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Trachelomonas (Euglenophyta) from a eutrophic reservoir in Central Mexico

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Abstract

This study provides valuable information on the ultrastructure and environmental conditions of the *Trachelomonas* Ehr. (Euglenophyceae) genus in the Guadalupe Dam, a eutrophic reservoir located in the suburbs of Mexico City, which receives a considerable volume of wastewaters. Specimens were collected at surface level between November 2005 and May 2006. Using LM and SEM twelve taxa from phytoplankton were identified of which, 9 are new records for Mexico. The reservoir is warm monomictic, with basic pH values (7.4-10.1), a high concentration of chlorophyll *a* (18-101 $\mu g\ l^{-1}$), a permanent anoxic bottom, specific conductivity (K_{25}) of 205 to 290 $\mu S\ cm^{-1}$, $N\text{-}NO_3$ 0.19-1.2 $mg\ l^{-1}$ and $P\text{-}PO_4$ 0.22-1.6 $mg\ l^{-1}$. Water temperature was 15.6-23.0°C. Most of the *Trachelomonas* species were found during the dry season, when concentrations of organic matter, nitrogen and phosphorus as well as the temperature were the highest. Higher species richness was also associated with the warmer months. This research contributes to increase our knowledge on *Trachelomonas* in Mexico and constitutes the first detailed description of lorica ultrastructure of 12 taxa that grow in a body of water with high concentration of nutrients and a moderate amount of mineral contents.

Key words

Lorica, Dam, Euglenoids, Phytoplankton, Ultrastructure

Introduction

There are many records of *Trachelomonas* species in the world, which are mostly based on observations carried out using light microscopy (LM). Unfortunately, with this method it is difficult to achieve a detailed study of the lorica structure, which is the most reliable basis for the taxonomy of the genus (Wolowski and Hindák, 2004). Some species are endemic; others are cosmopolitan or restricted to cold, temperate or warm regions (Couté and Tell, 2006).

More than 200 species are described, in the "Monographie du genre *Trachelomonas* Ehrenberg" (Deflandre, 1926). The most important studies on these organisms took into consideration the size, shape and ornamentation of lorica as the main characters for their classification (Conrad and Van Meel, 1952). Huber-Pestalozzi (1955) compiled 256 species, 190 varieties and 46 forms. Rosowski *et al*. (1975) were the first to study the details of the lorica surface using a scanning electron microscope (SEM). Ever since various species of *Trachelomonas* have been examined with this method. Some works on the study of *Trachelomonas* in the world have been carried out in South America (Tell and Couté, 1980; Couté and Thérézien, 1985, 1994; Conforti, 1993, 1999; Conforti and Nudelman, 1994; Conforti and Perez, 2000; Conforti and Tell, 1986), in North

America (Conforti and Joo, 1994; Wolowski and Walne, 2007), in Europe (Kocárková *et al*., 2004; Wolowski and Hindák, 2004; Wolowski and Grabowska, 2007), in Asia (Kim *et al*., 1999; Conforti and Ruiz, 2001), and in Africa (Couté and Iltis, 1981; Da *et al*., 2009).

It is important to note that there is not very much information available on the taxonomical, ecological and geographical distribution of the *Trachelomonas* genus in Mexico. A list of 27 taxa has been recorded in Mexico (Ortega, 1984; Díaz-Pardo *et al*., 1998; García-Rodríguez and Tavera, 2002; Schmitter-Soto *et al*., 2002; García-Rodríguez *et al*., 2003; Moreno-Ruiz, 2005; Quiroz-Castelán *et al*., 2007; Moreno-Ruiz *et al*., 2008). Two species were found to have been reported previously in Guadalupe Dam by Lugo *et al*. (1998, 2007). The present paper contributes to the knowledge of the *Trachelomonas* species composition in Mexico. For the first time our country, details of the lorica ultrastructure of the species are described, using LM and SEM. Likewise, the main physicochemical conditions found during the study are also provided.

Materials and Methods

Study area: The Guadalupe Dam is located outside Mexico City in the suburbs, in the State of Mexico (19° 48' 30'' N 99° 15' W, 2350 m.a.s.l., maximum volume 60 X 10^6 m^3, maximum depth 20 m). The dam was built to control and store the waters of the Cuautitlan River for irrigation (Fig. 1). Currently, a high percentage of the total annual inflow comes from sewage discharges from a highly populated area around the dam (Lugo *et al*., 2007).

The climate is temperate subhumid with a rainy summer. Mean annual temperature is 16°C, and annual precipitation is of 706 mm (Hidalgo-Wong and Pulido-Navarro, 2006). The reservoir is eutrophic with high concentrations of chlorophyll *a* (Lugo *et al*., 1998).

At the beginning of the 80's the dam was invaded by water hyacinth *Eichhornia crassipes*. The Department of Agriculture (SARH) together with the Mexican Institute of Water Technology (IMTA) in 1993 successfully implemented the first chemical control program, through the application of herbicides such as 2, 4 D and diquat. In 1995, the weed reappeared, and in 1997 it was eliminated again by chemical and mechanical weed control. Ever since, hyacinth has not invaded the water surface again, however other ecological problems such as blooms of algae and the death of fish during the winter seasons in 2004 and 2005 have arisen (Lugo *et al*., 2007).

Sample collection: Samples were collected with 20 μm mesh plankton net at surface level in November 2005, April and May 2006. The material was fixed in 4% formaldehyde. Detailed examination of the material was carried out with a Zeiss phase-contrast microscope (LM). On the other hand, for scanning electron microscopy (SEM) analysis, a concentrated subsample was filtered using Millipore® filters (0.45 μm pore size), and air-dried. The pieces of filters were adhered to aluminum stubs and coated with gold (Zalocar de Domitrovic and Conforti, 2005). A Hitachi S-2460N electron microscope, operating at 15kV at the Institute of Biology, UNAM and a JEOL JSM6380LV at the Electron Microscopy Service (FES Iztacala, UNAM) were used. The samples were deposited at the IZTA herbarium with reference numbers 1753-1764 (Holmgren *et al*., 1990).

Environmental conditions were recorded at the same time the samples of *Trachelomonas* were collected. Water temperature, dissolved oxygen and conductivity (K_{25}) were measured using a YSI multisonde mod. 85 (Yellow Spring Instruments Co. Ohio, USA). And transparency was measured using a Secchi disk.

The content of chlorophyll *a* was evaluated *in vivo* with a portable Aquafluor fluorometer (Turner Designs Co. California USA). N-NO_3, P-PO_4 and BOD_5 samples were obtained from five sites at the surface and sent to the laboratory to be analyzed. N-NO_3 as well as total dissolved phosphorous was measured using the cadmium - reduction method for the first and the ascorbic acid method for the latter. BOD_5 was evaluated with the bottle dilution method (APHA, 1989).

The identification and distribution of the species was based on the research works by Conrad and Van Meel (1952); Hüber-Pestalozzi (1955); Couté and Iltis (1981); Tell and Zalocar de Domitrovic (1985); Tell and Conforti (1986); Conforti and Tell (1986, 1989); De la Rosa and Sanchez-Castillo (1991); Conforti (1993, 1999); Conforti and Joo (1994); Conforti and Nudelman (1994); Couté and Thérézien (1994); Kim *et al*. (1999, 2000); Dillard (2000); Conforti and Ruiz (2001); Kocárková *et al*. (2004); Wolowski and Hindák (2004); Da *et al*. (2009) and *Algaebase.org*. The terminology used in this paper follows Conforti and Tell's criterion (1986). In average, 35 organisms were used for measurements. As far as scientific names and synonyms are concerned, same were verified in the *Integrated Taxonomic Information System* and *Index Nominum Algarum.*

Results and Discussion

Environmental conditions: The Guadalupe Dam is a warm monomictic water body. In November, at the beginning of the cold dry season, the water column was thermally homogeneous at around 17°C (16.9-18.8°C) which means that mixing conditions were prevalent. In March, and particularly during April (15.6-21°C) and May (17-23°C), there was a thermal stratification.

The high organic load at the bottom of the dam causes permanent anoxia in the deep water layer. In November, dissolved oxygen ranged from non detectable (n.d.) to 12 mg l^{-1} in the top 10 m of the water column; however during April and May oxygen was detected only in the top five meters. K_{25} values in November were lower (205-241 μS cm^{-1}) than in April and May (250-290 μS cm^{-1}). At the surface level pH was always basic, in November ranging from 7.4 to 9.3 and during April and May the values increased to 7.9-10.1. Water transparency varied between 0.2 and 0.5 m indicating a shallow euphotic zone.

Table - 1: List of taxa observed in Guadalupe Dam, the marked with an asterisk are new record for Mexico and P (presence).

Taxa	November 2005	April 2006	May 2006
**T. globularis* var. *gigas* Drezepolski 1923		P	
T. hispida var. *hispida* (Perty) Stein 1878	P	P	P
**T. hispida* var. *coronata* Lemmermann 1913		P	P
**T. nexilis* Palmer 1925			P
**T. rugulosa* var. *rugulosa* Stein 1878			P
**T. rugulosa* var. *meandrina* (Conrad) Conrad 1952			P
**T. rugulosa* var. *steinii* Deflandre 1927			P
**T. similis* var. *spinosa* Hüber-Pestalozzi 1955	P		P
**T. sydneyensis* Playfair 1915	P	P	P
**T. verrucosa* f. *irregularis* Deflandre 1926			P
T. volvocina var. *volvocina* Ehrenberg 1833	P	P	
T. volvocina var. *punctata* Playfair 1915		P	P
Total	4	6	10

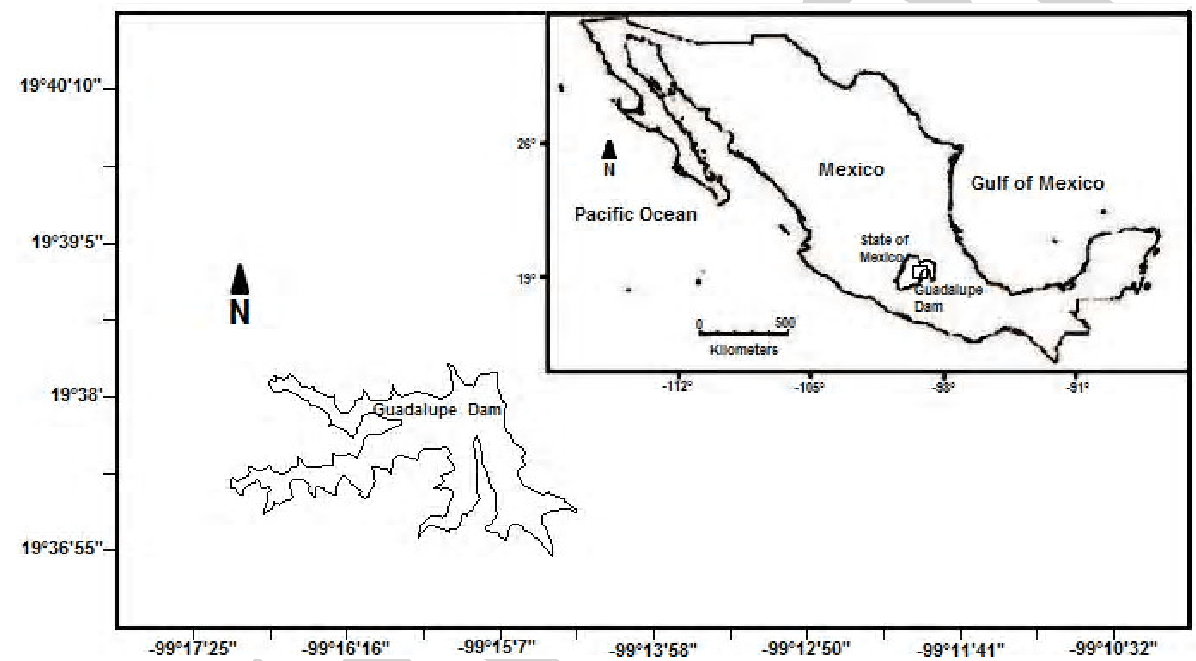


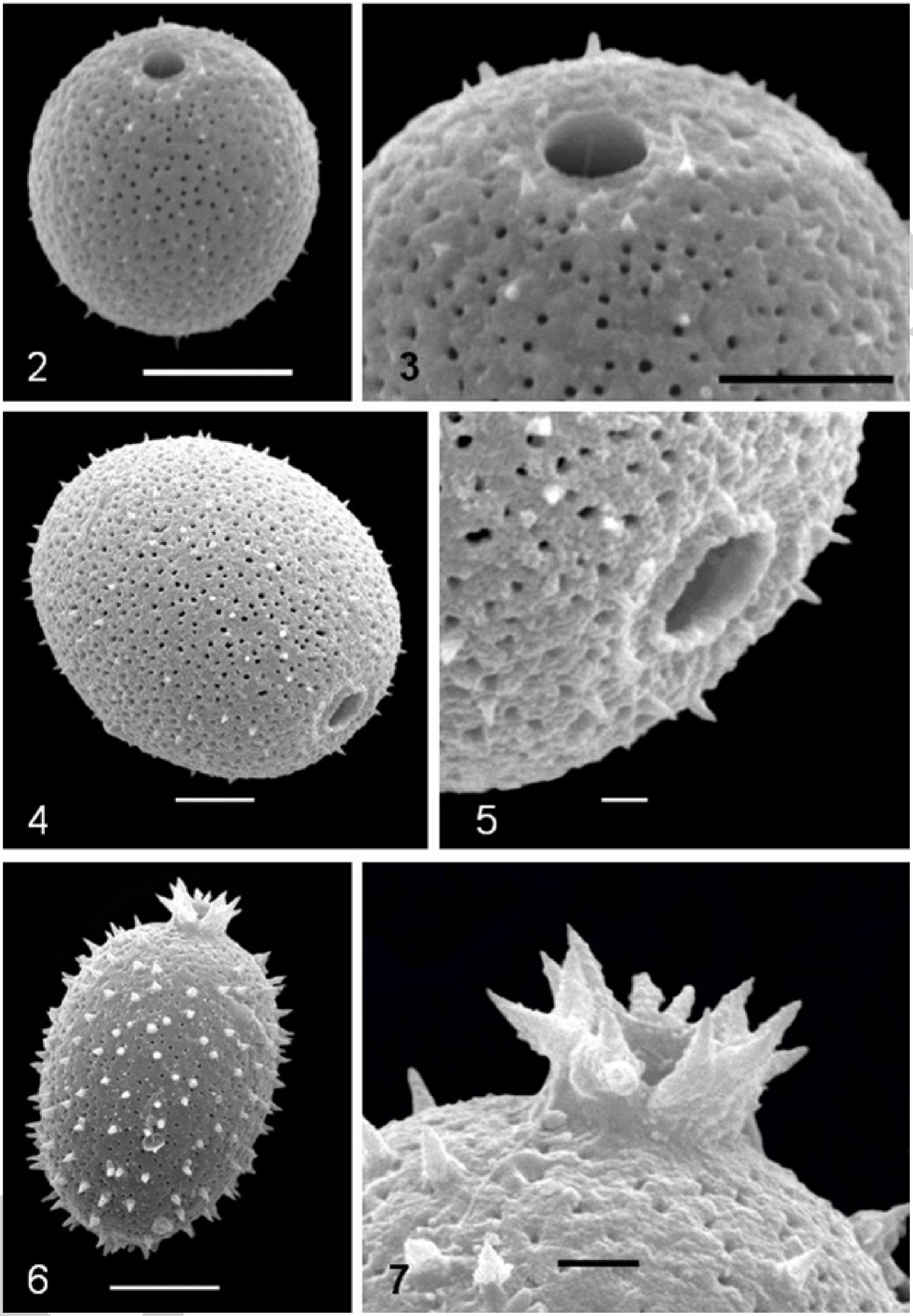
Fig. 1: Location of Guadalupe Dam, Mexico

Species richness of *Trachelomonas* was correlated with nutrient concentration. In November, when low N and P concentrations were measured (mean value: N-NO_3 0.186 mg l^{-1}; P-PO_4 0.216 mg l^{-1}), only four species were observed. On the other hand, during April and May with an increased N and P concentration, (mean value: N-NO_3 0.7-1.2 mg l^{-1}, P-PO_4 1.6 mg l^{-1}), the number of *Trachelomonas* species rose to six and ten respectively.

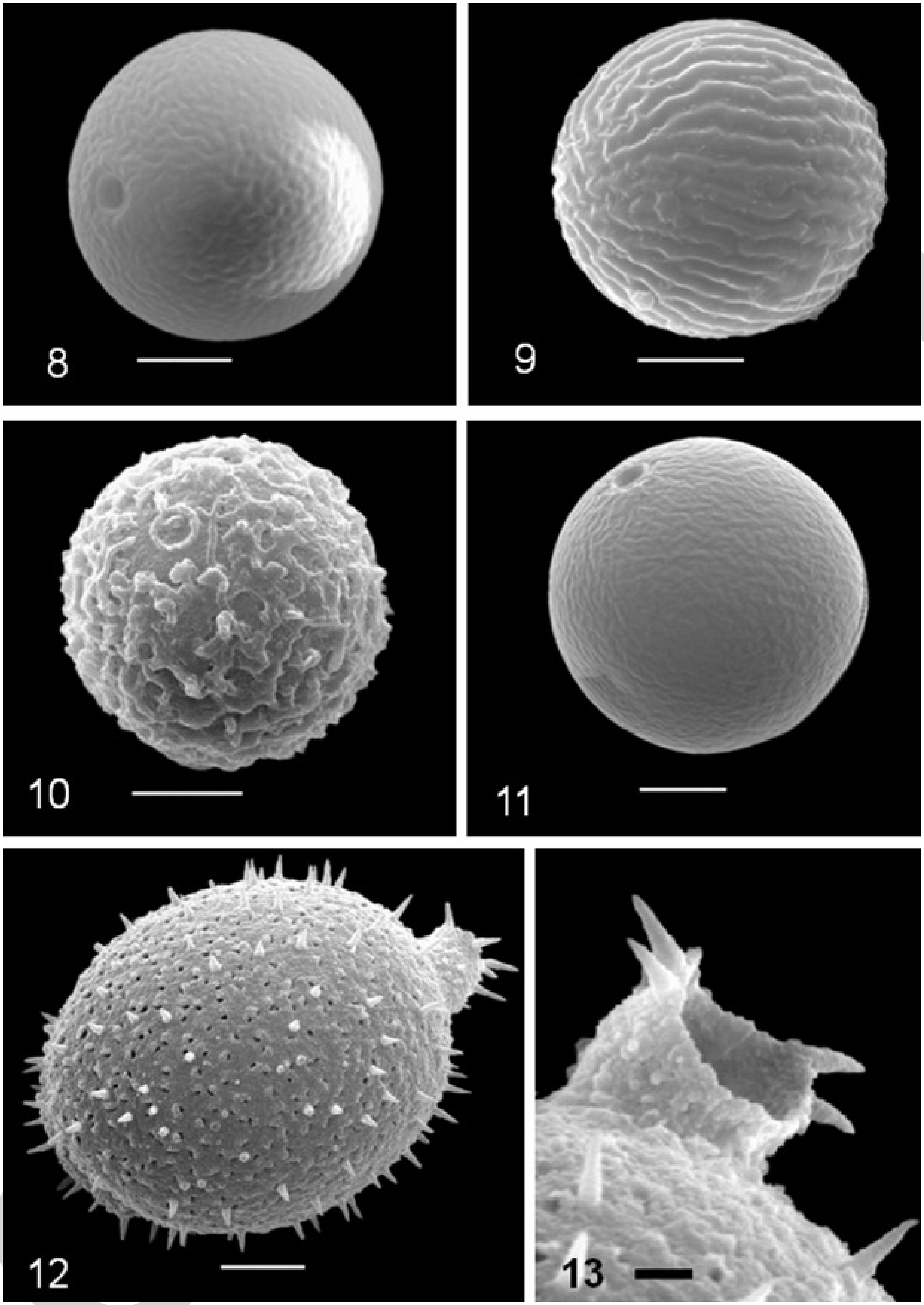
The concentration of Chlorophyll *a* was also related with nutrients. In November the measured values were low, within a range from 18 to 25 µg l^{-1}. April showed the highest values (72-101 µg l^{-1}) decreasing in May (30-45 µg l^{-1}). These average values are high and clearly show the eutrophic conditions of the dam (Margalef, 1983).

BOD_5 values measured occasionally in the dam, ranged between n.d. to 65 mg O_2 l^{-1}, indicating a moderate organic load in the surface level. Wolowski and Hindák (2004) found that *Trachelomonas* are generally resistant to organic pollution. Sládecek (1973) considered *Trachelomonas* as a typical indicator of medium to high organic matter concentrations in water, especially associated with high ammonium concentrations (Alves-da-Silva *et al*., 2008; Conforti and Tell, 1986; Conforti, 1986,1993; De la Rosa and Sanchez, 1991; Da *et al*., 2009).

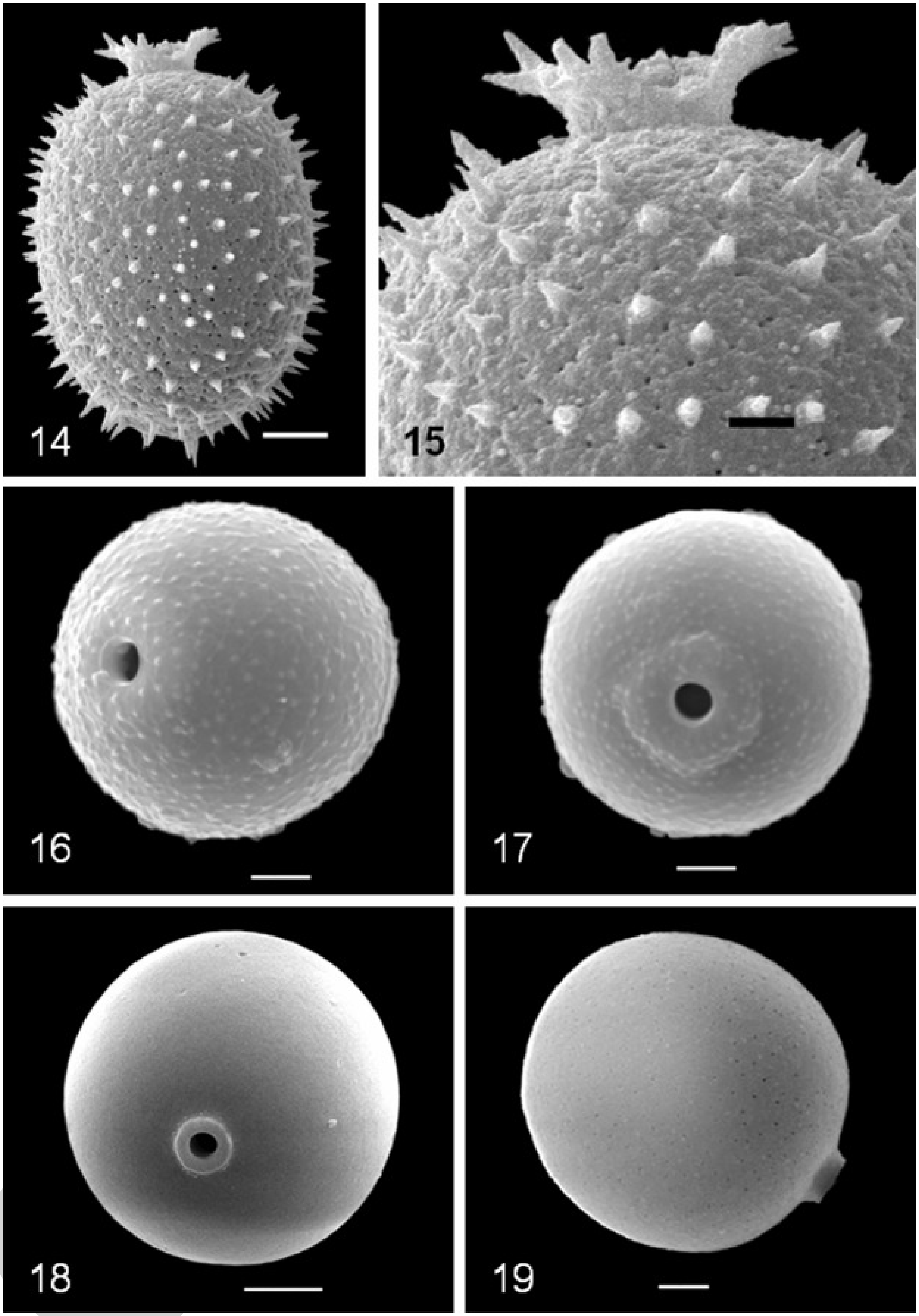
In temperate climates euglenoids are observed in the warmer months (spring, summer) and only a few species can be found in cold water or even under the ice (Starmach, 1983; Conforti and Tell, 1988). In this work we observed that the highest richness



Figs. 2-7. Figs. 2-3: *Trachelomonas globularis* var.*gigas*: **2-** general view, **3-** apical view, showing detail of the pore; Figs. **4-5:** *T. hispida* var. *hispida*, **4-** general view, **5-** apical view, showing detail of the pore surrounded by a short neck with spines **Figs. 6-7:** T. *hispida* var. *coronata,* **6-** general view, **7-** apical view, showing detail of the neck. Scale bars = 10 µm (Figs. 2, 6), 5 µm (Figs. 3, 4), 2µm (Fig. 7), 1µm (Fig. 5).



Figs. 8-13. Fig. 8: *T. nexilis* **Fig. 9**:*T. rugulosa* var. *rugulosa* **Fig**. **10:** *T. rugulosa* var. *meandrina* **Fig. 11:** *T. rugulosa* var. *steinii* **Figs. 12-13:** *T. similis var. spinosa*, **12-** general view, 13- detail of the neck. Scale bars 5 µm (Figs. 8, 9, 10, 11, 12), 1µm (Fig. 13)



Figs. 14-19. Figs. 14-15: *T. sydneyensis*, **14-** general view, **15-** detail of the neck **Figs. 16-17:** *T. verrucosa* fo. *irregularis*, **16-** general view, **17-** apical view, showing detail of the pore **Fig. 18:** *T. volvocina* var. *volvocina* **Fig. 19:** *T. volvocina* var. *punctata.* Scale bars 5 µm (Figs. 14, 18), 2µm (Figs. 15, 16, 17, 19).

Trachelomonas species was associated with the warmer months. Only *T. hispida* and *T. sydneyensis* were observed during the entire period that was (Table 1).

Taxonomical descriptions: During the study period 12 taxa of *Trachelomonas* were identified, including species, varieties and forms; nine of these taxa are new records for Mexico. Lugo *et al.* (1998) found *T. hispida* and *T. volvocinopsis* in a previous survey in the dam. This study confirms the presence of *T. hispida* and adds 10 taxa not observed previously. *Trachelomonas globularis* var. *gigas* Drezepolski 1923 Fig. 2, 3.

Lorica spherical with 19 μm in diameter, wall with 80 100^{-1} μm^2 punctae covered sparsely with 0.5-0.95 μm short conical spines. Apical pore with a 2.5-3.7 μm diameter, surrounded by an annular thickening (IZTA-1753).

Distribution: Argentina, Poland and the US.

The specimens studied here are smaller than those described by Conforti (1999) with a 31-32 μm diameter and Dillard (2000) 34 μm diameter. *Trachelomonas hispida* var. *hispida* (perty) Stein 1878 Fig. 4, 5.

Lorica elliptical; 22-25 μm long, 16.5-21 μm wide, with a wall covered with 72-100 100 μm^2 punctae, 0.5-1.3 μm short conical spines, uniformly distributed 8-20 100 μm^2. Apical pore with a 3.2-4.2 μm diameter, surrounded by an annular ring-like thickening (IZTA-1754).

Distribution: Cosmopolitan. It was previously reported in the Chapultepec and Xochimilco Lakes in Mexico City; the Guadalupe Dam in the State of Mexico; El Rodeo Lagoon in the State Morelos; in Tulancingo, in the State of Hidalgo; Tonatihua and Zempoala Lagoons in the State of Morelos; González River and Mandinga respectively in the States of Tabasco and Veracruz in Mexico. *Trachelomonas hispida* var *coronata* Lemmermann 1913 Fig. 6,7.

Lorica elliptical; 28-30 μm long, 21-21.5 μm wide, with a rounded or acuminate posterior end, wall 88 100 μm^2 punctae covered with conical spines 1.5-3 μm high with a diameter 0.75-1.5 μm at base, uniformly distributed 16 100 μm^2. Apical pore surrounded by a short 1-2.5 μm high and 5 μm wide neck, with sharp 1.5 μm long spines along its margin (IZTA-1755).

Distribution: Britain, Romania, Spain, Africa, Australia, Argentina, Portugal, New Zealand and US.

In the specimens described by Da *et al.* (2009) the punctae are 270-354 μm/100 μm^2. Our specimens showed 88/100 μm^2. *Trachelomonas nexilis* Palmer 1925 Fig.8.

Lorica spherical with 9-17 μm in diameter, wall with irregularly 0.15-0.17 μm wide vermicular lines and depressions. Apical pore 2 μm wide surrounded by an annular thickening (IZTA-1756).

Distribution: Argentina, Brazil, Spain and the US. *Trachelomonas rugulosa* var. *rugulosa* Stein 2878 Fig.9.

Lorica spherical with 14-15 μm in diameter, wall covered with distinct anastomosing ridges arranged longitudinally or spirally 0.65 μm thick and separated by an average of 0.7 μm, ten ribs by 10 μm, twisted in the anterior end. Apical pore 2 μm in diameter surrounded by an annular thickening (IZTA-1759).

Distribution: Britain, Romania, Spain, Argentina and the US. *Trachelomonas rugulosa* var. *meandrina* (Conrad) Conrad 1952 Fig.10.

Lorica spherical with 24 μm in diameter, wall covered with anastomosing, arranged folds. Apical pore with 2 μm in diameter surrounded by a ring-like thickening (IZTA-1764).

Distribution: Britain, Slovakia. *Trachelomonas rugulosa* var. *steinii Deflandre* 1927 Fig.11.

Lorica spherical with 15-18.5 μm in diameter, and densely or lightly ornamented wall with anastomosing ridges coming radially from the pore. Apical pore 1.5-2 μm diameter, surrounded by an 0.8 μm annular thickening (IZTA-1760).

Distribution: Colombia, Korea, France and Austria. *Trachelomonas similis* var. *spinosa* Hubar-Pestalozzi 1955 Fig.12, 13.

Lorica elliptical; 23.5-25 μm long and 19-20.5 μm wide, with a rounded or slightly acuminate posterior end, 45-80/100 μm^2 punctae covered with a few short 1.6-2 μm long conical spines with a diameter of 0.50-0.80 μm at the base, sparsely distributed 10-13/ 100 μm^2. Apical pore surrounded by a cylindrical neck usually curve towards one side, 2-4.5 μm wide and 2-5.0 μm high, 0.20-0.35 μm thick wall with spines irregularly distributed spines at the end of the neck which has a thick rim where there are conical spines up to 2 μm long (IZTA-1757).

Distribution: Africa, Argentina, Bolivia, Brazil, Colombia, the US and Venezuela. *Trachelomonas sydneyensis* Play fair 1915 Fig. 14, 15.

Lorica elliptical; 28-33 μm long, 20-24 ``μm wide, the rounded poles and covered with long and sharp conical spines in the poles and smaller ones 1-3 μm long, 12-24/100 μm^2 in the center; 56-94/100 μm^2 punctae. Apical pore surrounded by a conspicuously divergent neck 1-3.5μm high and 5-7.5 μm wide in its opening and spines in the rim (IZTA-1758).

Distribution: Africa, Asia, Argentina, Australia, Brazil, Britain, New Zealand, Romania and Spain.

In the specimens described by Da *et al.* (2009) the punctae are 145-181/100 μm^2. Our specimens showed 56-94/100 μm^2. *Trachelomonas verrucosa F. irregularis* Deflandre 1926 Fig. 16, 17.

Lorica spherical with 11-11.5 μm in diameter, ornamented wall with small uniformly distributed 300/100 μm^2 warts. Apical pore

1-1.5 μm diameter, surrounded by an annular thickening (IZTA-1761).

Distribution: Africa, Slovakia and US.

In the specimens described by Conforti and Joo (1994) the warts were more dense (520/100 μm^2) than in our material. *Trachelomonas volvocina* var. *volvocina* Ehrenbeig 1833 Fig. 18.

Lorica spherical with 10-22 μm in diameter and smooth wall. Apical pore with 1-2 μm in diameter, surrounded by an annular thickening. Two lateral chloroplasts have double sheathed pyrenoids. Flagellum three times longer than the lorica (IZTA-1762).

Distribution*:* Cosmopolitan. In Mexico it was recorded in the Chapultepec and Xochimilco Lakes in Mexico City; Lerma wetland in the State of Mexico; Tulancingo, State of Hidalgo; in the El Rodeo Lake, Tonatihua and Zempoala Lagoons in the State of Morelos; Labradores Lake in the State of Nuevo León; Tehuantepec River in the State of Oaxaca; Gonzalez River in the State of Tabasco and Apizaco in the State of Tlaxcala in Mexico. *Trachelomonas volvocina* var. *punctata* Play fair 1915 Fig.19.

Lorica spherical with 13-15 μm in diameter, 130-135/100 μm^2 punctae Apical pore with 2.5 μm diameter, surrounded by a low neck. Two lateral chloroplasts have double sheath pyrenoids (IZTA-1763).

Wolowski and Hindák (2004) observed 200-300/100 μm^2 punctae, our specimens had a lower number of 66-132/100 μm^2 punctae.

Distribution: Argentina, Australia, Romania, Spain, New Zealand, Denmark, Slovakia, Switzerland, Turkey, US, Venezuela. In Mexico it was reported at the Tehuantepec River in the State of Oaxaca.

All the studied species are widespread or cosmopolitan, although *T. rugulosa* var*. meandrina* has only been observed in Britain and Slovakia. In this work the geographical distribution is extended in North America.

The number of taxa of *Trachelomonas* in the Guadalupe Dam was intermediate, in comparison with Alves-da- Silva and Schüler- da- Silva (2007) who found only nine taxa *Trachelomonas* in 26 water bodies of the Jacuí Delta State Park of the Río Grande do Sul State in Brazil. In several shallow lagoons of Granada in Spain De la Rosa and Sanchez-Castillo (1991) observed only 5. Kocarková *et al*. (2004) found 25 taxa *Trachelomonas* in ponds and puddles of the north region of Moravia in the Czech Republic. On the other hand, Conforti (1993) reported the presence of 90 taxa in the Camaleao Lake in Manaos, Brazil, and Conforti and Ruiz (2001) found 41 taxa in the Chuman reservoir in South Korea.

In Mexico, the usual number of taxa found per water body is two with the exception of the Tehuantepec River where 8 taxa have been found (Moreno-Ruiz *et al*., 2008). In the present study the information obtained with the use of SEM allowed us to increase our knowledge and characterization of the observed taxa. On the other hand, in previous studies, where only LM was used, it is quite likely that the number of *Trachelomonas* taxa could have been subestimated.

The Guadalupe Dam was infested with water hyacinth *Eichhornia crassipes* for more than 12 years. In 1993 a program to remove hyacinth was carried out at reservoir including the use of herbicides and mechanical control. Changes in the habitat conditions and the disappearance of the hyacinth have promoted an increase in the diversity and abundance of phytoplankton (Lugo *et al*., 1998). There has been an increase in the number of species of *Trachelomonas* under the new environmental conditions, from two to twelve species. The present study shows an important increase in the diversity of the *Trachelomonas* species most likely associated with the presence of better environmental conditions for phytoplankton growth.

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