared to 400–610 × 110–160 µm) and has two to three contractile vacuoles (compared to only one in *F. paramagna* spec. nov.). Besides, *F. vernalis* is a marine species while *F. paramagna* spec. nov. is a freshwater species.

Sequences comparison and phylogenetic analyses. Trees constructed using different algorithms showed similar topologies in this investigation. The unstable topologies for the order Peniculida were mainly caused by the position instability of the species *Urocentrum turbo*. All of the species of genus *Frontonia* were positioned in the order Peniculida, although they did not form a monophyletic clade due to the split from *F. didieri* (Fig. 4). The clade formed by *Frontonia paramagna* spec. nov. /*Frontonia* sp./ *F. vernalis* cluster with *F. leucas* with full support, although even these four species have very different morphological characters (Table 2) and SSU rRNA sequences (Fig. 4, Table 3). Furthermore, we also carried out an Approximately Unbiased (AU) test on the SSU-rDNA dataset to assess the monophyly of *Frontonia* species. The test could not significantly reject the constrained topology where all the *Frontonia* species were forced to be monophyletic (AU=0.889, AU>0.05). And more molecular information may be needed in future work for a better understanding of this genus.

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