Morphology and systematic position of *Euplotes manganari* sp. n. (*Ciliophora, Euplotida*) isolated from the Sea of Marmara, Istanbul (Turkey)

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Summary

Morphology and phylogenetic relationships of a euplotid ciliate, *Euplotes manganari* sp. n., isolated from the Sea of Marmara (Istanbul, Turkey) were investigated based on live observations, silver nitrate impregnation and Feulgen staining methods. The new species is characterized by the following combination of features: small size (31 × 19 µm), six conspicuous dorsal ridges, 10 normal-sized frontoventral cirri, two or three caudal cirri, five dorsal kineties, and double-patella type of silverline system. The SSU rRNA sequence of *E. manganari* branches with good support in the *E. orientalis* and *E. plicatum* clade.

Key words: ciliates, *Euplotes*, morphological features, phylogenetic analysis, SSU rRNA gene, the Sea of Marmara

Introduction

Ciliates from the genus *Euplotes* Ehrenberg, 1831 are a diverse group of ciliated protists ubiquitous in freshwater, marine and soil biotopes (Curds, 1975; Chen et al., 2013; Yan et al., 2018; Lian et al., 2020). According to the modern estimates, the genus comprises more than 160 species (Lian et al., 2021). *Euplotes* spp. can be identified based on a combination of the following morphological characters: the size and shape of the body, the shape of the macronucleus, the type of dargyrome, the number of cirri on the ventral side of the cell and the presence and number of dorsal ridges. Despite a high species diversity and clear morphological characters, *Euplotes* is considered as one of the most taxonomically problematic genera of ciliates (Kouser et al., 2022). This is due to the following reasons: lack of information on the pattern of dargyrome in several early studies; similarity of morphological characters, such as the number of cirri and dorsal kineties, in many species; scarcity of molecular data; the fact that many nucleotide sequences were assigned to *Euplotes* spp. without providing light-optical and histochemical data (Schwarz et al., 2007; Jiang et al., 2010; Syberg-Olsen et al., 2016; Huang et al., 2021).

In this paper, I described a new species of *Euplotes, E. manganari* sp. n., isolated from the coastal biotope of the Sea of Marmara in Istanbul (Turkey). I employed an integrative approach combining traditional techniques and the methods of molecular biology.

Material and methods

Ciliates *Euplotes manganari* sp. n. were found in a sample of sandy littoral sediments collected on
14 July 2023 from a coastal habitat near Istanbul (Sea of Marmara, 40°57′54″ N; 28°47′48″ E). The water temperature in the habitat was 28 °C (Fig. 1). Average salinity in the Sea of Marmara near the southern end of the Bosporus is 23-25‰ (Sacu et al., 2020). Samples of water together with the bottom sediment layer were collected into plastic samplers with a volume of 100 ml. Monoclonal strain (Tu-1.1) was cultured in artificially prepared 25‰ sterile water, maintained in 18 °C incubator. Wheat grains were used as the food source.

The ciliates were examined in vivo using bright field and differential interference contrast (DIC) microscopy. Silver nitrate impregnation was used to reveal the infraciliature and the silverline systems (Foissner, 2014). Feulgen staining was used to reveal the nuclear apparatus. Morphometric data were obtained with ImageJ ver.2.1.0 and analyzed with RStudio ver.2021.09.1+build 372 (Ferreira and Rasband, 2012). Measurements were performed under magnifications of 10–40×. Classification of ciliates is given according to Lynn (2008).

Approximately 50 cells were used for DNA extraction. The primers used for PCR amplification were forward 18S F9 (5′–CTGGTTGAT CCTGCAG–3′) and reverse 18S R1513 Hypo (5′–TGATCCTTCGAGTTCC–3′) (Rosati et al., 2004). DNA amplification was done in T100 Thermal Cycler (BioRad, USA). PCR cycles were set as follows: 3 min 94 °C, 35 × (30 s 94 °C, 30 s 55 °C, 2 min 72 °C), 6 min 72 °C (Serra et al., 2020). The new sequence was deposited in the GenBank database with the accession number PP112207.

For estimating the phylogenetic relationships of E. manganari, its SSU rRNA sequence was aligned to homologous sequences of euplotid ciliates available in GenBank. Data set also included seven outgroup sequences of the genera: Aspidisca (Li et al., 2008; Yi et al., 2009); Euplotidium (Petroni et al., 2000); Stylonychia (Bernhard et al., 2001; Hewitt et al., 2003); Phacodinium (Shin et al., 2000); and Gastrocirrhus (Miao et al., 2007). To carry out phylogenetic analysis, we used the facilities of the computing server at the Zoological Institute RAS. Mafft ver.7.490 (Katoh and Standley, 2013) was used to align the sequences and trimAl ver.1.4.1 (Capella-Gutierrez et al., 2009) was used for tree trimming. Maximum likelihood (ML) analysis with 100 bootstrap replicates was calculated with the RAxML ver.8.2.12 (Stamatakis, 2014) using the GTRGAMMA model. Bayesian inference (BI) analysis was carried out with MrBayes ver.3.2.7 (Ronquist et al., 2012). Four Markov chain Monte Carlo (MCMC) were run with 10^6 generations and sampled every 100th cycle, with a burn-in of 25%.

**Results**

**TAXONOMIC SUMMARY**

**Phylum** Ciliophora Doflein, 1901  
**Class** Spirotrichea Butschli, 1889  
**Subclass** Hypotrichia Stein, 1859  
**Order** Euplotida Small et Lynn, 1985  
**Family** Euplotidaceae Ehrenberg, 1838  
**Genus** Euplotes Ehrenberg, 1831  
**Euplotes manganari** sp. n. (Fig. 2; Table 1).
Fig. 2. *Euplotes manganari* sp. n. after silver nitrate impregnation (A, F, G), *in vivo* (B–D), and after Feulgen staining (E). A – Scheme of argyrome and nuclear features drawn from fixed cells; B – population of *E. manganari*, arrows show the dorsal ridges; C – ventral view of a representative specimen; D – dorsal view, arrows show the dorsal ridges, DIC microscopy; E – nuclear apparatus stained after Feulgen; F – dorsal view of a representative specimen after silver nitrate impregnation; G – ventral view after silver nitrate impregnation. **Abbreviations:** AZM – adoral zone of membranelles; B – bristles; CC – caudal cirri; FVC – frontoventral cirri; K – kineties; Ma – macronucleus; MC – marginal cirri; Mi – micronucleus; Pm – paroral membrane; TC – transverse cirri; Vr – ventral ridges. Scale bars: 10 µm.

**Type location:** Sea of Marmara (40°57′54″ N; 28°47′48″ E) near Istanbul, Turkey. Water temperature about 28 °C.

**Type material:** Holotype is represented by of the type culture (accession No ZIN.2024.01), DNA sample (accession No C06), slides containing silver nitrate-impregnated cells (accession No SI-C06.1, SI-C06.2), and Feulgen-stained cells (accession No F-C06.1, F-C06.2) stored with the Culture Collection of Heterotrophic Protists at the Zoological Institute of the Russian Academy of Sciences.

**Data availability:** Slides with silver nitrate-impregnated cells and Feulgen-stained cells of the *E. manganari* sp. n. are available from Zoological Institute RAS (Laboratory of Cellular and Molecular Protistology). DNA sample is available from the author upon request.

**Etymology:** The new species is named in honour of Mikhail Manganari (1804–1887), sailor and researcher, who conducted the first hydrographical studies of the Sea of Marmara.

**18S rRNA gene sequence of the type strain:** GenBank number — PP112207.

**Zoobank LSID of the publication:** urn:lsid:zoobank.org:pub:BAF8F1D9-C0E5-4D99-8ACA-09718BBE4284.

**Zoobank LSID of the species:** urn:lsid:zoobank.org:act:93F69EA9-D5B3-4B15-9189-36F1A493B128.

**Morphological description of *Euplotes manganari* sp. n.**

The average cell size is 30.8 ± 2.5 × 19.6 ± 2.1 µm (*in vivo*), with a length/width ratio of about 1.5. The average cell size after silver nitrate staining is 31.7 ± 2.5 × 26.6 ± 3.4 µm. The body shape is oval in outline and the body ends are smoothly rounded (Fig. 2, B, C). Buccal field is wide and long, extending to 70–80% of body length (Fig. 2, A, C, G). There are 20–25 membranelles in the adoral zone (AZM),

### Table 1. Morphometric data on *Euplotes manganari* sp. n.

<table>
<thead>
<tr>
<th>Morphometric features</th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length (live view), µm</td>
<td>26.2</td>
<td>35.4</td>
<td>30.9</td>
<td>30.8</td>
<td>2.5</td>
<td>8.3</td>
<td>20</td>
</tr>
<tr>
<td>Body width (live view), µm</td>
<td>16.1</td>
<td>23.2</td>
<td>19.6</td>
<td>19.6</td>
<td>2.1</td>
<td>10.7</td>
<td>20</td>
</tr>
<tr>
<td>Body length (after silver nitrate impregnation), µm</td>
<td>26.4</td>
<td>37.3</td>
<td>31.4</td>
<td>31.7</td>
<td>2.5</td>
<td>7.9</td>
<td>20</td>
</tr>
<tr>
<td>Body width (after silver nitrate impregnation), µm</td>
<td>17.3</td>
<td>32.3</td>
<td>27.1</td>
<td>26.6</td>
<td>3.4</td>
<td>12.7</td>
<td>20</td>
</tr>
<tr>
<td>No of AM</td>
<td>20</td>
<td>25</td>
<td>22</td>
<td>22.1</td>
<td>1.4</td>
<td>6.3</td>
<td>13</td>
</tr>
<tr>
<td>No of FVC</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>20</td>
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<tr>
<td>No of DK</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>No of TC</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>No of CC</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2.2</td>
<td>0.4</td>
<td>19.7</td>
<td>20</td>
</tr>
<tr>
<td>No of MC</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Length of Mi, µm</td>
<td>1.0</td>
<td>1.3</td>
<td>1.1</td>
<td>1.1</td>
<td>0.08</td>
<td>7.5</td>
<td>20</td>
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<tr>
<td>Width of Mi, µm</td>
<td>0.8</td>
<td>1.2</td>
<td>0.9</td>
<td>0.9</td>
<td>0.1</td>
<td>9.8</td>
<td>20</td>
</tr>
<tr>
<td>No of bristles in central row</td>
<td>5</td>
<td>7</td>
<td>5.5</td>
<td>5.7</td>
<td>0.8</td>
<td>14.0</td>
<td>20</td>
</tr>
<tr>
<td>No of bristles in leftmost dorsal kinety</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>4.7</td>
<td>0.4</td>
<td>10.0</td>
<td>20</td>
</tr>
<tr>
<td>No of bristles in rightmost dorsal kinety</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4.2</td>
<td>0.4</td>
<td>10.4</td>
<td>20</td>
</tr>
<tr>
<td>Length of AZ, µm</td>
<td>23.7</td>
<td>26.2</td>
<td>24.8</td>
<td>24.8</td>
<td>0.7</td>
<td>2.9</td>
<td>20</td>
</tr>
<tr>
<td>Length of paroral membrane, µm</td>
<td>2.3</td>
<td>2.9</td>
<td>2.5</td>
<td>2.5</td>
<td>0.1</td>
<td>7.2</td>
<td>8</td>
</tr>
</tbody>
</table>

Notes: AM — adoral membranelles; AZ — adoral zone; CC — caudal cirri; CV — coefficient of variation, %; DK — dorsal kineties; FVC — frontoventral cirri; MC — marginal cirri; Max — maximum; Mi — micronucleus; Min — minimum; n — number of cells measured; SD — standard deviation; TC — transverse cirri.
starting at the top of the cell (where no peristomial collar is visible) and continuing down the left side in a regular curve (Fig. 2, G). Paroral membrane about 2.3–2.9 µm (Fig. 2, G). The contractile vacuole is not visible. Cytoplasm is colorless and transparent, without endosymbionts. The macronucleus (Ma) usually mirror-inverted, C-shaped, with rounded ends (Fig. 2, A, E). The micronucleus (Mi) is single (average size 1.1 × 0.9 µm) and spherical (compact type), usually localized in the cavity of Ma (Fig. 2, A, E). The dorsal argyrome (dargyrome) is of the double-patella type, with two unequal rows of roughly rectangular alveoli between each pair of dorsolateral kineties (Fig. 2, A, F). Five dorsal kineties (K), mid-dorsal kinety with five to seven bristles, leftmost and rightmost kineties consisting of four or five bristles (Fig. 2, F). The dorsal surface is decorated with six conspicuous ridges (Fig. 2, D). The ventral side with three ridges (Fig. 2, C). There are 10 normal-sized frontoventral cirri (FVC), five close-set transverse cirri (TC), two or three well-developed caudal cirri (CC), and two marginal cirri (MC) (Fig. 2, A, G). Euplotes manganari was collected from the sandy littoral sediments of a coastal habitat near Qingdao (China) is very similar to E. manganari from the sandy littoral sediments of a coastal habitat near Istanbul (Sea of Marmara).

**Molecular data and phylogenetic analysis**

The 18S rRNA gene sequence of E. manganari sp. n. obtained with the help of PCR was 1580 bp long with a G+C content of 45.8%. In the BLAST analysis, the E. manganari sequence shows the closest relatedness to the following sequences: E. plicatum (Accession number: EF094966) – 98.93%; E. orientalis (Accession number: FJ875138) – 98.55%; E. balteatus (Accession number: EF094966) – 98.93%; E. orientalis sp. n. – 98.17%. The SSU rRNA sequence shows the cloest relatedness with E. manganari sp. n. and warrants its description as a novel taxon (Table 2).

**Discussion**

As noted above, Euplotes spp. possess a number of clear morphological features, and euplotids can be easily identified in the ciliate assemblages even by a novice ciliatologist. However, according to Syberg-Olsen (2016), some of these features are of low taxonomic value. For instance, a C-shaped macronucleus is characteristic of all Euplotes spp. except E. woodruffi Gaw, 1939. The ventral cirri are usually represented by five transverse and four caudal cirri, including two marginal ones. Thus, the most reliable characters for identification of Euplotes spp. are the cell size, the type of dargyrome, the number of dorsal kineties, and the topography and the number of frontoventral cirri.

The combination of morphological features, namely, a small-size (about 30 µm), the type of dargyrome (double-patella), the number of dorsal kineties (five), the cirral pattern (10 normal-sized FVC and two or three CC) and the number of bristles in mid-dorsal row (five–seven) is unique to Euplotes manganari sp. n. and warrants its description as a novel taxon (Table 2).

**Comparison with related congeners**

Euplotes orientalis described by Jiang et al (2010) from the sandy littoral sediments of a coastal habitat near Qingdao (China) is very similar to E. manganari sp. n. in the cell size, the type of dargyrome (it is the only closely related species that has dargyrome of the same type), the number of membranelles in adoral zone, and the number of bristles in middle dorsal row. However, these two species can be distinguished by several morphological features, namely, the number of dorsal kineties (six–seven vs. five), the number of dorsal ridges (five–six vs. six), and the number of CC (two vs. two–three). Besides, the authors mention that E. orientalis has eight normal-sized FVC and two “highly reduced basal plaques” (Jiang et al., 2010). Euplotes manganari sp. n. has 10 normal-sized FVC.

Euplotes plicatum (Accession number: FJ875138) – 98.55%; E. balteatus (Accession number: EF094966) – 98.93%; E. orientalis sp. n. – 98.17%. The SSU rRNA sequence shows the cloest relatedness with E. manganari sp. n. and warrants its description as a novel taxon (Table 2).

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E. balteatus (Dujardin, 1841) Kahl, 1932 from the sandy littoral sediments of a coastal habitat near Qingdao (China) is very similar to E. manganari sp. n. in the cell size, the type of dargyrome (it is the only closely related species that has dargyrome of the same type), the number of membranelles in adoral zone, and the number of bristles in middle dorsal row. However, these two species can be distinguished by several morphological features, namely, the number of dorsal kineties (six–seven vs. five), the number of dorsal ridges (five–six vs. six), and the number of CC (two vs. two–three). Besides, the authors mention that E. orientalis has eight normal-sized FVC and two “highly reduced basal plaques” (Jiang et al., 2010). Euplotes manganari sp. n. has 10 normal-sized FVC.

Euplotes plicatum (Accession number: FJ875138) – 98.55%; E. balteatus (Accession number: EF094966) – 98.93%; E. orientalis sp. n. – 98.17%. The SSU rRNA sequence shows the cloest relatedness with E. manganari sp. n. and warrants its description as a novel taxon (Table 2).
Fig. 3. Phylogenetic position of *Euplotes manganari* sp. n. based on SSU rRNA gene sequence data. Numbers at nodes represent the bootstrap values of the ML analysis and the posterior probability of the Bayesian inference (BI) analysis. Fully supported (100%/1.00) branches are marked with solid circle. Values below 70/0.90 are not shown.

(31–39 vs. 20–25), more bristles in mid-dorsal kinety (10–13 vs. five–seven), and the type of dargyrome (double-*eurytomus* vs. double-*patella*). *Euplotes alatus* Kahl, 1932 is a very rare species. There is only one modern reinvestigation of *E. alatus* using silver impregnation (Alekperov et al., 2006). It should be noted that the modern morphological description of this species differs somewhat from the original description. It is indicated that the cell size is 20–30 µm, while in the original description it is 40×30 µm. The number of membranelles in the adoral zone is 40–45 vs. ~26 in the original description.
Table 2. Morphological comparison of *Euplotes manganari* sp. n. and five similar species.

<table>
<thead>
<tr>
<th>Features</th>
<th><em>E. manganari</em> sp. n.</th>
<th><em>E. plicatum</em></th>
<th><em>E. orientalis</em></th>
<th><em>E. balteatus</em></th>
<th><em>E. alatus</em></th>
<th><em>E. bisulcatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Size, μm (mean)</td>
<td>31×19</td>
<td>48×32</td>
<td>36×26</td>
<td>85×63</td>
<td>40×30 20×30</td>
<td>40×30</td>
</tr>
<tr>
<td>Shape</td>
<td>oval</td>
<td>oval</td>
<td>oval</td>
<td>ellipsoidial</td>
<td>oval</td>
<td>oval</td>
</tr>
<tr>
<td>Peristome</td>
<td>3/4 of body length</td>
<td>2/3 of body length</td>
<td>2/3 of body length</td>
<td>3/4 of body length</td>
<td>2/3 of body length</td>
<td>2/3 of body length</td>
</tr>
<tr>
<td>No of Dr</td>
<td>6</td>
<td>7–8</td>
<td>5–6</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>No of bristles in central row</td>
<td>5–7</td>
<td>14</td>
<td>6–8</td>
<td>10–13</td>
<td>10–12</td>
<td>5–7</td>
</tr>
<tr>
<td>No of FVC</td>
<td>10</td>
<td>10</td>
<td>8+2</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>No of CC</td>
<td>2–3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4–5</td>
<td>3</td>
</tr>
<tr>
<td>Type of dargyrome</td>
<td>double-patella</td>
<td>double-eurystomus</td>
<td>double-patella</td>
<td>double –eurystomus</td>
<td>double –eurystomus</td>
<td>double –eurystomus</td>
</tr>
</tbody>
</table>

**Notes:** AM – adoral membranelles; CC – caudal cirri; DK – dorsal kineties; Dr – dorsal ridges; FVC – frontoventral cirri; ND – not described.

However, in both descriptions *E. alatus* differs from *E. manganari* in the following characters: the type of dargyrome (double-eurystomus vs. double-patella), the number of dorsal kineties (eight vs. five), and the number of bristles in the mid-dorsal kinety (10–12 vs. five—seven). Besides, both descriptions of *E. alatus* do not provide information on the number of dorsal ridges.

*Euplotes bisulcatus* Kahl, 1932 is similar to *E. manganari* in the cell size and the number of bristles in the middle dorsal row. It differs from *E. manganari* in the number of FVC (nine vs. eight), the number of dorsal kineties (eight vs. five), the number of membranelles in adoral zone (~17 vs. 20–25), and the type of dargyrome (double-eurystomus vs. double-patella).

Acknowledgements

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