Frontonia anatolica n. sp., a new peniculid ciliate (Protista, Ciliophora) from Lake Van, Turkey

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Received: 27.03.2012 ● Accepted: 26.07.2012 ● Published Online: 24.12.2012 ● Printed: 21.01.2013

Abstract: The morphology, ciliature, and silverline system of a new ciliate, Frontonia anatolica n. sp., isolated from the bottom sediment of the eastern shore of Lake Van, a large alkaline lake in Eastern Anatolia Turkey, were investigated using live and silver impregnation methods. Frontonia anatolica n. sp. is characterised by an elliptical body shape; by an in-vivo body size of 101–134 × 47–67 µm; by dorsoventral flattening of about 2:3 to 1:2; by 2 contractile vacuoles located in the anterior and posterior body parts, each with 6–7 collecting canals and 1 excretory pore; by about 93 somatic kineties; by 3 vestibular and 3–5 postoral kineties; and by peniculi 1 and 2 each having 4 ciliary rows, and peniculus 3 having 2 ciliary rows.

Key words: Frontonia, morphology, new species, alkaline habitat

1. Introduction
The genus Frontonia was originally described by Ehrenberg in 1838, and so far more than 40 nominal species have been recognised and described (Kahl, 1931; Burkovsky, 1970; Roque and Puytorac, 1972; Carey, 1992; Foissner et al., 2002; Long et al., 2005, 2008; Fan et al., 2011). The members of this genus are commonly found as benthic or pelagic members of both freshwater and marine biotopes and in the moist soils of terrestrial habitats (Kahl, 1931; Roque, 1961; Borror, 1963; Dragesco and Dragesco-Kernéis, 1986; Alekperov and Carey, 1992; Foissner et al., 1994, 2002; Sadikhova, 2006; Fokin, 2008). The identification of species belonging to the genus Frontonia relies on a combination of the following characteristics: body shape and size; oral and somatic infraciliature; morphology of the oral apparatus; characters of the contractile vacuole (number of contractile vacuoles, location within the cell, presence or absence of collecting canals, and number of excretory pores); number of macronuclear nodules; and habitat (Foissner et al., 1994; Long et al., 2005, 2008; Fan et al., 2011).

In recent investigations carried out on ciliated protozoa on the eastern shore of Lake Van, we observed an unusual Frontonia population in the bottom sediment of the lake, and collected morphological data. After detailed investigations on this population, we conclude it proves to be a previously unknown member of the genus Frontonia. The results are documented below.

2. Materials and methods
Frontonia anatolica n. sp. was collected from the bottom sediment of a small marina (0.5–3 m depth) on the eastern shore of Lake Van, Turkey. Lake Van, or Van Gölü in Turkish, is located in the highlands (1646 m above sea level) of eastern Anatolia. The salinity of the lake is about 19‰ (brackish water), the water temperature is 10–20 °C, and the pH is 9.8. Lake Van is also the largest soda lake on earth, and has served as a model of the possible highly alkaline chemistry of the early ocean (Reimer et al., 2009).

The material was collected from the upper 5 cm layer of sediment together with some water from September 2008 to May 2010. Specimens were either observed immediately or maintained for several days in the laboratory (20–24 °C) for further studies. Cells were first observed in vivo under a stereo microscope, and then by phase-contrast and differentiation interference-contrast (DIC) compound microscopes (×100–1500). The infraciliature was revealed by silver carbonate, silver nitrate, and protargol impregnation methods (Foissner, 1991; Foissner et al., 1999; Ma et al., 2003). Drawings of live cells were based on free-hand sketches and micrographs, and those of impregnated ones were made by computer with camera lucida and micrographs. Terminology and taxonomy are according to Dragesco and Dragesco-Kernéis (1986), Foissner et al., (2002), Kahl (1931), and Lynn (2008).
3. Results
3.1. Class Oligohymenophorea de Puytorac et al., 1974
3.2. Order Peniculida Fauré-Fremiet in Corliss, 1956
3.3. Family Frontoniidae Kahl, 1926
3.4. Genus Frontonia Ehrenberg, 1838
3.4.1. Frontonia anatolica n. sp.
(Figures 1a–h, 2a–m; Table 1, 2)

Diagnosis: Size about 101–134 × 47–67 μm in vivo, body shape ellipsoidal, dorsoventrally slightly flattened of about 2:3 to 1:2. Oral cavity in body midline occupying about 18% of body length. 78–105 somatic, 3 vestibular, and 3–5 postoral kineties. Peniculi 1 and 2 each have 4 rows, whereas peniculus 3 has 2 ciliary rows. Macronucleus ellipsoidal to ovoidal. One elliptical to spherical micronucleus attached to macronucleus. Two contractile vacuoles each with 6–8 collecting canals, located in anterior and posterior body parts. Extrusomes spindle shaped.

![Diagram of Frontonia anatolica n. sp.](image)

**Figure 1.** Line diagrams of *Frontonia anatolica* n. sp. from life (a–d, h), after silver nitrate impregnation (e–g). a) Ventral view of representative specimen. b–d) Ventral, dorsal, and apical views showing sutures and location of contractile vacuoles. e) Oral apparatus showing 4 vestibular kineties of which VK1 is very near to or on paroral membrane. Arrowheads mark postoral kineties. f, g) Ciliary pattern of ventral and dorsal side, same specimen. h) Optical section of cortex showing arrangement of extrusomes. CV = Contractile vacuole; Di = Diatom; EP = Excretory pore; Ma = Macronucleus; Mi = Micronucleus; OA = Oral apparatus; P1–3 = Peniculi 1–3; PeS = Preoral suture; PM = Paroral membrane; PoS = Postoral suture; VK1–3 = Vestibular kineties 1–3.
Type location: Bottom sediment of small marina on eastern shore of Lake Van, Turkey (38°33′38″N, 43°16′39″E).

Type slides: The holotype (slide number: 2012/H1) and 2 paratype slides (slide number: 2012/P01, 2012/P02) with silver nitrate impregnated specimens are deposited in the Laboratory of Protozoology, Faculty of Science, Yüzüncü Yıl University, Turkey.

Etymology: The species name anatolica refers to the place, Anatolia, the Asian part of Turkey, where it was discovered.

Description: Size 101–134 × 47–67 μm in vivo, 83–114 × 40–63 μm in silver nitrate impregnated specimens (Table 1). Ovoidal with broadly rounded anterior and posterior ends in live specimens (Figures 1a–c, 2a–d), the anterior more broadly than the posterior in silver nitrate preparations (Figures 1f, g, 2g). Dorsoventral flattening of 2:3 to 1:2 (Figure 1d), width/length ratio about 41%–60% in both live and impregnated individuals. Nuclear apparatus usually located in mid-body, sometimes observed in anterior or posterior body half (Figures 1a, 2a). Macronucleus ellipsoidal, about 26 × 15 μm. Micronucleus compact, ellipsoidal to globular, located in indentation of macronucleus (Figures 1a, 2a, h), about 7 × 5 μm in size. Two contractile vacuoles each with ca. 7 conspicuous long collecting canals, positioned dorsally on both anterior and posterior body half (Figures 1a–d, 2b–e). Each vacuole with one excretory pore, located left of dorsal side on both body parts (Figures 1g, 2e, g). Cytopyge extends in ventral portion of postoral suture. Resting extrusomes fusiform, 6–8 μm long, narrowly located and attached perpendicularly to cortex between kinetids (Figures 1a, h, 2f, j). Cytoplasm colourless, without pigment granules. However, some large indeterminable lipid-droplet–like

### Table 1. Morphometric data of Frontonia anatolica n. sp. Data based on live and silver nitrate impregnated specimens; all measurements in μm.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>SD</th>
<th>SE</th>
<th>CV</th>
<th>N</th>
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<td>63.00</td>
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<td>34.00</td>
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<td>21.00</td>
<td>3.22</td>
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<td>45.00</td>
<td>68.00</td>
<td>7.19</td>
<td>1.44</td>
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<td>18.00</td>
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<td>0.84</td>
<td>0.37</td>
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*Upper rows obtained from silver nitrate impregnated specimens, lower rows obtained from live specimens.

Abbreviations: CV = Coefficient of variation (%); Max = Maximum value of examined specimens; Mean = Arithmetic mean; Min = Minimum value of examined specimens; N = Number of individuals examined; SD = Standard deviation; SE = Standard error of arithmetic mean.
structures were observed in a few individuals (Figure 2a). Feeds mainly on diatoms and bacteria (Figures 1a, 2a, b). Swims moderately rapidly with clockwise rotation about main body axis. Somatic cilia about 8 µm long in vivo, on average 93, mainly arranged in 78–105 ciliary rows (kineties) (Figures 1f, g, 2g, h; Table 1). Somatic ciliary rows form preoral and postoral sutures in ventral midline of cell (Figures 1b, f, 2h). Preoral suture commences at vertex of oral apparatus and extends to anterior pole, terminating subapically on dorsal side (Figures 1c, g). Postoral suture commences at right posterior corner of oral apparatus and extends to posterior pole, and ends subterminally on dorsal side (Figures 1c, g). Ventral somatic kineties shorten gradually on preoral and postoral sutures. There are 3–5 postoral kineties commencing from underneath the oral apparatus and terminating on the left side of the postoral suture (Figures 1e, f, 2m). Dorsal ciliary rows are bipolar (Figures 1g, 2g).

Oral cavity shallow, triangular in outline, about 15% back from anterior end, occupies 18% of body length (Table 1). Oral apparatus as shown in Figures 1a, e, f, 2a, h, k–m. Three short vestibular kineties (VK1–3) run from anterior vertex of oral cavity to postoral suture, with densely arranged kinetosomes. Anterior part of first vestibular kinety (VK1), which is on the right side of the oral apparatus, is located very close to the paroral membrane and is difficult to distinguish from it (Figures 1e, 2l, m). Three slightly curved peniculi (P1–3) located on left wall of oral cavity. Peniculus 1 and 2 almost equal in length (20 µm and 18 µm respectively), positioned close to each other, each composed of 4 rows of kinetosomes. Ciliary rows in peniculus 1 posteriorly gradually shortened from left to right (Figures 1e, 2m). Peniculus 3 about 15 µm long and composed of 2 rows of kinetosomes (Table 1). Paroral membrane commences at anterior right edge of peniculus 1 and extends along right margin of oral cavity, composed of one row of very closely spaced basal bodies (Figures 1e, 2m).

Silverline system composed of rectangular, longitudinally orientated meshes, similar to congeners (Figure 2i).

4. Discussion

Recent investigations have demonstrated that the most valuable criteria for species identification and separation in *Frontonia* are the structure of peniculi 1–3, body size and shape, the number of somatic kineties, the position of the contractile vacuole and the presence or absence of collecting canals, the appearance of the nuclear apparatus, and the habitat (Foissner et al., 1994; Long et al., 2005, 2008; Fan et al., 2011).

With regard to the contractile vacuole, *Frontonia anatolica* n. sp. closely resembles *F. elliptica* Beardsley, 1902 and *F. fusca* (Quennerstedt, 1869) in having 2 contractile vacuoles with collecting canals. All other congeners differ from the new species in having a single contractile vacuole.
Figure 2. Photomicrographs of Frontonia anatolica n. sp. from live specimens (a–f, j), after silver nitrate (g, i, l, m), and silver carbonate impregnation (h, k). a–d) General morphology of live specimens. e) A view of contractile vacuole. Arrow marks excretory pore, arrowheads mark collecting canals. f) Surface view showing extrusomes (arrowheads). g) Ciliary pattern of dorsal side. h) Ventral view of silver carbonate impregnated specimen showing somatic and oral structures in same focal plane due to the strong body flattening. i) Silverline system. j) Extrusomes beneath pellicule. k) Oral apparatus in early divisional stage. l, m) Oral ciliature showing 3 almost parallel, slightly curved peniculi on the left wall of the oral cavity and the paroral membrane vestibular kineties on the right. Arrowheads mark postoral kineties. CV = Contractile vacuole; Di = Diatom; E = Extrusome; EP = Excretory pore; LD = Lipid droplets; Ma = Macronucleus; Mi = Micronucleus; OA = Oral apparatus; PM = Paroral membrane; P1–3 = Peniculus 1–3; PeS = Preoral suture; PoS = Postoral suture; VK1–3 = Vestibular kineties 1–3.
with or without collecting canals (Figures 3a–g, Table 2) (Kahl, 1931; Roque, 1961; Borror, 1963; Dragesco and Dragesco-Kernéis, 1986; Carey, 1992; Foissner et al., 1994, 2002; Long et al., 2005, 2008; Fokin, 2008; Fan et al., 2011).

In addition to identical contractile vacuoles, at first glance our population shows some similarities with *F. elliptica*, especially in having elliptical body outline and body size (Figures 3 b–d). However, in our population the anterior region is never wider than the posterior as in *F. elliptica*. In vivo, the new form rather has a constant elliptical outline with almost equally rounded anterior and posterior body ends (Figures 1a, 2a–d). The new species,
“F. anatolica” differs from it in the following features: (1) different habitat (brackish, alkaline soda lake vs. brackish water in “F. elliptica”); (2) fewer vestibular kineties (3 vs. 4 in “F. elliptica”); fewer kinety rows in peniculi 1–3 (4 + 4 vs. 5–6 + 5–6 + 5–6 in “F. elliptica”); (3) excretory pore in each contractile vacuole (1 vs. 1–2 in “F. elliptica”); there is only one excretory pore per contractile vacuole in more than 50 specimens of “F. anatolica” examined. Hence, the 2 organisms can be clearly distinguished (Kahl, 1931; Dragesco and Dragesco-Kernéis, 1986; Carey, 1992; Foissner, 1996; Foissner and Wenzel, 2004). “F. anatolica” also may be easily confused with “F. fusca”, which has a similar body shape and size (100–170 × 45–75 µm in vivo), number of contractile vacuoles, and somatic kineties (75–90) (Fokin, 2008) (Figures 3e–g, Table 2). However, the new species can be easily distinguished from “F. fusca” due to the absence of pigment granules located anteriorly. In addition, the present species is different from “F. fusca” in the following characters: (1) different habitat (brackish, alkaline soda lake vs. brackish water in “F. fusca”); (2) fewer kinety rows in peniculus 3 (2 vs. 3 in “F. fusca”); (3) number of excretory pores per contractile vacuole (1 vs. 2–3 in “F. fusca”); (4) number of micronuclei (1 vs. 2 in “F. fusca”) (Fokin, 2008).

To date, no species has been found in the literature that was identical to our population. Therefore, this population is evaluated as a new species and named “Frontonia anatolica.”

Acknowledgements
This work is a part of project supported by Yüzüncü Yıl University, Office of Science Research Project (2007 FED B40).

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