

Free-living ciliates from a perturbed marsh in Central Mexico: some notes about taxonomy and ecology

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| Submitted September 27, 2018 | Accepted October 5, 2018 |

Summary

Ciliates are a morphologically diverse group of protists inhabiting aquatic and terrestrial ecosystems. We studied the taxonomy and ecology of free-living ciliates from a freshwater marsh in Central Mexico during the period from 2012 to 2013 during both dry and rainy seasons. We found 30 ciliate species recorded for the first time from Atarasquillo marsh in Mexico, most of which are common species in freshwater ecosystems. The species richness was higher during the dry season than rainy season. Five trophic groups were observed: bacterivorous, bacteria-algae-heterotrophic flagellate consumers (BAF-consumers), omnivorous, predators, and mixotrophic species. The trophic group composition changed according to the season and the environmental conditions, e.g. vegetation and water level. Generally bacterivorous ciliates were more frequent during the rainy season, meanwhile the omnivorous during the dry season; thus, the feeding strategies in ciliates were different between both seasons. Due to the fact that Atarasquillo marsh is in a deterioration process, ciliate records could be used to assess water quality.

Key words: Ciliophora, marsh wetland, Mexico, seasonal variation, trophic role

Introduction

Free-living ciliates inhabit diverse aquatic and terrestrial environments (Foissner et al., 2008; Lynn, 2008), and have an important role in food webs (Finlay and Esteban, 1998; Weisse, 2002, 2017; Lynn, 2008). Heterotrophic ciliates feed on a

diversity of prey, including bacteria, sulphur bacteria, cyanobacteria, algae, diatoms, heterotrophic flagellates, protists including ciliates, and small metazoans (Foissner and Berger, 1996). In addition, mixotrophic ciliates are important contributors to primary production (Johnson, 2011). They and other protists are good indicators of trophic status in

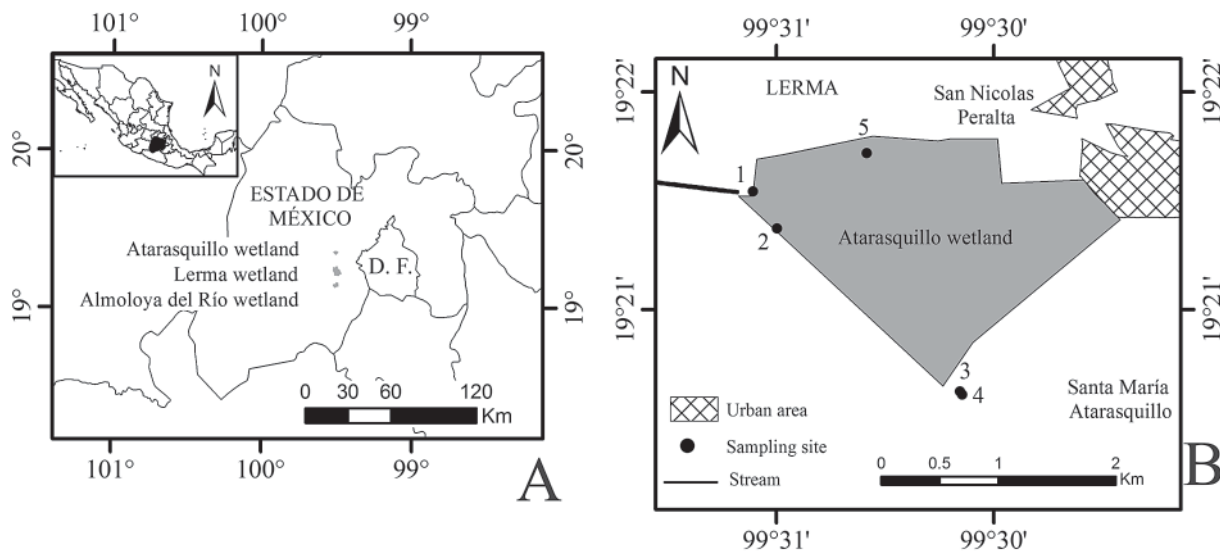


Fig. 1. Location of Lerma marshes (A) and Atarasquillo (Chignahuapan) wetland (B), showing the sampling sites in black circles. Sites 3 and 4 are outside of the protected area.

lakes (Beaver and Crisman, 1989) and bioindicators of water quality (Madoni, 2005; Jiang et al., 2011; Debastiani et al., 2016), mainly due to the fact that their species richness and biomass are affected by changes in the physicochemical conditions in the water (Mieczan, 2007a, 2007b; 2009).

Despite the importance of ciliates, knowledge about their diversity in wetlands is scarce. Henebry et al. (1981) studied the composition and structure of some protozoan communities, including ciliates, in seven different wetlands (bogs, fens, marshes, swamps) in the USA and found 2 to 12 ciliate species. Mieczan (2007a, 2007b) observed 7-37 species in peatbogs in Poland, and Lopes and Heckman (1996) found 55 ciliate species in the largest wetland of the world, Pantanal Mato Grosso in Brazil. The wetland where the highest ciliate diversity was recorded is a group of *Sphagnum* ponds in Simmelried, Germany, with 202 species identified (Kreutz and Foissner, 2006).

In Mexico, ciliate species diversity in wetlands is poorly understood, and only *Glaucoma dragescui* Corliss, 1971 has been reported from Lerma marshes in the Central region of the country (Madrado-Garibay and López-Ochoterena, 1985). However, Mexican wetlands have been mainly used for agricultural purposes causing perturbation and loss of biodiversity (Guerra and Ochoa, 2006; Zepeda-Gómez et al., 2012b). This is the case of Lerma marshes that comprise three wetlands: Chignahuapan, Chimaliapan and Chiconahuapan, that are considered as RAMSAR sites and are under

Mexican protection laws (SEMARNAT, 2002; Ceballos, 2003).

The goal of this study is to record free-living ciliate species communities and provide data on their trophic role in a perturbed marsh from Central Mexico.

Material and methods

STUDY AREA

Atarasquillo wetland, also known as Chignahuapan Lake (Fig. 1), is a freshwater marsh near Lerma town in Mexico State, Central Mexico at 2,600 meters above sea level, with 346 ha. The weather is temperate with an annual average temperature of 19 °C (maximum 30 °C and minimum 7 °C). The annual rainfall (1,700 mm total, on average) occurs in summer (June-September) while the dry season occurs in the rest of the year (Ceballos, 2003).

SAMPLING

We sampled at five sites (sites 1-5, Figs 2 a-j), where sites 3 and 4 were located outside the protected area (Fig. 1; SEMARNAT, 2002). A total of 39 water samples were manually collected by using plastic jars of 250 ml, and extracting a volume of 200 ml. Twenty samples were taken during the dry season and 19 during the rainy season. The pH, water temperature and depth were registered *in situ*.

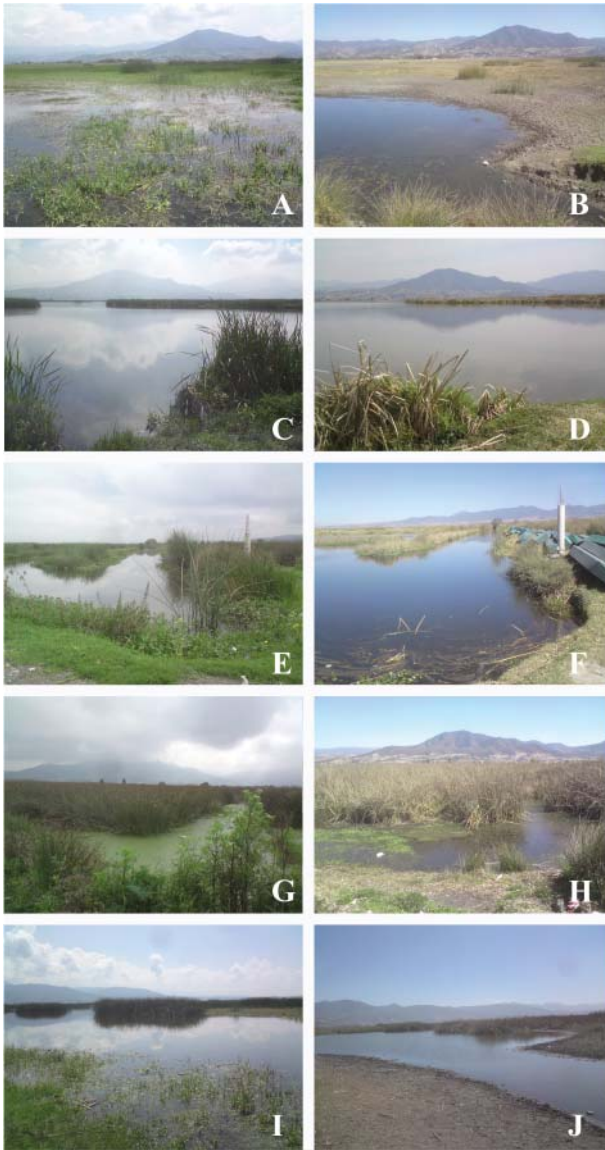


Fig. 2. Photographs of sampling sites during rainy (left picture) and dry season (right picture). Site 1 (A, B), site 2 (C, D), site 3 (E, F), site 4 (G, H), site 5 (I, J).

CILIATES IDENTIFICATION

Water samples were immediately transported to the Laboratorio de Ictiología y Acuicultura, Universidad Autónoma del Estado de México (UAEMex). Ciliates were observed with bright field (Motic BA200) and phase contrast (Carl Zeiss) microscopy. Microphotographs and morphometric data were obtained with Motic Image Plus 2.0 software. Harris hematoxylin and nigrosine staining, and also dry silver nitrate impregnation techniques were used to reveal cytological structures (Klein,

1958; Borror, 1969; Lee et al., 1985; Foissner, 2014). For species identification, Foissner et al. (1991, 1992, 1994, 1995), Borror and Hill (1995), and Berger (1999) were consulted. Taxonomy was based on Lynn (2008).

For each species, their main food, as proposed by Foissner and Berger (1996), was considered, and then categorized into five trophic groups: bacterivorous, omnivorous, mixotrophic, bacteria-algae-heterotrophic flagellate consumers (BAF-consumers), and predators.

STATISTICAL ANALYSIS

The species richness (number of species) registered in each sampling site (Magurran, 2004) was estimated and compared using U-Mann Whitney test to determine the temporal variation between rainy and dry seasons.

We used presence-absence data of ciliate species, regarding the trophic groups, in each site during rainy and dry seasons to calculate frequency, and to identify changes in trophic structure of ciliate communities. Water temperature, pH and water depth of each sampling site were compared between rainy and dry seasons by using U-Mann Whitney test to probe any factor that could be involved in the ciliate composition. All analyses were carried out in StatGraphics XVII software by using a confidence interval of 95%.

Results

ABIOTIC FACTORS

Temperature, pH and water depth data are shown in Table 1. Water temperature and pH were not significantly different between seasons ($p > 0.05$) in each sampling site, but water depth was greater in the rainy season than in the dry season ($p < 0.05$).

SPECIES RICHNESS AND SPECIES COMPOSITION

Thirty ciliate species were identified (Table 2, Figs 3 and 4) belonging to two subphyla, nine classes, 14 orders and 22 families.

Species richness was significantly different between dry (mean 13, total 29) and rainy (mean 6, total 24) seasons ($U = 45.5$, $p < 0.05$), with dry season having higher species richness in all sampling sites (Fig. 5).

Site 1 showed the highest number of species (26

Table 1. Abiotic data of Atarasquillo wetland from Mexico (2012-2013).

Site	Location	Season	pH	Water temperature (°C)	Depth water (cm)
1*	19°21'32.5" N, 99°31'6.3" W	R	5.5±1	19±2.4	40
		D	6.5±1	19.7±5.7	10
2*	19°21'23.1" N, 99°31'0" W	R	5.5±0.5	20.5±1.9	60
		D	6±0.8	18.2±3.5	40
3	19°20'36.7" N, 99°30'9.1" W	R	5.5±0.5	18±2.1	150
		D	5	17±7.1	50
4	19°20'36.7" N, 99°30'8.7" W	R	5.5±0.5	15.7±1.5	120
		D	5	15.2±6.8	50
5*	19°21'43.4" N, 99°30'32.8" W	R	6.3±0.5	24.3±4.5	20
		D	6.5±0.5	23±6.6	10

Notes. R - rainy season, D - dry season, * protected area sampling sites.

species), and the lowest number (17 species) was registered in site 5 (Table 2).

The community composition of ciliates in each sampling site was different during rainy and dry seasons (Table 2).

SEASONAL COMPOSITION OF TROPHIC GROUPS

We observed eight bacterivorous species, eight BAF-consumers, seven omnivorous species, five predators and two mixotrophic species (Table 2).

The frequency of trophic groups in all sampling sites was different between rainy and dry seasons (Table 3; Fig. 6); however, bacterivorous and omnivorous species were more frequent than predator species during both seasons. Mixotrophic species were only found in two sites: 4 and 1.

In sites 1 and 5, during the rainy season, bacterivorous ciliates were more frequent (42.9 % in site 1, 51.6 % in site 5), as compared with dry season (18.4 % in site 1, 28.5 % in site 5), and omnivorous ciliates were more frequent during dry season (44 % site 1, 45.3 % site 5). In site 5, during rainy season about 22.6 % of species were predator ciliates, but in dry season those were less frequent, as compared to the other sampling sites, both in rainy and dry season (except for the mixotrophic ciliate, *Paramecium* cf. *bursaria* from site 1). In site 3, omnivorous ciliates were more frequent (36 %) during rainy season than in dry season (25.5 %), and bacterivorous species were more frequent during dry season (36.6 %).

In site 4, omnivorous ciliates were more frequent in both seasons than other ciliates (35.4 % at rainy and 42.1 % at dry season); mixotrophic ciliates were present in both seasons, but were more frequent during the dry season (14.8 %) than in rainy season (10.8 %).

BAF-consumers ciliates in sites 1, 2, 4 and 5 were more frequent during dry season than rainy season, while in site 3 they were more frequent during rainy season (Table 3).

Discussion

DIVERSITY OF CILIATES IN ATARASQUILLO MARSH

Atarasquillo marsh provided the conditions for colonization of 30 free-living ciliate species during the 2012-2013.

The ciliate species richness in Atarasquillo marsh was greater than in two marshes in Florida, USA where only 3-5 ciliate species were recorded (Henebry et al., 1981), was similar to some peatbogs from Poland (Mieczan, 2007a, 2009), and was lower than in Pantanal Mato Grosso in Brazil (55 species) (Lopes and Heckman 1996), suggesting the appropriate environmental conditions were present for ciliates.

All species have been previously recorded in other aquatic ecosystems, including wetlands (Lopes and Heckman, 1996; Kreutz and Foissner, 2006; Mieczan, 2008, 2009; Tirjaková and Vďačný, 2013). With the exception of *Glaucoma dragescui* Corliss, 1971, recorded by Madrazo-Garibay and López-Ochoterena (1985) in Lerma marshes, all species are recorded for the first time in Mexican wetlands.

SPECIES RICHNESS AND COMPOSITION

The composition of trophic groups and species richness during dry and rainy seasons was different in each sampling site (Figs 5 and 6). These results agree with studies of ciliate assemblages in some

Table 2. Ciliate species composition, seasonal and spatial distribution, and their trophic role in Atarasquillo wetland from Mexico.

Species	Trophic group	Rainy season					Dry season				
		Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5
<i>Loxodes</i> sp.*	O	—	—	—	+	—	+	+	+	+	+
<i>Blepharisma lateritium</i> (Ehrenberg)	BAF	—	—	—	—	—	+	—	—	—	—
<i>Spirostomum minus</i> Roux*	B	—	+	+	—	—	—	—	+	+	—
<i>Spirostomum teres</i> Claparède & Lachmann*	BAF	—	+	+	+	—	+	+	+	+	+
<i>Spirostomum</i> sp.*	BAF	—	—	—	—	—	+	+	—	—	—
<i>Stentor coeruleus</i> (Pallas)*	O	+	—	+	—	—	+	+	—	+	—
<i>Stentor roeselii</i> Ehrenberg*	O	+	+	+	+	—	+	+	+	+	+
<i>Aspidisca</i> sp. *	B	+	+	+	+	+	+	+	+	+	+
<i>Euplotes moebiusi</i> Kahl	BAF	+	+	+	—	+	+	+	+	—	+
<i>Euplotoides</i> sp.	M	—	—	—	+	—	—	—	—	+	—
<i>Euplotoides eurytomus</i> (Wrzesniowski)	O	—	—	+	+	—	+	+	+	+	+
<i>Halteria</i> sp.*	BAF	+	+	—	+	+	+	+	+	+	+
<i>Stylonychia</i> sp.*	O	+	+	+	—	—	+	+	+	+	+
<i>Caenomorpha</i> sp.	B	—	—	—	—	—	—	—	—	+	—
<i>Brachonella</i> sp.	B	—	—	—	+	—	—	—	—	+	—
<i>Didinium</i> sp.*	P	—	—	—	—	—	+	—	—	—	—
<i>Lacrymaria</i> sp.*	P	—	—	—	—	—	+	+	—	—	—
<i>Litonotus lamella</i> (Müller)*	P	+	+	+	+	+	+	+	+	+	+
<i>Litonotus</i> sp.*	P	+	—	—	—	+	+	—	+	—	+
<i>Chilodonella uncinata</i> (Ehrenberg)*	B	+	—	—	+	+	+	+	+	+	+
<i>Coleps hirtus</i> (Müller)*	O	+	+	+	+	+	+	+	+	+	+
<i>Prorodon</i> sp.*	P	—	—	—	—	—	+	+	—	+	—
<i>Plagiopyla nasuta</i> Stein	BAF	+	+	—	+	—	—	—	—	—	—
<i>Frontonia</i> sp.*	O	+	—	—	+	—	+	+	—	+	+
<i>Paramecium aurelia</i> complex	B	+	+	+	—	+	+	+	+	+	+
<i>Paramecium</i> cf. <i>bursaria</i> *	M	—	—	—	—	—	+	—	—	+	—
<i>Paramecium caudatum</i> Ehrenberg*	BAF	+	+	+	+	—	+	+	+	+	+
<i>Urocentrum turbo</i> (Müller)*	BAF	+	—	+	—	—	+	—	+	+	—
<i>Cyclidium glaucoma</i> Müller*	B	+	+	+	+	+	+	+	+	+	+
<i>Tetrahymena pyriformis</i> complex	B	+	+	+	+	+	+	+	+	+	+
Species richness		17	14	15	16	10	25	20	18	23	17

Notes. * Previously recorded in wetlands (Henebry et al. 1981; Lopes and Heckman 1996; Mieczan 2007a, 2009); B - bacterivorous, BAF - consumers of bacteria, algae and small heterotrophic flagellates, M - mixotrophic, O - omnivorous, P - predators, + present, — absent.

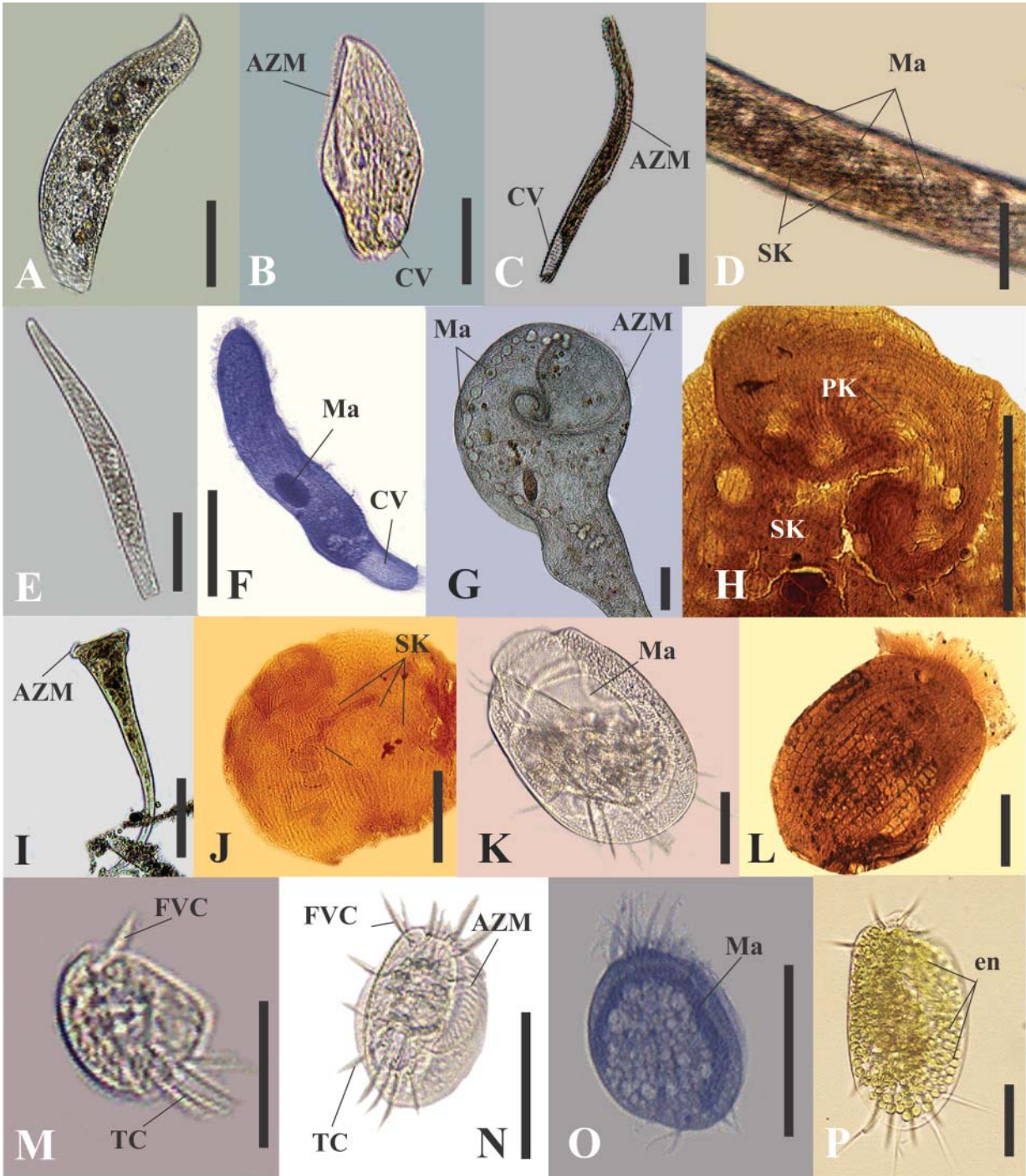


Fig. 3. Ciliates from Atarasquillo wetland. A – *Loxodes* sp.; B – *Blepharisma lateritium*; C, D – *Spirostomum minus*; E, F – *Spirostomum teres*; G, H – *Stentor coeruleus*; I, J – *Stentor roeselii*; K, L – *Euplotoides eurystomus*; M – *Aspidisca* sp.; N, O – *Euplotes moebiusi*; P – *Euplotoides* sp. Abbreviations: AZM – adoral zone of membranelles, CV – contractile vacuole, SK – somatic kineties, Ma – macronucleus, PK – perioral kineties, FVC – frontoventral cirri, TC – transversal cirri, en – endosymbiotic algae. Scale bars: 50 µm.

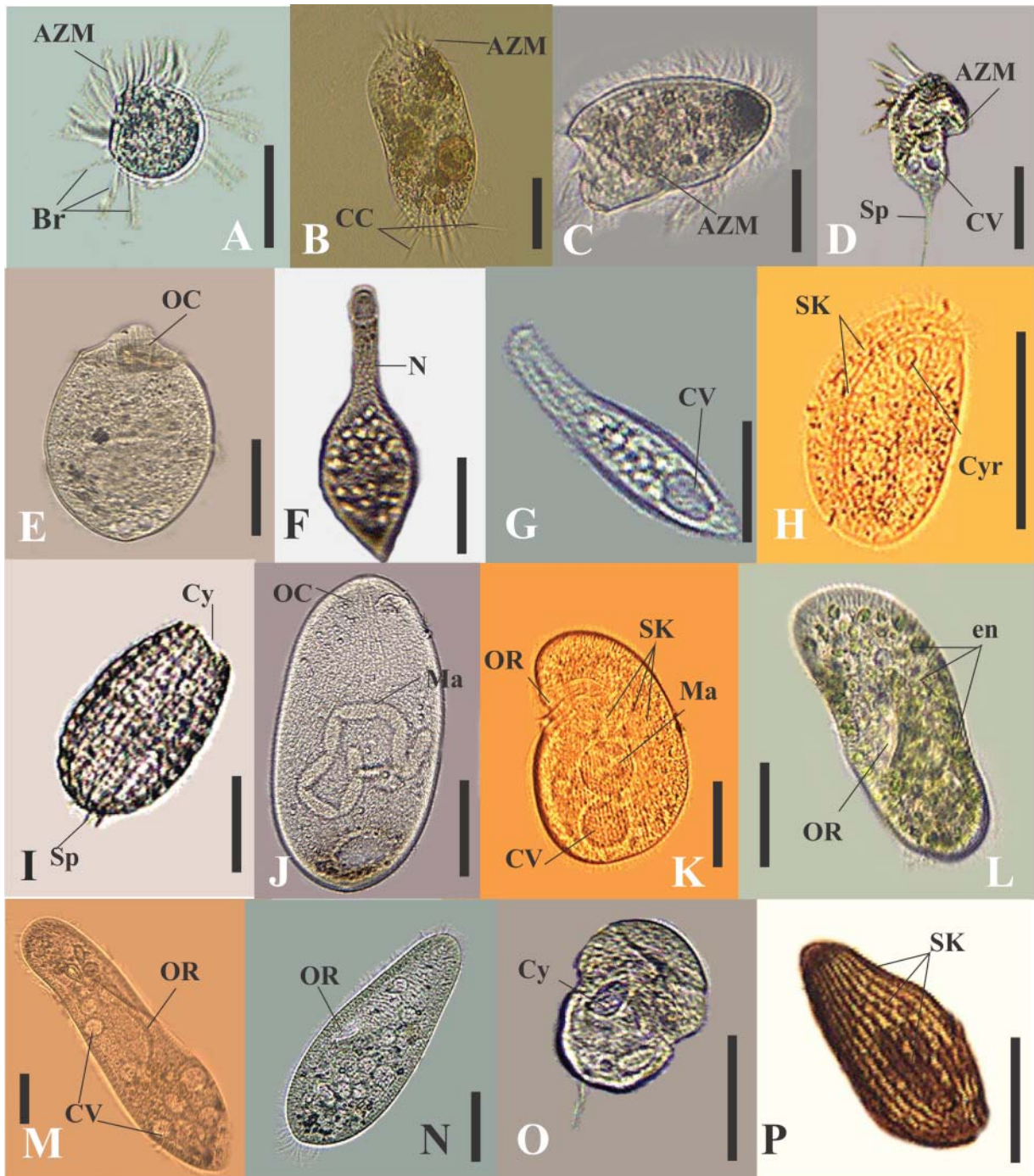


Fig. 4. Ciliates from Atarasquillo wetlands. A – *Halteria* sp.; B – *Stylonychia* sp.; C – *Brachonella* sp.; D – *Caenomorpha* sp.; E – *Didinium* sp.; F – *Lacrymaria* sp.; G – *Litonotus lamella.*; H – *Chilodonella uncinata*; I – *Coleps hirtus*; J – *Prorodon* sp.; K – *Plagiopyla nasuta*; L – *Paramecium* cf. *bursaria*; M – *Paramecium caudatum*; N – *Paramecium aurelia*; O – *Urocentrum turbo*; P – *Tetrahymena pyriformis*. Abbreviations: Br - bristles, CC - caudal cirri, Sp - spine, OC - oral cone, N - neck, Cyr - cyrtos, Cy - cytostome, OR - oral region, for explanation of other symbols see Fig. 3. Scale bars: 50 μ m.

Table 3. Frequency (%) of ciliates trophic groups in every site during rainy and dry seasons.

Site	1*		2*		3		4		5*	
	R	D	R	D	R	D	R	D	R	D
Bacterivorous	42.9	18.4	39.3	19.6	22.7	36.2	23.1	14.8	51.6	28.5
BAF-consumers	18.2	23.1	32.8	36.9	32.0	28.2	23.1	26.3	9.7	16.8
Omnivorous	28.6	44.0	21.3	31.3	36.0	25.5	35.4	42.1	16.1	45.3
Predators	10.4	13.2	6.6	12.1	9.3	10.1	7.7	1.9	22.6	9.5
Mixotrophic	0.0	1.3	0.0	0.0	0.0	0.0	10.8	14.8	0.0	0.0

Notes. R - rainy season, D - dry season, * protected area sampling sites.

peatbogs from Poland, which changed when reeds were removed, suggesting the involvement of physicochemical processes in determining community assemblages (Mieczan et al., 2018). In Atarasquillo wetland marsh some macrophytes, e.g. *Hydrocotyle* sp., *Typha* sp., are removed during the dry season by human activities, which may contribute to changes in ciliate richness and assemblage composition. In addition, heavy metals have been reported from *Hydrocotyle* sp. in surrounding areas of Atarasquillo wetland (Ceballos, 2003; Zarazúa et al., 2013), and it has been reported that heavy metals can be toxic to ciliates (Madoni and Giuseppa, 2006).

Most of the identified species: i.e., *Spirostomum minus*, *Stentor coeruleus*, *S. roeselii*, *Chilodonella uncinata*, *Coleps hirtus*, *Paramecium caudatum* (bacterivorous, BAF-consumers and omnivorous), were found in more than three sampling sites, including both or one season, and have been considered as freshwater common species due to their capacity to tolerate changes in their habitats (Foissner et al., 1991, 1992, 1994, 1995; Foissner and Berger, 1996; Pfister et al., 2002). Regarding their wide distribution, *Spirostomum minus*, *S. teres*, *Paramecium aurelia*, *P. caudatum*, *Coleps hirtus*, have been recorded in the five continents (Fokin, 2010; Boscaro et al., 2014), and *Stentor coeruleus*, *S. roeselii*, *S. minus*, *Euplotoides eurystomus* in four continents (Foissner et al., 1991, 1992).

In site 5, where the vegetation coverage was lesser or absent relative to the other sites, the ciliate species richness was lower as well. Madoni (1991), Lugo et al. (1998), Song (2000) and Babko et al. (2010) concluded that habitats with macrophyte presence are appropriate for ciliate assemblages, with high content of organic matter favoring bacteria growing. Thus, we agree that macrophyte presence is an important factor for the assemblages of ciliates in wetlands, setting a microbial food web with bacterivorous ciliates and BAF-consumers, that are the

food resources for omnivorous and predator ciliates.

SEASONAL VARIATION OF TROPHIC GROUPS

Seasonally we observed changes between bacterivorous and omnivorous ciliates (Fig. 6). In general, in sites 1, 2 and 5, during rainy season, most bacterivorous ciliates were found, in comparison to the dry season where the water level dropped off (Table 1), causing some changes in the composition of vegetation (Zepeda-Gómez et al., 2012a). Moreover, Ceballos (2003) reported in Lerma marshes that the total density of coliforms was lower during dry season than in rainy season. Indirectly we could assume that total coliforms are changing between seasons because our data showed the bacterivorous ciliates decreased in the dry season. Also, Debastiani et al. (2016) found a positive correlation between the presence of bacterivorous ciliates and total coliforms. During the dry season, omnivorous ciliates were more frequent than the remaining groups, likely due to the advantages for a wider diet feeding on algae, protists and small metazoan (Foissner and Berger, 1996; Dias and D'Agosto, 2006).

Regarding ciliate composition in site 4, we observed that omnivorous ciliates were more frequent than other groups, and in addition, two mixotrophic ciliates were also observed, *Paramecium* cf. *bursaria* and *Euplotoides* sp. Mieczan (2009) observed a major percentage of mixotrophic and a relatively high percentage of omnivorous ciliates in *Sphagnum*-peatlands, similarly to our results. Furthermore, we found some anaerobic ciliates, i.e. *Brachonella* sp., *Caenomorphia* sp. and *Plagiopyla nasuta* (Foissner et al., 1994, 1995; Foissner and Berger, 1996; Guhl et al., 1996), and microaerophilic ciliates, i.e. *Spirostomum teres* (Madoni, 1991), probably due to low oxygen concentrations. This finding agrees with Headley and Tanner (2012) who showed that low dissolved oxygen concentrations are common in wetlands dominated by free-floating macrophytes,

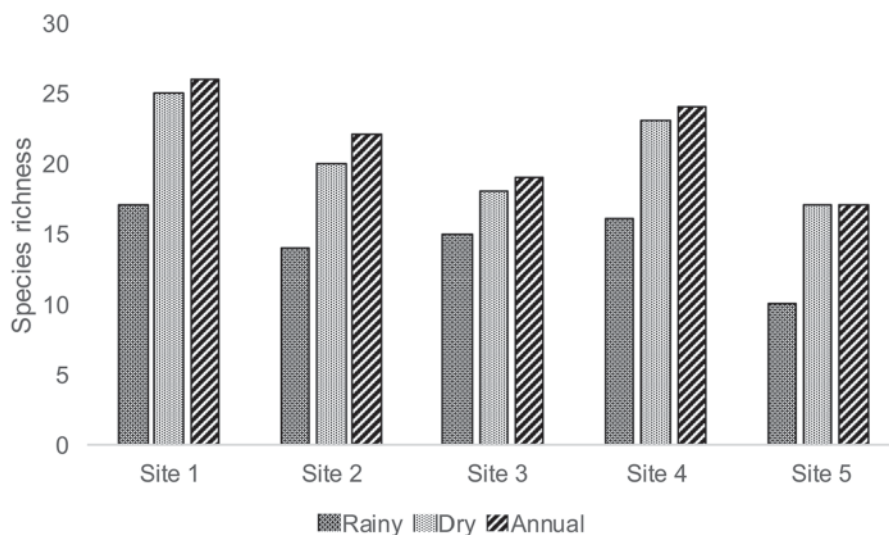


Fig. 5. Ciliate species richness during rainy and dry seasons and for an annual period, for each sampling site.

as in site 4, which was covered in whole sampling period with free-floating macrophytes i.e. *Marsilea mollis* and *Lemna* sp., providing a barrier against aeration from the atmosphere. Sipaúba-Tavares and Dias (2014) mentioned that shallow waters with macrophytes promote good conditions to maintain a highly diverse planktonic community and macrofauna associated with plants.

Only four species were categorized as predator ciliates and they were less frequent, as has been observed in other aquatic ecosystems (Mieczan, 2009; Babko et al., 2010).

ABIOTIC FACTORS

Pfister et al. (2002) argued that the ecological preferences of some common ciliates seem to be wider, and that temperature, pH and oxygen seem to have only weak influence on the distribution of some ciliate species. All species from Atarasquillo wetland have been previously recorded in a wide range of temperature (Foissner et al., 1991, 1992, 1994, 1995; Pfister et al., 2002), and because our physicochemical results were not significantly different between seasons at each sampling site ($p > 0.05$), we conclude that there was no influence of these factors on the ciliate species distribution (Figs 5 and 6, Table 1).

FURTHER CONSIDERATIONS

Most species that we found have been categorized as key species of α -mesosaprobic and β -mesosaprobic waters (Foissner and Berger, 1996);

however, we also found, mainly in site 4, species known as indicators of polysaprobic, metasaprobic and isosaprobic waters, i.e. *Loxodes* sp., *Paramecium caudatum*, *Brachonella* sp., *Caenomorpha* sp., *Plagiopyla nasuta* (Foissner and Berger, 1996; Luna-Pabello, 2006). Moreover, *Brachonella* sp., *Caenomorpha* sp. and *Plagiopyla nasuta* produce gas methane, thus, those ciliates could be used to determinate water quality in Lerma marshes, as in other aquatic ecosystems (Beaver and Crisman, 1989; Foissner and Berger, 1996; Jiang et al., 2011; Tirjaková and Vďačný, 2013; Debastiani et al., 2016).

Furthermore, our results showed that seasonality could be an important factor for the assemblages of ciliated trophic groups through changes in water level and vegetation composition.

Atarasquillo marsh is the most diverse and the most heterogeneous in composition of macrophyte communities than the other two Lerma marshes (Zepeda-Gómez et al., 2012a), and despite this wetland is under legal protection, it has been altered by anthropogenic activities resulting in lower water quality where some heavy metals have been found threatening the biota (Ceballos, 2003). Wetlands are important ecosystems playing a role as soil and minerals recyclers, climate stabilizers, and providing habitat for the biota (Mitsch and Gosselink, 2015), where ciliates are mineral recyclers in water as part of microbial food webs and could be used in further studies in monitoring the restoration of wetlands (Mieczan et al., 2018) like Lerma marshes, which are threatened of area reduction (Zepeda-Gómez et al., 2012b).

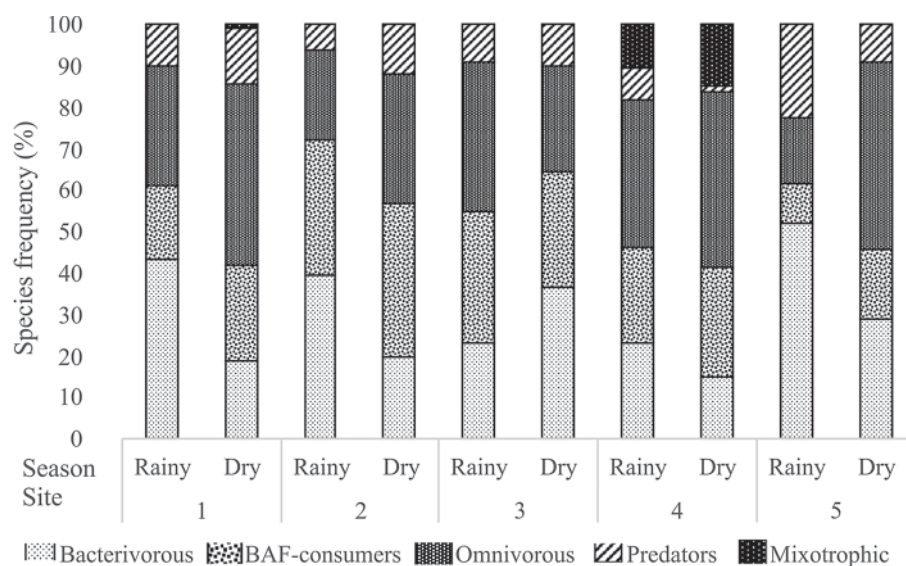


Fig. 6. Frequency of ciliate trophic groups during rainy and dry seasons in each sampling site (numbers 1-5).

Acknowledgements

The authors acknowledge Universidad Autónoma del Estado de México Project 3429/2013CHT for financial support of field research and Biól. M. Reyes-Santos, Lab. de Protozoología, Universidad Nacional Autónoma de México, for her assistance in staining and impregnations techniques. We thank to Dr. Rebecca Zufall, Department of Biology and Biochemistry, University of Houston, USA, for assisting with the English.

References

- Babko R., Fyda J., Kuzmina T. and Hutorowicz A. 2010. Ciliates on the macrophytes in industrially heated lakes (Kujawy Lakeland, Poland). *Vestn. Zool.* 46 (6), 1–11.
- Beaver J.R. and Crisman T.L. 1989. Analysis of the community structure of planktonic ciliated protozoa relative to trophic state in Florida lakes. *Hydrobiologia.* 174, 177–184.
- Berger H. 1999. Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). Kluwer Academy Pub., London.
- Borror A.C. 1969. Application for stain-fixative nigrosin-HCl₂-formalin to fragile or contractile ciliates. *Trans. Am. Microsc. Soc.* 88, 454–458.
- Borror A.C. and Hill B.F. 1995. The order Euplotida (Ciliophora): taxonomy, with division of *Euplotes* into several genera. *J. Eukaryot. Microbiol.* 42, 457–466.
- Boscaro V., Carducci D., Barbieri G., Senra M.V.X., Andreoli I., Erra F., Petroni G., Verni F. and Fokin S.I. 2014. Focusing on genera to improve species identification: Revised systematics of the ciliate *Spirostomum*. *Protist.* 165, 527–541.
- Ceballos G. 2003. Ficha informativa de los humedales Ramsar (FIR): Ciénegas de Lerma. In: Ramsar sites information service. Available via pdf <https://rsis.ramsar.org/ris/1335> accessed 28 November 2016.
- Debastiani C., Meira B.R., Lansac-Tõha M.N., Velho L.F.M. and Lansac-Tõha F.A. 2016. Protozoa ciliates community structure in urban streams and their environmental use as indicators. *Braz. J. Biol.* 76, 1043–1053.
- Dias R.J.P. and D'Agosto M. 2006. Feeding behavior of *Frontonia leucas* (Ehrenberg) (Protozoa, Ciliophora, Hymenostomatida) under different environmental conditions in a lotic system. *Rev. Bras. Zool.* 23, 758–763.
- Finlay J.B. and Esteban G.F. 1998. Freshwater protozoa: biodiversity and ecological function. *Biodivers. Conserv.* 7, 1173–1186.
- Foissner W. 2014. An update of 'basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa'. *Int. J. Syst. Evol. Microbiol.* 64, 271–292.
- Foissner W. and Berger H. 1996. An user friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. *Freshwater Biol.* 35, 375–482.

- Foissner W., Berger H. and Kohmann F. 1994. Taxonomische und Ökologische Revision der Ciliaten des Saprobiensystems. Band III: Hymenostomata, Prostomatida, Nassulida. Informationsberichte des Bayer. Landesamts für Wasserwirtschaft. 1/94, 1–548.
- Foissner W., Blatterer H., Berger H. and Kohmann F. 1991. Taxonomische und Ökologische Revision der Ciliaten des Saprobiensystems. Band I: Cytrophorida, Oligotrichida, Hypotrichia, Colpodea. Informationsberichte des Bayer. Landesamts für Wasserwirtschaft. 1/91, 1–478.
- Foissner W., Blatterer H., Berger H. and Kohmann F. 1992. Taxonomische und Ökologische Revision der Ciliaten des Saprobiensystems. Band II: Peritrichia, Heterotrichida, Odontostomatida. Informationsberichte des Bayer. Landesamts für Wasserwirtschaft. 5/92, 1–502.
- Foissner W., Blatterer H., Berger H. and Kohmann F. 1995. Taxonomische und Ökologische Revision der Ciliaten des Saprobiensystems. Band IV: Gymnostomatea, *Loxodes*, Suctorina. Informationsberichte des Bayer. Landesamts für Wasserwirtschaft. 1/95, 1–540.
- Foissner W., Chao A. and Katz L.A. 2008. Diversity and geographic distribution of ciliates (Protista: Ciliophora). *Biodivers. Conserv.* 17, 345–363.
- Fokin S.I. 2010. *Paramecium* genus: biodiversity, some morphological features and the key to the main morphospecies discrimination. *Protistology.* 6 (4), 227–235.
- Guerra V.M. and Ochoa G.S. 2006. Evaluación espacio-temporal de la vegetación y uso del suelo en la Reserva de la Biósfera Pantanos de Centla, Tabasco (1990–2000). *Invest. Geog.* 59, 7–25.
- Guhl B.E., Finlay B.J. and Schink B. 1996. Comparison of ciliate communities in the anoxic hypolimnion of three lakes: general influence features and the influence of lake characteristics. *J. Plankton Res.* 18, 335–353.
- Headley T.R. and Tanner C.C. 2012. Constructed wetlands with floating emergent macrophytes: an innovative stormwater treatment technology. *Crit. Rev. Env. Sci. Tec.* 42, 2261–2310.
- Henebry M.S., Cairns J.J., Schwintzer C.R. and Yongue W.H.J. 1981. A comparison of vascular vegetation and protozoa communities in some freshwater wetlands of Northern Lower Michigan. *Hydrobiologia.* 83, 353–375.
- Jiang Y., Xu H., Hu X., Zhu M., Al-Rasheid K.A.S. and Warren A. 2011. An approach to analyzing spatial patterns of planktonic ciliates communities for monitoring water quality in Jiaozhou Bay, northern China. *Mar. Pollut. Bull.* 62, 227–235.
- Johnson M.D. 2011. Acquired phototrophy in ciliates: a review of cellular interactions and structural adaptations. *J. Eukaryot. Microbiol.* 58, 185–195.
- Klein B.M. 1958. The “dry” silver method and its proper use. *J. Protozool.* 5, 99–103.
- Kreutz M. and Foissner W. 2006. The sphagnum ponds of Simmelried in Germany: a biodiversity Hot-Spot for microscopic organisms. *Protozoological Monographs.* 3, 1–267.
- Lee J.J., Small E.B., Lynn D.H. and Bovee E.C. 1985. Some techniques for collecting, cultivating and observing protozoa. In: *An illustrated guide to the protozoa* (Eds: Lee J.J., Hutner S.H. and Bovee E.C.). Society of Protistologists, Lawrence, Kansas, pp. 1–7.
- Lopes H.E. and Heckman C.W. 1996. The seasonal succession of biotic communities in wetlands of the Tropical wet-and-dry climatic zone: IV. The free-living sarcodines and ciliates of the Pantanal of Mato Grosso, Brazil. *Int. Rev. gesamten Hydrobiol. Hydrogr.* 81, 367–384.
- Lugo A., Alcocer J., Sánchez M.A. and Escobar E. 1998. Littoral protozoan assemblages from two Mexican hyposaline lakes. *Hydrobiologia* 381, 9–13.
- Luna-Pabello V.M. 2006. Atlas de ciliados y otros microorganismos frecuentes en sistemas de tratamiento aerobio de aguas residuales. Facultad de Química, Universidad Nacional Autónoma de México, México.
- Lynn D.H. 2008. The ciliated protozoa. Characterization, classification, and guide for the literature. Springer, New York.
- Madoni P. 1991. Community structure and distribution of the ciliate protozoa in a freshwater pond covered by *Lemna minor*. *Boll. Zool.* 58, 273–279.
- Madoni P. 2005. Ciliated protozoan communities and saprobic evaluation of water quality in the hilly zone of some tributaries of the Po River (northern Italy). *Hydrobiologia.* 541, 55–69.
- Madoni P. and Giuseppa M. 2006. Acute toxicity of heavy metals towards to freshwater ciliated protists. *Environ. Pollut.* 141, 1–7.
- Madrado-Garibay M. and López-Ochoterena E. 1985. Protozoarios ciliados de México XXV. Estomatogénesis en *Glaucoma dragescui* Corliss,

- 1971 (Oligohymenophorea, Hymenostomatida). *Rev. Lat-amer. Microbiol.* 27, 45–48.
- Magurran A.E. 2004. *Measuring biological diversity*. Blackwell Publishing, Oxford.
- Mieczan T. 2007a. Epiphytic protozoa (testate amoeba and ciliates) associated with *Sphagnum* in peatbogs: relationship to chemical parameters. *Pol. J. Ecol.* 55, 79–90.
- Mieczan T. 2007b. Seasonal patterns of testate amoeba and ciliates in three peatbogs: relationship to bacteria and flagellates (Poleski National Park, Eastern Poland). *Ecohydrol. Hydrobiol.* 7, 79–88.
- Mieczan T. 2008. Diversity and vertical distribution of planktonic ciliates in a stratified mesotrophic lake: relationship to environmental conditions. *Oceanol. Hydrobiol. Stud.* 37, 83–95.
- Mieczan T. 2009. Ciliates in *Sphagnum* peatlands: vertical micro-distribution, and relationship of species assemblages with environmental parameters. *Zool. Stud.* 48, 33–48.
- Mieczan T., Adamczuk M. and Pogorzelec M. 2018. Ciliates as restoration indicators in peatbogs – 10 years of study. *Eur. J. Protistol.* 62, 11–23.
- Mitsch W.J. and Gosselink J.G. 2015. *Wetlands*. Wiley, New Jersey.
- Pfister G., Auer B., Arndt H. 2002. Pelagic ciliates (Protozoa: Ciliophora) of different brackish and freshwater lakes - a community analysis at the species level. *Limnologica.* 32, 147–168.
- SEMARNAT. 2002. Decreto por el que se declara área natural protegida, con el carácter de área de protección de flora y fauna, la región conocida como Ciénegas de Lerma en el Estado de México, con una superficie total de 3,023-95-74.005 hectáreas. *Diario Oficial de la Federación* 27 de Noviembre 2002, Secretaría de Medio Ambiente y Recursos Naturales, México. pp. 4–12.
- Sipaúba-Tavares L.H. and Dias S.G. 2014. Water quality and communities associated with macrophytes in a shallow water-supply reservoir on an aquaculture farm. *Braz. J. Biol.* 74 (2), 420–428.
- Song B. 2000. A comparative study on planktonic ciliates in two shallow mesotrophic lakes (China): species composition, distribution and quantitative importance. *Hydrobiologia.* 427, 143–153.
- Tirjaková E. and Vďačný P. 2013. Analysis and evolution of water quality of the upper Váh river (northern Slovakia) by long-term changes in the community structure of ciliates (Protista: Ciliophora). *Biologia.* 68, 667–678.
- Weisse T. 2002. The significance of inter- and intraspecific variation in bacterivorous and herbivorous protists. *Antonie van Leeuwenhoek.* 81, 327–341.
- Weisse T. 2017. Functional diversity of aquatic ciliates. *Eur. J. Protistol.* 61, 331–358.
- Zarazúa G., Ávila-Pérez P., Tejeda S., Valdivia-Barrientos M., Zepeda-Gómez C. and Macedo-Miranda G. 2013. Evaluación de los metales pesados Cr, Mn, Fe, Cu, Zn y P en sombrero de agua (*Hydrocotyle ranunculoides*) del curso Alto del Río Lerma, México. *Rev. Int. Contam. Ambient.* 29, 17–24.
- Zepeda-Gómez C., Lot-Helgueras A., Nemiga X.A. and Madrigal-Uribe D. 2012a. Florística y diversidad de las Ciénegas del Río Lerma Estado de México, México. *Acta Bot. Mex.* 98, 23–49.
- Zepeda-Gómez C., Nemiga X.A., Lot-Helgueras A. and Madrigal-Uribe D. 2012b. Análisis del cambio del uso de suelo en las ciénegas de Lerma (1973–2008) y su impacto en la vegetación acuática. *Invest. Geog.* 78, 48–61.

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