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On the importance of attentive reading of research articles: the case study of *Frontonia* (Peniculia, Oligohymenophora, Ciliophora) species descriptions and redescriptions

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Summary

A commonly encountered problem of ignoring or inattentive reading of scientific articles by some protistologists of the past and present has been revealed using the case study of taxonomy of the ciliate genus *Frontonia*. The comparison between materials from literature and own investigations allowed the author to conclude that *F. vernalis* Ehrenberg, 1833 cannot to be a valid species and assume that it is rather a cluster of closely related freshwater ciliates with one contractile vacuole and a stable ability to accommodate green algae as the cytoplasmic symbionts. "*F. vernalis*", as described by Bullington (1939), was shown to have little in common with the original description by Ehrenberg and most likely is a brackishwater ciliate similar to *F. fusca*. *F. oculiaris* described by the same author (Bullington, 1939) is obviously *F. fusca*, and the name "*F. oculiaris*" should be considered as a younger synonym of the latter. Thus, redescription of *F. oculiaris* (Pan et al., 2013b) should be treated just as a morphological study of the local Chinese population of *F. fusca*. The redescription of *F. canadensis* from brackish waters by the same authors can be considered as a description of a new species that has little in common with the original description of this freshwater ciliate (Roque and Puytorac, 1972). Recent phylogenetical reconstructions indicate the necessity to split *Frontonia* into several genera.

Key words: *Frontonia*, morphology, phylogeny, species redescription

Introduction

Among Oligohymenophorea (Ciliophora, Protista), the subclass Peniculia is one of the best know and, in some aspects, intensively studied groups of ciliates. A few of these organisms became good and promising model objects for laboratory investigations. At the same time, peniculines are important components of natural ciliate communities being numerous in many different biotopes, mainly due to the representatives of two related genera – *Frontonia* and *Paramecium* (Corlis, 1979; Puytorac et al., 1987; Strüder-Kypke et al., 2000; Lynn, 2008; Foissner et al., 1994, 2002; Fokin and Trivashkevitch, 2004;

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Fokin et al., 2004, 2006; Fokin, 2008; Gao et al., 2008; Long et al., 2008; Fokin, 2010/2011; Pan et al., 2013b; Cai et al., 2018). *Frontonia* is the largest genus of the group, comprising more than 40 species according to published data. However, only about 10 of them are the relatively common species (Kahl, 1931, 1943; Dragesco, 1970; Roqui and Putorac, 1972; Dragesco and Dragesco-Kernéis, 1986; Carey, 1992; Foissner et al., 1994, 2002; Fokin and Trivashkevitch, 2004; Gao et al., 2008; Fokin et al., 2017; Cai et al., 2018; Fokin et al., 2019). *Frontonia* representatives are widely spread in many types of water bodies (freshwater, brackishwater and marine) as well as in soil (Foissner et al., 2002).

At the first glance, ciliates of the *Frontonia* group look quite similar to each other from the morphological point of view and the whole genus looks like a solid entity. These ciliates are characterized by the same type of stomatogenesis (bucco-kinetal) with a distinctive set of oral membranelles (oligohymenimum): 3 penniculi on the left side of the buccal cavity and paroral membrane on the right buccal margin. Topographically, somatic ciliature is uniformly organized with some exceptions at the oral region (vestibular and postoral kineties) and distinctive pre- and postoral sutures; typical trichocyst-like extrusomes are always present in the cortex. At the same time, some morphological features (e.g., number and structure of contractile vacuoles and micronuclei, as well as the cell size) are strongly variable within the group. It is quite probable that *F. leucas* (Ehrenberg 1838), one of the first described *Frontonia* species (type species of the genus has not yet been established), which manifests huge size variation (from 100 to 500 μm), in fact is a group of sibling species (Foissner et al., 1999; Fokin et al., 2006). The first phylogenetic analysis using 18S rRNA gene sequences of a number of the genus' representatives showed that *Frontonia* is a non-monophyletic group (Andreoli et al., 2007a, 2007b). This conclusion was confirmed using an another set of different frontoniids (Gao et al., 2008), and later this fact was demonstrated several times again (e.g., Fan et al., 2013; Fokin et al., 2017, 2019; Cai et al., 2018).

During the recent decade, a number of articles dealing with *Frontonia* spp. were published, but in some of those articles several mistakes which probably could be just due to the insufficient knowledge of old (and also not so old) literature were revealed (Fokin et al., 2017).

In fact, starting from the middle of the XIX century (Dujardin, 1841; Claparède and Lachmann, 1858), then one century later (Bullington, 1939) and, finally, in the last decade numerous cases were revealed when researchers were demonstrating ignorance or misinterpretation of the previously published results. These phenomena sometimes caused significant taxonomic errors and instead of clarifying the situation were making it even more confusing. As an example, let's take a closer look at the initial stages of description of the members of the genus *Frontonia*.

The history of description and redescription of some frontoniids

The first case is *F. vernalis*. *Frontonia* as a particular 'type' of ciliate protists was first mentioned by Ch. G. Ehrenberg as early as in 1833 under the name of *Bursaria vernalis* (Ehrenberg, 1833, p. 235–236), and then in 1838 under the same name, but with the indication that this species belonged to subgenus *Frontonia* (Ehrenberg, 1838, p. 329). According to the author's "Polygastric theory" proposed in 1838 in his monograph entitled "Die Infusionthierchen als Vollkommene Organismen" ("The Infusion Animals as Comprehensive Organisms"), all those "animalcula" – protozoa, bacteria, diatoms, desmids and rotifers – were considered as tiny though perfect creatures organised in the same way as higher animals. In his monograph (Atlas, Table 34, Fig. VII), Ehrenberg simply reproduced the main drawing of *F. vernalis* made in 1833 (p. 383, Plate III). Despite quite primitive description of *Frontonia* made in both abovementioned publications, the scientist indicated several important features of the new ciliate: size about 200–250 μm (1/10–1/12 of line=2,54 mm); body, which was uniformly elongated, ovoid, dorso-ventrally flattened with some reduction of size at the end of the cell, with elongated oral aperture (around 1/5–1/6 of body – according to the drawings); 2 contractile vacuoles (CV – he treated these structures as seminal vesicles) with particular location (anterior and posterior third of the cell, dorsally), and numerous green particles of 4–5 μm in diameter in the cytoplasm. Food vacuoles were mainly filled with *Oscillatoria* (cyanobacteria) and *Navicula* (diatoms). Ciliates moved with rotation along the body axis, and some of them were recorded as dividing longitudinally. The latter observation apparently indicated that Ehrenberg saw the conjugants that very often appear in the just collected samples. Importantly, the new ciliate was collected by the scientist nearby Berlin (at the zoo), in fresh water. It was not indicated by

Ehrenberg how many cells were investigated; thus, we can doubt that 2 CV is a stable feature. Later on, several times *F. vernalis* was synonymized with *F. leucas* (Dujardin, 1841, Claparède and Lachmann, 1858; Kent, 1880-1882; Schewiakoff, 1889, 1896), and then again these frontoniids were accepted as two different species (Kahl, 1931, 1943).

Meanwhile, comparison of the materials presented as *F. vernalis* at the very beginning of its history (Ehrenberg, 1833, 1838) with the characteristics of a ciliate "redescribed" as *F. vernalis* by W. Bullington (1939) clearly indicates that the latter scientists dealt with the absolutely different ciliate species.

First of all, Ehrenberg (1833, 1838) personally worked with the freshwater "green" ciliates (containing numerous green "albuminous" particles in the cytoplasm) that he collected repeatedly near Berlin in 1832, 1833, and 1835. He also just mentioned (Ehrenberg, 1838) that some, probably similar but not identical, organisms were found by O. Müller and J.B. Bory in the marine environment near Copenhagen and Cadix. Moreover, he made a clear statement: "**I have no rights to combine them into one**" (Ehrenberg, 1838, p. 329). Bullington, apparently, could not translate the German text properly and therefore started his description in a wrong way: "Ehrenberg (1838) found a ciliate which he described under the name Bursaria, subgenus *Frontonia* (*vernalis*) **in sea water near Berlin, Copenhagen, and Cadix**" (Bullington, 1939, p. 36). Further he wrote: "...***Parophrys fusca* (Quennerstedt, 1869) which seems to me undoubtedly belongs here**" (Bullington, 1939, p. 36). So, definitely, Bullington's "*F. vernalis*" should be treated as a brackishwater species related to *F. fusca* (Quennerstedt, 1869; Kahl, 1931; Fokin, 2008). In the same article, Bullington also described *F. oculiaris* (Bullington, 1939, p. 42–46), which likewise reminds *F. fusca*.

Further on, in several recent publications the authors accepted those wrong descriptions of Bullington and until 2018 treated *F. vernalis* as a brackishwater species (Chen et al., 2014; Cai et al., 2018). Additionally, a redescription of "*F. oculiaris*" was made (Pan et al., 2013b) which, according to morphology and the sequences presented, is definitely *F. fusca* (Fokin, 2008; Fokin et al., 2019). Moreover, the same researchers made another redescription of *F. canadensis* as a brackishwater species (Pan et al., 2013a), not paying attention to the important fact that in the original description (Roque and Puytorac, 1972) this species was characterized as a freshwater ciliate. It is especially confusing that Pan with co-authors claimed that "i**n the original description of *F. canadensis* Roque and Puytorac gave neither a clearly outlined diagnosis nor details of the living morphology**" (Pan et al., 2013a, p. 69), as if the assignment of the newly found ciliate to the name *F. canadensis* by Roque and Puytorac (1972) was not justified at all.

Meantime, already in 1931 A. Kahl briefly discussed taxonomical situation with *F. vernalis* and logically compared it with *F. leucas*, as both of them were freshwater species (Kahl, 1931, p. 317). He recommended to accept *F. vernalis* as a distinctive species name because the ciliate contained zoochlorellae (which could not be experimentally kept in *F. leucas*) and used a different type of food (particularly diatoms). Unfortunately, he did not make a redescription of this green *Frontonia*. A. Kahl not only proposed to accept *F. vernalis* as a separate species, but also indicated 2 'types' of such ciliates that differed from each other by the cell shape.

The situation was briefly recorded by W. Foissner who wrote 2 decades ago that "status (of green frontoniid) is not yet clear" (Foissner et al., 1999, p. 416). He also highlighted the similarity of oral kinetom in *F. leucas* and *F. vernalis* ("green *F. leucas*") and remarked that "whether the chlorotic populations are conspecific with the apochlorotic ones needs detailed investigations" (Foissner et al., 1999, p. 423). This comment is of special importance because earlier J. Dragesco indicated for "*F. leucas*" containing *Chlorella* sp. a different from the classical *F. leucas* number of vestibular kineties (Dragesco and Dragesco-Kerneis, 1986, p. 319).

Current taxonomy of *Frontonia vernalis*

In all recently published molecular trees concerning *Frontonia*, the 18S rRNA gene sequence with accession number U97110, which is shown in GenBank database as *F. vernalis*, is present. This sequence is the only one available for the species; it was deposited by Hirt and the colleagues in 1997, but has not yet been supported by any publication. It is known that Hirt and his colleagues worked with the freshwater frontoniid hosting *Chlorella*-like cytoplasmic symbionts (e.g. Berninger et al.,1986; Finlay et al.,1987; Esteban et al., 2010), which was collected in a small productive pond (Priest Pot, Like District, Cumbria, England). It looks like the identification of that ciliate as *F. vernalis* was done at that time by Dr. C. Curds (Berninger et al.,

1986, p. 557), and thereafter the ciliate was never characterized morphologically in the large set of the English-language publications where the name "*F. vernalis*" was utilized (see: Esteban et al., 2010). Meanwhile, this frontoniid does not match the original morphotype of green *F. vernalis* described by Ehrenberg (1833, 1838) since there are two CVs vs one in the cells from the UK population. So, several articles were published using in fact the unknown (i.e. not yet described) *Frontonia* sp.

Outlook

The comparative analysis of some old and new publications dealing with taxonomy of the genus *Frontonia* regretfully witnesses for inattentive reading or ignoring of the previously published literature by some protistologists of the past and the present.

As a result of comparison between the materials from literature and own data, the author proposes to reject *F. vernalis* as a valid species' name and assume that this is a cluster of closely related freshwater ciliates with one contractile vacuole and a stable ability to accommodate green algae as the cytoplasmic symbionts. One of such species was just recently described from the North of China – *F. shii* (Cai et al., 2018), and description of two others – *F. paravernalis* and *F. apovernalis* (briefly mentioned in Fokin et al., 2017) is currently in progress.

The green frontoniid used by the colleagues from the UK (Finlay et al., 1987 and references therein) should be precisely described as a different ciliate (for instance – *F. pseudovernalis*) using a multidisciplinary approach. "*F. vernalis*" described by Bullington (1939) has nothing in common with the original description of Ehrenberg (1833, 1838) and most likely was a brackishwater ciliate similar to *F. fusca* (Fokin, 2008).

F. oculiaris, also described by Bullington (1939), was obviously *F. fusca*; therefore, the name *F. oculiaris* should be considered as a younger synonym of *F. fusca*. Thus, a redescription of *F. oculiaris* made by Pan with colleagues (Pan et al., 2013b) should be treated just as a morphological study of the local Chinese population of *F. fusca*. The redescription of *F. canadensis* (Roque and Putorac, 1972) from brackish waters made by the same authors (Pan et al., 2013b) should be considered as a description of a new species that has nothing in common with the original description of a freshwater ciliate made in the 1970ies (Roque and Puytorac, 1972).

At present, we have got at our disposal more than 70 sequences of the representatives of the genus *Frontonia*. The composition of various groups in both freshwater and brackish sublinages of *Frontonia* allows splitting the genus phylogenetically into 3 clusters (Fokin et al., 2019), most likely corresponding to the generic level. Therefore, obviously, a comprehensive revision of the genus *Frontonia* is required, including segregation of a number of new genera, like it has already been done for several large ciliate taxa – for example, spathidiids and dileptids (Foissner and Xu, 2007; Vdačnŷ and Foissner, 2012).

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