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Free-living Heterotrophic Flagellates (Protista) from Two Hypersaline Lakes in Turkey

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Abstract: This study was carried out in two hypersaline lakes (Acı and Meke Lakes) in Turkey to understand the diversity and geographic distribution of free-living heterotrophic flagellates. Heterotrophic flagellates of hypersaline environments have not previously been studied in Turkey. We found seventeen morphospecies of heterotrophic flagellates with one unidentified protist. The observed species belong to Craspedida, Heterolobosea, Apusomonadida, Neobodonida, Bicosoecida and Protista *incertae sedis*. Of the 17 species, ten species were new records for Turkey. All of the morphospecies described here except one unidentified protist were previously reported elsewhere and appear to be cosmopolitan.

Key words: Protista, heterotrophic flagellates, hypersaline lakes, biodiversity, cosmopolitan.

INTRODUCTION

Microbial communities in aquatic ecosystems have an important role in carbon and nutrient cycling (Azam et al. 1983, Pomeroy et al. 2007, Özen et al. 2018). Among these eukaryotes, heterotrophic flagellates serve as an important trophic linkage to higher foodweb (Azam et al. 1983, Arndt et al. 2000, Lee and Patterson 2002). Heterotrophic flagellates are also known to be of great importance in the evolutionary history of eukaryotes (Fenchel 1986, Cavalier-Smith 2000).

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Recently, there has been an increasing trend of published articles on the heterotrophic flagellates systematics and diversity (e.g., Al-Qassab et al. 2002; Lee 2002, 2006a, 2006b, 2008, 2012, 2015; Schroeckh et al. 2003, Weitere and Arndt 2003, Lee et al. 2005; Tikhonenkov et al. 2006, 2015; Tikhonenkov and Mazei 2008, Aydın and Lee 2012, Jeuck and Arndt 2013; Prokina et al. 2017a, b). Compared to the marine and freshwater ecosystems, the studies of hypersaline environments are relatively limited. There has been several debates about the existence of eukaryotes in hypersaline environments (e.g., Ramos–Cormenzana 1991, Pedrós–Alió et al. 2000). However, several studies proved that many protozoa exist in hypersaline habitats and have an important role as primary consumers (Namyslowski 1913, Kirby 1932, Ruinen 1938, Ruinen and Bass-

Becking 1938, Volcani 1944, Post et al. 1983, Patterson and Simpson 1996, Hauer and Rogerson 2005; Park et al. 2006, 2007, 2009; Cho et al. 2008; Bardavid et al. 2008; Oren 2008, 2011; Park and Simpson 2010).

Hypersaline environments have a sporadic distribution both in the northern and eastern hemispheres (Javor 2012). Thus, the knowledge of the diversity and systematics of saline habitat protists were based on the sporadically performed studies over the past years. Systematic studies from hypersaline environments were restricted to Namyslowski (1913), Ruinen (1938), Post et al. (1983) and Patterson and Simpson (1996) till 2000s. Further studies were conducted by Park and coworkers (Park et al. 2006, 2007, 2009, 2010, 2011; Jhin and Park 2018). These were reviewed by Cho, Harding and Simpson, Hauer and Rogerson and Park and Simpson (Cho 2005, Hauer and Rogerson 2005, Park and Simpson 2015, Harding et al. 2018).

However, the endemism level of protists is a controversial topic (Finlay and Fenchel 2004, Foissner 2006, Boenigk et al. 2012). Providing data from previously unstudied geographies is quite important to fill the gaps in the field of protist endemism. Within this context, a series of studies were carried out in Turkey one of which was published in 2012 (Aydın and Lee 2012). The main objectives of this study were to document the diversity of heterotrophic flagellates in hypersaline habitats and to use that information to address issues of endemism in this group.

MATERIALS AND METHODS

The study was carried out in two hypersaline maar inland lakes (Meke and Acı Lakes), which are located in the Konya Closed Basin in Central Anatolia of Turkey for a year (May 2010 to April 2011) (Fig.1). These lakes were mostly affected by the semi-arid climatic conditions according to the Köppen Climate Classification (Kottek et al. 2006). The basin was "extremely dry" according to Standardized Precipitation Index (SPI) (https://www.mgm.gov.tr/FILES/genel/makale/standartyagis.pdf).

Lake Meke (37°41'07.3"N 33°38'17.8"E) is a crater lake with double cone surrounded by a heavy black ash type soil around 0.5 km^2 surface area with a maximum depth of 12 m (İnandık 1965, Anonymous 2000) and contains high magnesium, sodium and sulfate ions. The lake is listed in the RAMSAR Index in 2005 as the 1st Degree Natural/ Historic Site because of its unique geological formation. Unfortunately, due to the misutilization of ground waters, water level drops triggering the decrease in water quantity (Hekimoğlu and Altındeğer 2008).

Acı Lake (37°42'45.0"N 33°39'55.8"E) has an area of 1.1 km^2 and fills the depression of a vertically edged maar (İnandık 1965). Although, the depth of the lake is not clear, it is thought to be approximately 100 m (Biricik 1992). The lake is fed only by groundwater dripping off calcareous rocks and there is no surficial outflow. Thus, it contains saline water (Cirik and Cirik 1999, Tavsanoglu et al. 2015). We sampled one station per lake monthly from May 2010 to April 2011 (n = 12).

The physical variables were determined *in situ*. The temperature and salinity, the dissolved oxygen concentration and the pH of the water were measured with YSI 33 probe, YSI 55 probe and Thermo Orion 230A, respectively. During the study, the water temperature was 1–23.5 °C (Avg. 16.4 °C) in Acı Lake and 0–23 °C (Avg. 15.6 °C) in Meke Lake. The salinity was 24–67.5 psu (Avg. 53.9 psu) in Acı Lake and 108.5–215 psu (Avg. 168 psu) in Meke Lake. The dissolved oxygen concentrations were 3.75–13.2 mg/L (Avg. 6.22 mg/L) in Acı Lake and 0.25–5.18 mg/L (Avg. 1.42 mg/L) in Meke Lake. The pH of the Meke Lake was 6.9–7.3 and Acı Lake was 6.8–7.9.

The samplings were performed on the subsurface sediments at around 1 cm depth of a 1x1 m quadrat. The samples were processed according to Lee and Patterson (2000). After the collection of the sediments, macrofauna and plant material were removed and the residues were placed in 1 cm depth trays to settle down for several hours. Excess water drained from the residues and lens cleaning tissues (Filter-Lab Cod. CP148295135) were laid on the subsurface of the sediments and placed under coverslips (No.1 22 × 22 mm). After 12–24 hrs, coverslips were removed and observations were carried out. The samples were maintained at room temperature (~ 20 °C) for 5–7 days. Coverslips were removed for the observation of flagellates every day. A Leica DMR microscope equipped with a digital camera (Nikon D90 Model) was used to take the images and the movies of the flagellates. Specimens were also drawn.

RESULTS

Nomenclature follows the International Code of Zoological Nomenclature (International Commission of Zoological Nomenclature, 1999).

Craspedida Cavalier-Smith 1997, emend. Nitsche et al. 2011

Salpingoecidae Kent 1880–1882, emend. sensu Nitsche et al. 2011

Codosiga botrytis (Ehrenberg 1838) Kent 1880 (Figs 2f, 3f)

Cells are 5.5–6 μm long and ovoid with a short collar and a long stalk. The pseudopodia are symmetrical and longer than the cell. The thickened flagellum is 2–2.5 times the cell length and acronematic. The cells may attach to the substrate by the stalk and swim. Observations based on 3 colonies with 12 cells. Occurance: every month at Acı Lake, temperature 1–23.5 °C, salinity 24–67.5 psu, dissolved oxygen 3.75–13.2 mg/L.

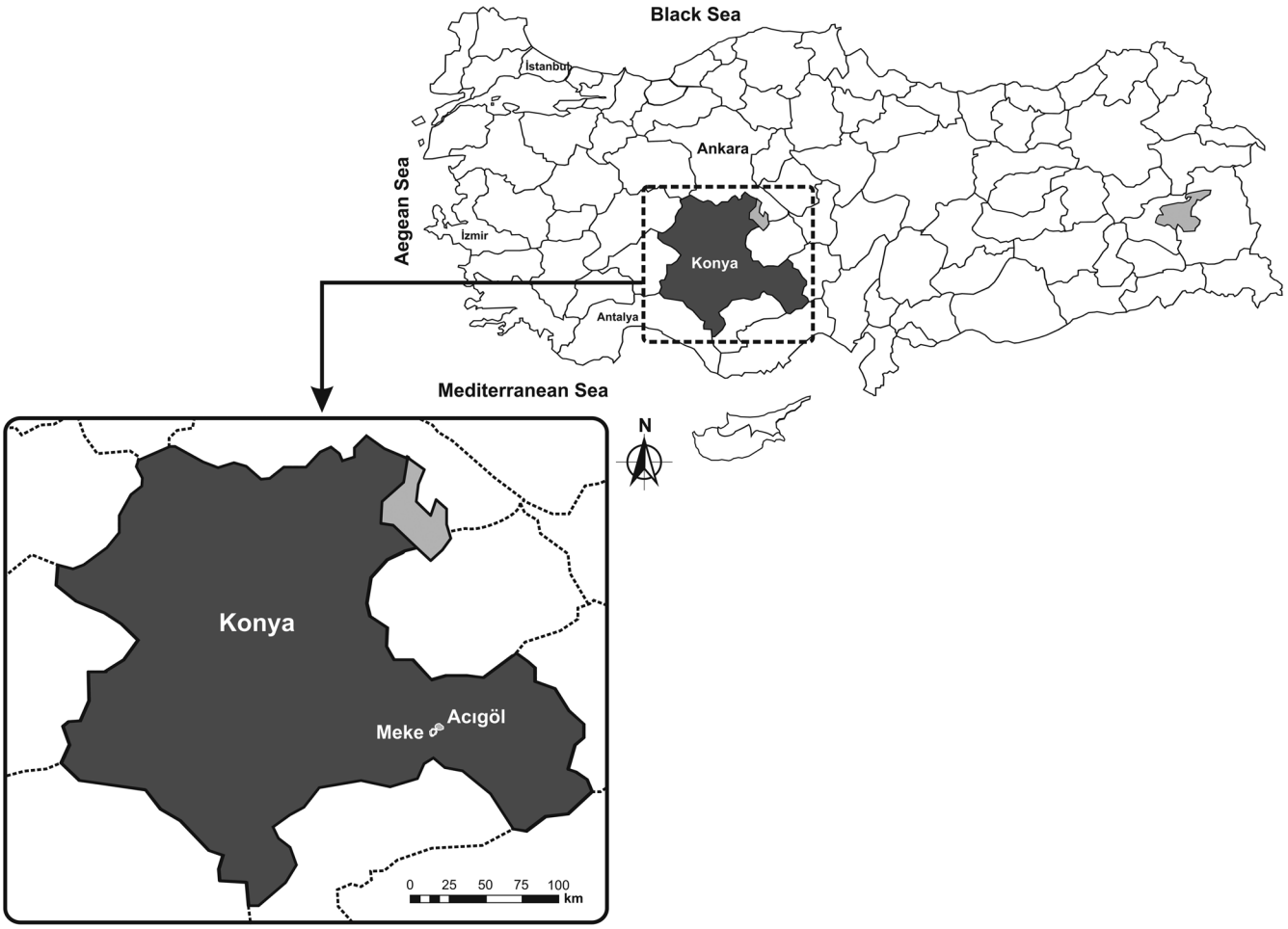

Fig. 1. Map of study sites.

Remarks: Choanoflagellates have been revised based on the molecular phylogenetic studies (Carr et al. 2008, Nitsche et al. 2011). The genera of Codosigidae were placed under Salpingoecidae *sensu* Nitsche et al. (2011). Generally, the observations are in accordance with those of Hibberd (1975) and Vørs (1992a). It was reported that the taxonomy of *Codosiga* is likely to be confused (Vørs 1992a) with *Monosiga*. *Codosiga botrytis* includes taxa with thin and thick stalks, but is distinguished primarily by the formation of unique colonies. Single celled forms of the genera may be indistinguishable from the genus *Monosiga* (Leadbeater and Morton 1974). This species was also found at marine sites in eastern North America, Denmark, Gulf of Finland, Germany and Italy (Griessmann 1913, Norris 1965 as *Codosiga pyriformis*, Hibberd 1975; Vørs 1992a, b).

***Monosiga brevicollis* Ruinen 1938 (Figs 2o, 3o)**

Cells are 4–6 μm long and spherical in the resting state. Swimming cells are more ovoid with a sharpened posterior end and a spread out collar. The collar is about 0.5 times the cell length, while the flagellum is 4–5 times the cell length. The cytoplasm is fine-grained to hyaline and contains strongly retractile grains with a vacuole lying in the posterior part of the cell. Description based on the observations of 16 cells. Occurance: every month with the exception of July and August at Meke Lake, temperature 0–21 °C, salinity 107.5–215 psu, dissolved oxygen 0.25–5.18 mg/L.

Remarks: As mentioned before by Tong et al. (1997), the species identities of this genus are not clear. Their shapes and lengths may change according to swimming or resting states (Tong et al. 1997). In general, our observations are in agreement with the origi-

nal description of Ruinen (1938). Four species, of three by Ruinen (1938) and one by Post et al. (1983), were reported from hypersaline environments: i) *Monosiga consociatum* Kent, ii) *M. ovata* Kent, iii) *M. brevicollis* Ruinen, and iv) *Monosiga* sp. *Monosiga brevicollis* differs from *M. consociatum* with its unique cell shape, short collars and more or less shorter flagellum. The extension of the collar is wider at *M. brevicollis* compared to *M. consociatum*. *Monosiga brevicollis* resembles *M. ovata* in general cell shape, but the length of flagellum and the collar are quite different. The collar is shorter and the flagellum is longer than that of *M. brevicollis'* (Throndsen 1974). *Monosiga brevicollis* is reported from Portugal to India (Ruinen 1938), as well as eastern and south-eastern Norway (Throndsen 1974).

***Salpingoeca marina* James–Clark 1867 (Figs 2j, 3p)**

Cells are 5–7 µm long with a pedicel, which is about 1.5 times the cell length. The thin lorica is ovoid and slightly pointed at the posterior end which is connected to the pedicel, and has a short neck at the anterior end. Cells are filling out the posterior part of the lorica. The thickened flagellum is 1–1.5 times the cell length and is surrounded by the pseudopodial tentacles. The lorica attaches to the substrate by the pedicel. Observations based on 19 cells. Occurance: every month at Acı Lake, temperature 1–23.5 °C, salinity 24–67.5 psu, dissolved oxygen 3.75–13.2 mg/L.

Remarks: The genus *Salpingoeca* differs from the other loricated flagellates by having a single theca, which comprises only of the cell, lacking silicified costae, by being closed at posterior end, by being sedentary and by not forming colonies (Trondsen 1974, Thomsen and Buck 1991). Species of the genus is discriminated according to the morphology of theca (Vørs 1992a). *Salpingoeca marina* is very similar to *S. infusionum* Kent 1880, but it is distinguished by the stiffness of the lorica and because the lorica of *S. infusionum* is slightly wider anteriorly than that of *S. marina* (Tong 1997a). *Salpingoeca marina* is reported from Antarctica, North Atlantic, subtropical Australia, England, Denmark, Gulf of Finland, France, Germany, Turkey and USA (James-Clark 1867, Griessmann 1913; Wailes 1929, 1939; Ruinen 1938; Vørs 1992a, b; Patterson et al. 1993; Tong 1997a, b; Tong et al. 1998, Aydın and Lee 2012).

Heterolobosea Page and Blanton 1985

***Pleurostomum flabellatum* Ruinen 1938 (Figs 2h, 3l-m)**

Cells are 18–30 µm long, flattened, somewhat elongate and narrower posteriorly. The synchronously moving two flagella are 1.5–2 times the cell length and emerge apically beneath the rostrum. Because of the rostrum, flagella seemed to emerge subapically. Both flagella move in close contact with lamella. The cytostomal structure originates near the point of flagellar insertion and extends in a spiral to the posterior end of the cell. The extension of the cytostomal structure gives the cell its spiral shape. A vacuole is located posteriorly. The cells swim slowly by making spirals. Description based on observations of 16 cells. Occurance: every month with the exception of July and August at Meke Lake, temperature 0–21 °C, salinity 107.5–215 psu, dissolved oxygen 0.25–5.18 mg/L.

Remarks: The genus *Pleurostomum* contains 5 species and is characterized by two parallel homodynamic subapically (and also apically in regard to former descriptions) emerging flagella and by its cytostomal groove. Generally, our observations are in accordance with the original description of Ruinen (1938) with respect to cell length and general appearance of the cell. The only difference is the point of flagellar insertion. According to Ruinen (1938) both flagella emerge apically, whereas in our cells the flagella emerged subapically. *Pleurostomum flabellatum* is similar to *P. salinum*, which was reported with the cell length of 20–22 µm and with a cytostomal groove extending 2/3 of the cell (Namyslowski 1913, Ruinen 1938), but can be distinguished because the cytostomal groove of *P. flabellatum* extends almost to the end of the cell and is relatively longer. The cells of *Pleurostomum flabellatum* reported by Patterson and Simpson (1996) and Park et al. (2007) are smaller than the original cells (16–30 µm) of Ruinen (1938) and the cells (18–30 µm) observed here: Patterson and Simpson, 11–14 µm; Park et al., 10–14 µm. Their cells resemble *P. salinum* Namyslowski 1913 because of the cell length and general appearance. Further studies are required to establish the identities of these taxa.

Park et al. (2009) reported a new amoeboid species *Tulamoeba peronaphora*, a close relative of *Pleurostomum flabellatum* in the 18S rRNA gene tree, but can be easily distinguished because of their morphology and because *Tulamoeba peronaphora* does not have a flagellated stage. *Pleurostomum flabellatum* and *Tu-*

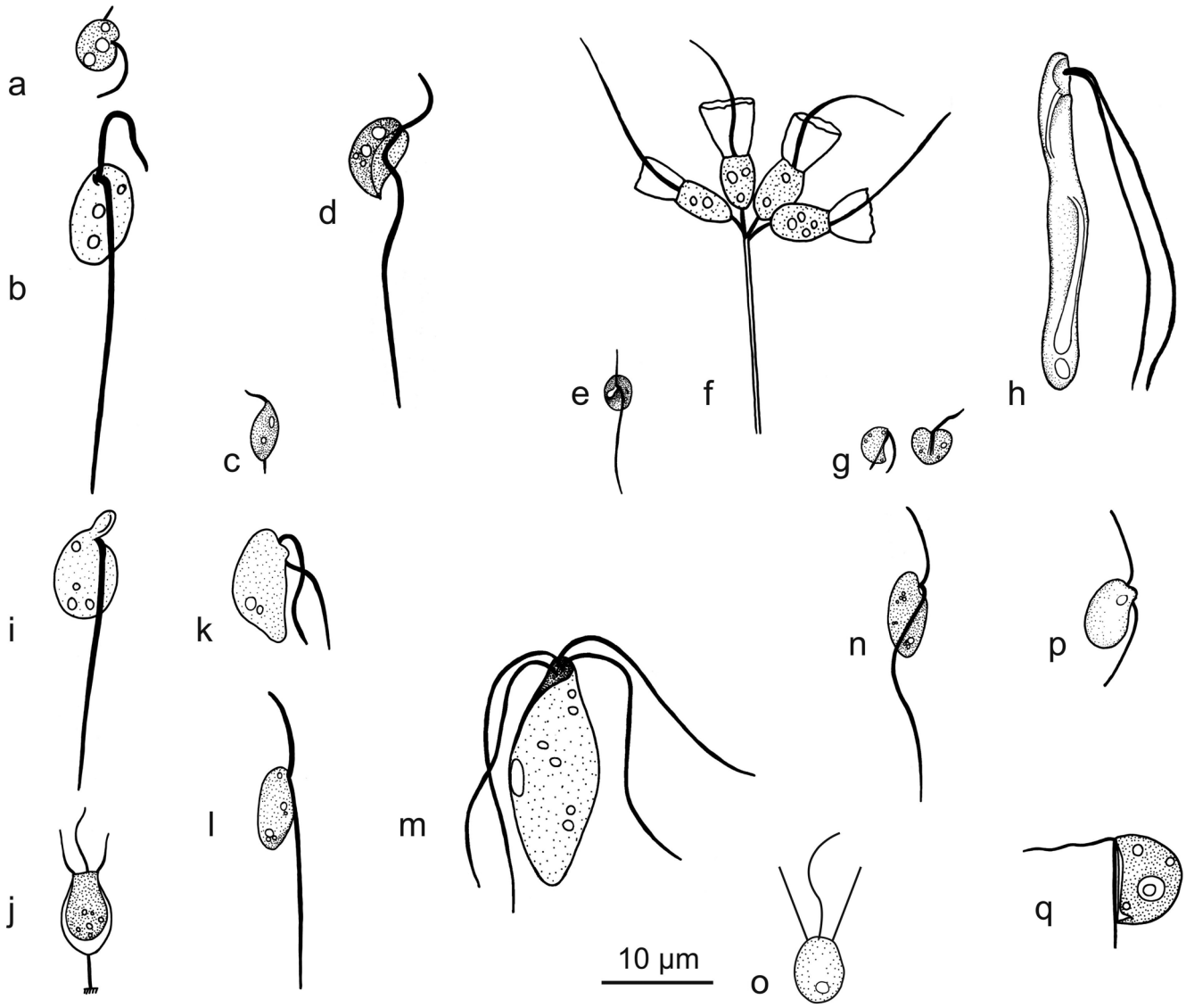

Fig. 2. (a) *Ancyromonas sigmoides*, (b) *Neobodo curvifilus*, (c) *Chelonemonas* sp., (d) *Carpediemonas membranifera*, (e) *Caecitellus parvulus*, (f) *Codosiga botrytis*, (g) *Cafeteria roenbergensis*, (h) *Pleurostomum flabellatum*, (i) *Rhynchomonas nasuta*, (j) *Salpingoeca marina*, (k) *Pendulomonas adriperis*, (l) *Neobodo saliens*, (m) unidentified protist, (n) *Neobodo designis*, (o) *Monosiga brevicollis*, (p) *Halocafeteria seosinensis*, (q) *Cantina marsupialis*.

lamoeba bucina Kirby et al. 2014's flagellated stage resemble each other, but can be distinguished by the paths of their cytostome: *P. flabellatum* has a cytostome extending along the body spirally and *T. bucina* has a cytostome curling around the lateral axis. *Pleurostomum flabellatum* has been reported from hyposaline habitats in Australia, India, Korea and Poland (Namyslowski 1913, Ruinen 1938, Patterson and Simpson 1996, Park et al. 2007).

Apusomonadida Karpov and Mylnikov 1989

***Chelonemonas* sp. (Figs 2e, 3e)**

Cells are 5–7 µm long, dorso-ventrally flattened and flexible. A laterally directed sleeve like protrusion extending anteriorly is 2–3 µm long and anterior flagellum emerges from the tip of the sleeve and beats in a small angle. The posterior flagellum is slightly longer than the cell length, lying in a groove along the margin of the cell, trailing under the cell and on occasion

protruding behind the cell. Cytoplasmic extension like strand occurs posteriorly which may be drawn out behind the cell. The nucleus is located at the anterior left of the cell. Description based on observations of 4 cells. Occurance: July to October at Acı Lake, temperature 18–23.5 °C, salinity 37–54 psu, dissolved oxygen 3.75–5.3 mg/L.

Remarks: The genus *Chelonemonas* was created by Heiss et al. (2015) and consists of 2 nominal species (*C. geobuk, C. masanensis*) (see Heiss et al. 2015 for more details). These two species can not be distinguished by light microscopy, alone.

Neobodonida Vickerman in Moreira *et al.* 2004

***Neobodo curvifilus* (Greissmann 1913) Moreira *et al*. 2004 (Figs 2b, 3h)**

Cells are 8–10 µm long and bean shaped. Two flagella insert subapically: one extends anteriorly and the other one extends posteriorly. The anterior flagellum is curved inward at the tip and the acronematic posterior flagellum is about 2.5 times the cell length. The cells move by gliding. Description is based on the observations of 13 cells. Occurance: every month at Acı Lake, temperature 1–23.5 °C, salinity 24–67.5 psu, dissolved oxygen 3.75–13.2 mg/L.

Remarks: The description shows compatibility with Lee and Patterson (2000). *Neobodo curvifilus* is distinguished from other species of *Neobodo* and *Bodo* by the curved shape and the paddling beat of the anterior flagellum. This species has similar features with *Bordnamonas tropicana* Larsen and Patterson 1990, but it can be distinguished by its much curved anterior flagellum, the acronematic posterior flagellum, quick movement, and by the visible mouth. A detailed discussion of *Neobodo curvifilus* is given in Tikhonenkov et al. (2016), in which the similarities of *N. curvifilus* with *Procryptobia sorokini* Frolov et al. 2001 and *Parabodo caudatus* are discussed. *Procryptobia sorokini* and *N. curvifilus* resemble in general cell shape, cell length, having curved shape and paddling beat of anterior flagellum. The morphological differences mentioned by Frolov et al. (2001) are not enough to differ these two species because *Neobodo curvifilus* was also reported with an attached posterior flagellum. We are of the view that *Procryptobia sorokini* is a junior synonym of *Neobodo curvifilus*. *Parabodo caudatus* and *N. curvifilus* also resemble each other. Compared to *N. curvifilus*, *Parabodo caudatu*s is more plastic and bigger. The anterior flagellum of *P. caudatus* beats with a paddling motion over the cell, while the anterior flagellum of *N. curvifillus* makes a movement like that of a beckoning index finger, as reported by Lee et al. (2003). Furthermore, *P. caudatus* has a prominent apical mouth and *N. curvifilus* also has a visible mouth, not prominent (Lee et al. 2003, 2005). *Neobodo curvifilus* has been described in marine sites in Antarctica, Arctic, north Atlantic, northeast Atlantic, Australia, Denmark, West Greenland and Norway, with cell lengths ranging from 4 to 12 µm mostly under the name of *Bodo curvifilus* (Griessmann 1913, Throndsen 1969, Turley and Carstens 1991; Vørs 1992a, b; Patterson et al. 1993, Tong et al. 1997, Lee 2015).

***Neobodo designis* (Skuja 1948) Vickerman 2004 (Figs 2n, 3i)**

Cells are 4–7 µm long, somewhat flexible and usually elliptical with two unequal flagella emerging from a subapical pocket. The anterior flagellum is about the length of the cell or slightly shorter and curved back over the rostrum. The anterior flagellum is folding around the anterior part of the cell when the cell is feeding. The acronematic posterior flagellum is 2–4 times the length of the cell and somewhat curved while swimming. The nucleus is located close to the midline of the cell. The cells rotate around their longitudinal axes while swimming. Description based on observations of 22 cells. Occurance: every month at Acı Lake, temperature 1–23.5 °C, salinity, 24–67.5 psu, dissolved oxygen 3.75–13.2 mg/L.

Remarks: *Neobodo designis* has been characterized by the rotating behavior of swimming cells, but *Neobodo cygnus* reported by Patterson and Simpson (1996) and *N. platyrhynchus* reported by Lee and Patterson (2000) also have a rotating swimming movement. *Neobodo cygnus* may also wrap the anterior flagellum around the anterior end of the cells just like *Neobodo designis,* may be distinguished from *N. designis* by having a spiral groove. Further study is required to establish the identities of these taxa. This species has been reported from the world in marine and freshwater environments.

***Neobodo saliens* (Larsen and Patterson 1990) Moreira *et al.* 2004 (Figs 2l, 3j)**

Cells are 4–12 µm long, elongate elliptical and somewhat inflexible. Two flagella of unequal in length emerge subapically form a shallow pocket. The anterior flagellum seems to be inactive, along the cell and helds forward with a single anterior curve. The acronematic posterior flagellum is typically directed straight behind

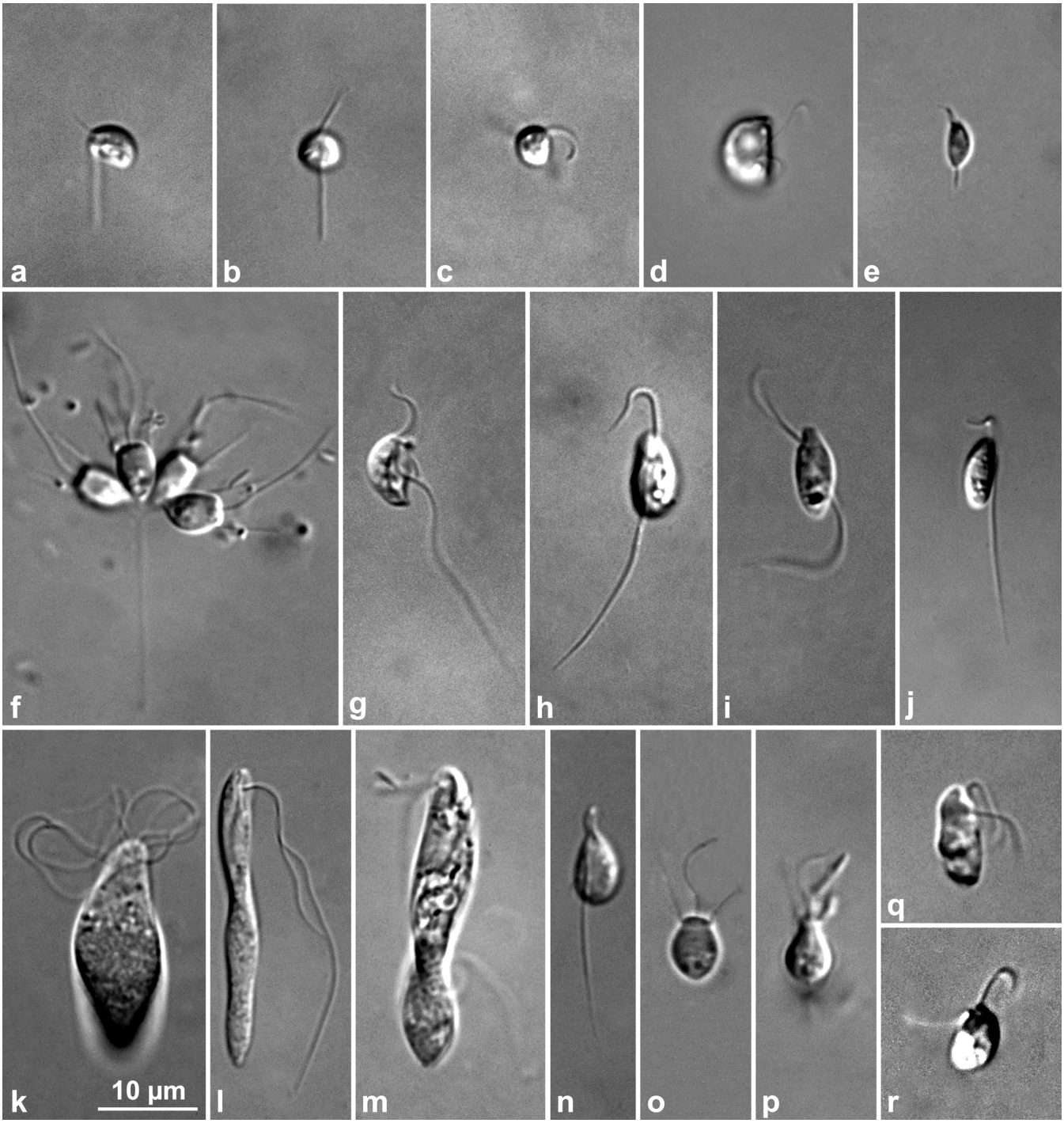

Fig. 3. (a) *Ancyromonas sigmoides*, (b) *Caecitellus parvulus*, (c) *Cafeteria roenbergensis*, (d) *Cantina marsupialis*, (e) *Chelonemonas* sp., (f) *Codosiga botrytis*, (g) *Carpediemonas membranifera*, (h) *Neobodo curvifilus*, (i) *Neobodo designis*, (j) *Neobodo saliens*, (k) unidentified protist, (l)-(m) *Pleurostomum flabellatum*, (n) *Rhyncomonas nasuta*, (o) *Monosiga brevicollis*, (p) *Salpingoeca marina*, (q) *Pendulomonas adriperis*, (r) *Halocafeteria seosinensis*. All micrographs are DIC images. Scale bar in (k) represents for all figures.

the cell and is 2.2–3.5 times longer than the cell length. The cells swim in straight lines with rapid darts as mentioned elsewhere. Description based on observations of 20 cells. Occurance: every month at Acı Lake, temperature 1–23.5 °C, salinity 24–67.5 psu, dissolved oxygen 3.75–13.2 mg/L.

Remarks: *Neobodo saliens* is distinguished from other species of the genus *Neobodo* by its rapid darting movement and the unique posterior flagellum directed in a straight line while swimming. This species is similar to *Neobodo curvifilus*, but is easily distinguished by the unique paddling anterior flagellum of *N. curvifilus*, which is curved along its entire length. *Neobodo saliens* also differs from *Neobodo designis*. *Neobodo designis* has both an anterior flagellum which coils up around the anterior part of the cell and a posterior flagellum, which has a sinuous form while swimming. *Neobodo saliens* has an anterior flagellum held forward, curved back at the tip with an angle and a posterior flagellum directed straigth behind the cell. While swimming, *N. designis* rotates around its longitudinal axes, whereas *N. saliens* makes sudden darts in a straight line. This species appears to be cosmopolitan.

***Rhynchomonas nasuta* Klebs 1893 (Figs 2i, 3n)**

Cells are 3.5–6 μm long, flattened and flexible. The cells are with a bulbous motile snout which beats slowly. The mouth is located on the bulbous motile snout. The anterior flagellum lies alongside the snout and is hard to see, and the acronematic, trailing posterior flagellum is 2–3 times the cell length. The cells move by gliding. Description based on observations of 45 cells. Occurance: every month at Acı Lake, temperature 1–23.5 °C, salinity 24–67.5 psu, dissolved oxygen 3.75–13.2 mg/L.

Remarks: This is a well-known species with a bulbous snout. This species has been reported from diverse environments worldwide (e.g., Lee and Patterson 1998, 2000; Lee 2015), including hypersaline habitats in Australia (Post et al. 1983, Patterson and Simpson 1996).

Bicosoecida Grasse´ 1926, emend. Karpov 1998

***Caecitellus parvulus* (Figs 2e, 3b)**

Cells are 3–4 μm long and somewhat triangular or rounded. The mouth is protruding ventrally on the left side of the cell. The cell is two flagella of unequal length. The acronematic anterior flagellum is beating slowly while inserting apically. It is slightly longer than the cell length. The non-acronematic posterior flagellum is about 2.5 times the cell length, and is merging from the ventral face of the cell and trailing posteriorly. The cells are gliding slowly by the anterior flagellum in close contact with the substrate. Description is based on the observations of 30 cells. Occurance: every month at Acı Lake, temperature 1–23.5 °C, salinity 24–67.5 psu, dissolved oxygen 3.75–13.2 mg/L.

Remarks: Generally, the observations are consistent with the findings of Griessmann (1913) and Larsen and Patterson (1990) under the name *Bodo parvulus* which was transferred to *Caecitellus parvulus* by Patterson et al. (1993). Later, Hausmann et al. (2006) carried out an ultrastructural, molecular study and established two new species – *Caecitellus paraparvulus* and *C. pseudoparvulus* – that can only be distinguished from *Caecitellus parvulus* by ultrastructural and molecular characters (Hausmann et al. 2006 for more details). Thus, in this study, we prefer to follow the criteria described by the previous works (Larsen and Patterson 1990, Lee and Patterson 2000, Al-Qassab et al. 2002) for this morphospecies. *Caecitellus parvulus* is characterized by the protruding mouth on the ventral side of the cell, the beating pattern of the anterior flagellum and the flagellar insertion (Larsen and Patterson 1990, Lee and Patterson 2000). This species is similar to *Glissandra innurende* Patterson and Simpson 1996 with the protruding mouth on the ventral side, but can be distinguished by its anterior flagellum's beating pattern and the insertion of the flagella. *Caecitellus parvulus* has been reported in Australia, North Atlantic, Brazil, Danish Wadden Sea, England, equatorial Pacific and Turkey, with the reported size range 2 to 7 μm (Larsen and Patterson 1990, Patterson et al. 1993, Ekebom et al. 1996, Patterson and Simpson 1996, Tong 1997b, Tong et al. 1998, Lee and Patterson 2000, Aydin and Lee 2012; Lee 2015, 2019).

***Cafeteria roenbergensis* (Figs 2g, 3c)**

Cells are 7–8 μm long, D-shaped, laterally compressed and with a shallow groove on the left side. Two flagella of similar length emerging subapically and are slightly longer than the cell. In the attached cells the anterior flagellum is directed perpendicular to the ventral face of the cell and the posterior flagellum is reflexed, passing over one face of the cell and then attaching to the substrate by the tip. In the swimming cells the anterior flagellum is directed forwards and is beating with a sine wave. The posterior flagellum is directed backwards. And moves fast following a spiral path. Description is based on the observations of 23 cells. Occurance:

every month at Acı Lake, temperature 1–23.5 °C, salinity 24–67.5 psu, dissolved oxygen 3.75–13.2 mg/L.

Remarks: Generally, the observations are consistent with the descriptions of Fenchel and Patterson (1988) and Larsen and Patterson (1990)'s *Cafeteria roenbergensis*. Cavalier-Smith and Chao (2006) and Schoenle et al. (2020) carried out molecular studies and separated the *Cafeteria* into two known species – *C. roenbergensis* and *C. mylnikovii* – and with the same study six new species (*C. maldiviensis*, *C. biegae*, *C. loberiensis*, *C. chilensis*, *C. graefeae*, *C. burkhardae*) established respectively. These *Cafeteria* species closely resemble to *Cafeteria roenbergensis* and cannot be easily distinguished each other without molecular studies. Thus, in this study, we prefer to follow the criteria described by previous works (Larsen and Patterson 1990, Lee and Patterson 2000, Al-Qassab et al. 2002, Lee 2015) for these morphospecies. This species resembles to *Cafeteria minuta* (Ruinen 1938) Lee and Patterson 1990 in general appearance, but is distinguishable by the longer anterior flagellum of *C. minuta*. *Cafeteria roenbergensis* resembles *Cantina marsupialis* (Larsen and Patterson 1990) (Yubuki et al. 2015) in general appearance and in having a short anterior flagellum, but *C. marsupialis* is slightly larger and has a more strongly developed ventral groove with a posterior channel leading into the cell. Previously reported studies for *Cafeteria roenbergensis* gave the size range to be 1.5 to 10 µm (Fenchel and Patterson 1988, Larsen and Patterson 1990; Vørs 1992a, b; Patterson et al. 1993, Vørs et al. 1995, Ekebom et al. 1996, Patterson and Simpson 1996; Tong 1997b, c; Tong et al. 1997, 1998; Bernard et al. 2000, Lee 2015). *Cafeteria roenbergensis* has a worldwide distribution (Lee and Patterson 1998, Lee 2002, Al-Qassab et al. 2002, Lee et al. 2003, Aydın and Lee 2012).

Cantina marsupialis (Larsen and Patterson 1990) Yubuki *et al.* 2015 (Figs 2q, 3d)

Cells are 7–8 µm long, 6–5 µm wide and D-shaped. The anterior flagellum is 1.5–2 times the cell length and directed normal to a deep ventral groove. The posterior flagellum lies in the ventral groove and is slightly longer than the cell. The cells attach to the substrate via the tip of the posterior flagellum. A single nucleus with a rounded nucleolus lies just below the insertion of the flagella. The cell body may or may not include many – sometimes large – food vacuoles. Description based on observations of 18 cells. Occurance: every month at Acı Lake, temperature 1–23.5 °C, salinity 24–67.5 psu, dissolved oxygen 3.75–13.2 mg/L.

Remarks: Yubuki et al. (2015) carried out an ultrastructural and molecular study which demonstrates that *Cafeteria marsupialis* should be re-named at the genus level since it is not in the same clade with the species of the genus *Cafeteria*. Thus, they established the new genus *Cantina* with the type species *Cantina marsupialis*. The genus *Cantina* resembles *Cafeteria* in having a D-shaped cell body, an anterior flagellum drawing water towards the cell body and a posterior flagellum adhering to the substrate by its tip, but differs from *Cafeteria* by having a large ventral groove and a posteriorly curved food ingestion region. *Cantina marsupialis* was reported in Australia, Brazil, United Kingdom, Japan, Canada and Rusia (White Sea) (Larsen and Patterson 1990, Ekebom et al. 1996; Tong 1997b, c; Tong et al. 1997, Lee and Patterson 1998, Lee et al. 2003; Lee 2006b, 2019; Tikhonenkov et al. 2006, Yubuki et al. 2015).

Carpediemonas membranifera (Larsen and Patterson 1990) Ekebom et al. 1996 (Figs 2d, 3g)

Cells are 6–7.5 µm long and elliptical with a longitudinal ventral groove extending to the posterior end of the cell. Two flagella unequal in length are emerging anteriorly; the anterior flagellum bent over backwards is as long as the cell and beating stiffly. The acronematic posterior flagellum is 2.5–4 times the cell length, beating actively and usually lying in the depression. Description is based on the observations of 7 cells. Occurance: every month at Acı Lake, temperature 1–23.5 °C, salinity 24–67.5 psu, dissolved oxygen 3.75–13.2 mg/L.

Remarks: The species was first described as *Percolomonas membranifera* by Larsen and Patterson (1990) but later renamed as *Carpediemonas* by Ekebom et al. (1996) since non-dividing cells of this species were observed with two flagella. Our observations are consistent with the description of Ekebom et al. (1996). It has a relatively long posterior flagellum, moves by skidding with the anterior flagellum beating in a paddling motion. This species is distinguished from *Kipferlia bialata* (Ruinen 1938) Kolisko et al. 2010 and from *Ergobibamus cyprinoides* (Park et al. 2010) by its smaller size, by the absence of the moving membrane and relatively longer posterior flagellum. *Carpediemonas membranifera* has been described in marine sites of Australia, Brazil, Korea and Rusia (White Sea) with the previously reported size range from 3 to 9 µm (Larsen and Patterson 1990, Ekebom et al. 1996, Simpson and Patterson 1999, Bernard et al. 2000, Lee and

Patterson 2000, Al-Qassab et al. 2002; Lee 2002, 2019; Tikhonenkov et al. 2006).

***Halocafeteria seosinensis* Park *et al.* 2006 (Figs 2p, 3r)**

Cells are 5–7 μm long and somewhat flexible with a shallow depression located laterally. The cells have a hyaline granule located posteriorly. Two flagella are 1.5–2 times the cell length, and emerge subapically at an acute angle like a small snout like protrusion. The cells swim with both flagella beating. Description based on observations of 6 cells. Occurance: every month with the exception of July and August at Meke Lake, temperature 0–21 °C, salinity 107.5–215 psu, dissolved oxygen 0.25–5.18 mg/L.

Remarks: Our observations are consistent with the description of Park et al. (2006). This species resembles *Cafeteria minuta.* However, unlike *Cafeteria minuta, Halocafeteria seosinensis* displays a jumping movement. Having this unique jumping movement *Halocafeteria seosinensis* also closely resembles *Bodo saltans*, but differs from *B. saltans* morphologically by its relatively shorter posterior flagellum. This species was first reported in Korea with 3–5 μm size range and later observed in the cultures from Australia, USA and in saline environments of Poland (Park et al. 2006, Park and Simpson 2015).

Protista *incertae sedis*

***Ancyromonas sigmoides* Kent 1880 (Figs 2a, 3a)**

Cells are 3–5 μm long, oval shaped and dorsoventrally flattened. The thin anterior flagellum is emerging from an anterior depression and is beating slowly. The non-acronematic trailing posterior flagellum is about 1.5 times the cell length. The cells move by gliding. Description is based on the observations of 19 cells. Occurance: every month at Acı Lake, temperature 1–23.5 °C, salinity 24–67.5 psu, dissolved oxygen 3.75–13.2 mg/L.

Remarks: Detailed studies of the genus *Ancyromonas* were published by two separate groups (Cavalier-Smith et al. 2008; Heiss et al. 2010, 2011). According to the work of Heiss et al. (2010), *Planomonas* Cavalier-Smith et al. 2008 has been concluded as a junior synonym of *Ancyromonas*. According to the previous studies (Heiss et al. 2010, 2011; Lee 2015), the genus *Ancyromonas* consists of 6 nominal marine (*A. sigmoides*, *A. cephalopora*, *A. impulusive*, *A. melba*, *A. sinistra*, *A. micra*) and 2 nominal freshwater (*A. howeae*, *A. limna*) species. When compared to *Ancyromonas sigmoides*, *A. melba* is larger, *A. sinistra* has a margin with the presumptive extrusomes and having a flatter cell body. *Ancyromonas micra* has a thicker anterior flagellum and a bigger rostrum, whereas *A. howeae* and *A. limna* have a contractile vacuole. As mentioned by Aydın and Lee (2012) due to their morphological similarities, *Ancyromonas micra* might have been reported elsewhere under the name of *A. sigmoides*. *Ancyromonas sigmoides* is similar to *Metopion fluens*, but is distinguished by the anteriorly directed flagellum. In addition, the second flagellum of *M. fluens* is thicker and is directed to the rear. This species has a worldwide distribution.

***Pendulomonas adriperis* Tong 1997 (Figs 2k, 3q)**

Cells are 4–9 μm long, ovoid or droplet-shaped, somewhat flexible and not flattened. The cells have two flagella similar in length that emerge subapically. The non-acronematic flagella are slightly longer than the cell. The anterior flagellum projects in front of the body and beats with an asymmetric pattern, and the posterior flagellum trails behind the cell and might be held in a curve or obliquely. Sometimes, the posterior flagellum beats stiffly and rapidly in non-swimming cells. The cells usually swim by slow rotating movements and do not wag. The cells may attach to the substrate by the tip of the trailing flagellum. In attached cells, the cells may wag or tremble rapidly. Description based on observations of 16 cells. Occurance: May to October at Acı Lake, temperature 1–23.5 °C, salinity 24–67.5 psu, dissolved oxygen 3.75–13.2 mg/L.

Remarks: Our observations are consistent with the original description of Tong (1997c). The genus *Pendulomonas* resembles the genus *Cafeteria*, but differs by the orientation and beating pattern of the flagella. The genus *Pendulomonas* also resembles the genus *Phyllomitus* in general cell shape, but differs by the two flagella of *Phyllomitus* insert together to an anterior pocket. Flagella of *Pendulomonas* emerge separately from a subapical pocket. A morphologically very similar species (*Wobblia lunata*) was reported by Moriya et al. (2000), but they didn't compare with *Pendulomonas adriperis* despite of the striking similarities in cell shape and length. Therefore, *Wobblia lunata* has been regarded as the junior synonym of *Pendulomonas adriperis* (Karpov et al. 2001, Al-Quassab et al. 2002, Lee et al. 2003, Lee 2006b). This species has been reported from Australia, England, Japan (as *Wobblia lunata*) and Black Sea (Kazachya Bay) (Tong 1997c, Lee and Patterson 2000, Moriya et al. 2000, Al-Qassab et al. 2002; Lee 2006b, 2019; Prokina et al. 2017b).

Unidentified Protist (Figs 2m, 3k)

The cell is about 21 μm long and 11 μm wide, and sac-shaped with a pointed posterior end. The cell has four flagella of almost equal length and each flagellum is about the cell length. Each flagellum is fluctuating separately. The cell has a slit like mouth at the left hand side of the anterior part where the flagella emerge. One cell observed. Occurance: October at Meke Lake, temperature 18 °C, salinity 210 psu, dissolved oxygen 0.3 mg/L.

Remarks: This unidentified protist cell resembles *Pharyngomonas kirbyi* in general cell appearance. *Pharyngomonas kirbyi* was reported from saline environments with two forward-pointing and two backward-pointing flagella, whereas our cell is with four flagella anteriorly pointed. *Pharyngomonas kirbyi* has a curving cytopharynx and a ventral groove, but we did not observe a curving cytopharynx and a ventral groove. In our cell, there is a slit like mouth opening under the flagellar insertion point. Having four flagella and a slit like mouth opening under the flagellar insertion point, this cell is also similar to the species of the genus *Tetramitus*, but differs by the truncated anterior flagellar stage of the genus *Tetramitus* and by the flexible cells. Unidentified protista also resembles to *Trimastigamoeba philippinensis* (Whitmore 1911), *Tetradimorpho tetramastix* (Penard 1921) Siemensma 1991, *Collodictiyon* and to *Liouamonas quinquiflagellata* (Skortzov 1960) in having four flagella. But the general cell shapes, flexibilities of the cells are quite different (Skuja 1956, Bovee 1959, Skvortzov 1960, Hamar 1973, Klaveness 1995, Mikrjukov 2000). With limited information available we are not able to identify this cell, but its presence as an unidentified species is recorded to establish the protista fauna in hypersaline sediments further.

DISCUSSION

Salinity is one of the most important restricting abiotic factors on the diversity of heterotrophic flagellates as stated in former studies (Post et al. 1983, Patterson and Simpson 1996, Al-Qassab et al. 2002, Cho 2005, Hauer and Rogerson 2005). Our results showed that the species diversity was lower in Meke Lake (hypersaline environment) than in Acı Lake and no same species or genus was found from both lakes. These might be due to the striking salinity difference between the two lakes. In addition, at Meke Lake, some species such as *Halocafeteria seosinensis* and *Pleurostomum flabellatum* were not found in July and August when the lake is dry and salty with the high evaporation rates resulting in salt plains. During the period, these species may be transformed into a cyst stage, but cysts of these species have not been reported yet. Otherwise, the absence of these species during the period may be influenced by the extrinsic factors such as under-sampling and under-reporting, which may be reduced by a long term, intensive study (Patterson and Lee 2000).

Several studies (e.g., Post et al. 1983, Patterson and Simpson 1996) reported the low species diversity of heterotrophic flagellates in hypersaline environments supporting our results. Post et al. (1983) reported 12 morphospecies of heterotrophic flagellates from a hypersaline lagoon and salt cultures. Patterson and Simpson (1996) studied on the diversity of heterotrophic flagellates at several sites with different salinity concentrations in Australia, and found that the species diversity varied significantly along the salinity gradient: 33 species (Little lagoon, about 41‰ salinity), 17 species (Hamelin pool, 60–65‰ salinity), 7 species (Hypersaline pond, about 150‰salinity) and 5 species (saturated puddle).

Studies carried out to understand the geographical distribution of these organisms demonstrate that most morphospecies of free-living heterotrophic flagellates have a cosmopolitan distribution (e.g. Larsen and Patterson 1990; Vørs 1992a, b; Patterson et al. 1993, Ekebom et al. 1996, Patterson and Simpson 1996, Lee and Patterson 2000, Patterson and Lee 2000, Al-Qassab et al. 2002; Lee et al. 2003, 2005; Lee 2002b, 2006a, 2008, 2012, 2015, 2019; Schroeckh et al. 2003). Our results also suggest that all of the morphospecies encountered here (except the unidentified protist) appear to be cosmopolitan. The establishment of novel species like ‘unidentified protist’ of this study and the unidentified organism ‘Aegoni’ from Saros Bay (Aydın and Lee 2012) is quite important in figuring out the endemism level of heterotrophic flagellates. Thus, understanding the geographic distribution of heterotrophic flagellates requires long term and intensive studies from previously unstudied geographic locations worldwide.

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