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Morphology and Ecology of Freshwater-blooming *Durinskia baltica* (Dinophyceae: Peridiniales) in Xochimilco, Mexico

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Authors' contributions

This work was carried out in collaboration between all authors. Author RT designed the study, performed the statistical analysis, wrote the first draft of the manuscript and managed literature searches. Authors BL, MWP and RT wrote the protocol, managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Framework and Aims: A dinoflagellate identified as *Durinskia baltica* has caused early spring blooms since 2007 in a eutrophic freshwater channel system influenced by agriculture and livestock in central Mexico. The goals of this study were to document the morphology of this freshwater strain of *D. baltica,* and describe its bloom formation in relationship with local environmental conditions.
Study Design, Place and Duration: The species blooming in Xochimilco was identified through light and electron microscopy, recording cellular structures that distinguish it from other Dinophyceae. Environmental and climatic variables were monitored during one-year collections of biological material to document the ecology of the species.

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Methodology: Biweekly and monthly surveys (September 2010 to January 2011 and fortnightly from February to August 2011) were conducted (calibrated field sensors and laboratory analysis) and population density of flagellate cells and cysts were performed (Sedgwick-Rafter chamber). For data analysis, descriptive statistics, correlations between variables and a Categorical Principal Components Analysis were performed.
Results: Morphological study of *D. baltica* revealed the typical plate 1a, sulcal plates, endosymbiont nucleus and stigma. Pearson correlations confirmed a significant relationship between flagellate cells and environmental temperature (P = .02) and pH (P = .003). Cysts were correlated with annual rainfall (P = .001). Total variance explained by the two-dimensional CATPCA (> 76%) model showed a strong association between the maximum and minimum species densities, with ambient conditions and trophic status indicating that nutrients, pH and climatic factors were parameters responsible for bloom formation and cyst occurrence.
Conclusions: *D. baltica* is indeed a freshwater species and is able to live at relatively high temperature in shallow, eutrophic columns. A seasonal effect and hence an association with temperature suggests that water bodies in urban-influenced tropical latitudes may be well suited as environments for *D. baltica* blooms.

Keywords: Dinophyceae; Durinskia; freshwater blooms; thecal morphology; tropical ecosystems.

1. INTRODUCTION

Durinskia baltica (Levander) Carty & Cox 1986 is a dinoflagellate species that has been reported in Canada [1], the United States [2], the Pacific Coast of Mexico [3], Brazil [4,5], Africa [6], Asia [7,8,9] Australia [10], and Europe [11]. These reports correspond to a broad ecological spectrum, both in lacustrine (freshwater and brackish water) and coastal marine environments [1-11]. Based on morphology, *D. baltica* may be easily mistaken with *Durinskia occulata* Hansen & Flaim and also with other genera such as *Kryptoperidinium foliaceum* (Stein) Lindemann and *Glenodiniopsis steinii* (Lemmermann) Woloszynska when identified only with light microscopy [12,6]. This gross morphological similarity with other taxa is reflected in a complicated systematic history [2,13] including its original identification as *Peridinium balticum* (Levander) Lemmermann [14]. *Durinskia baltica* and the similar-appearing dinoflagellates stated above have been reported as occurring in freshwater, such as shallow pools in Texas, USA [2], in eutrophic reservoirs in Brazil [4], and recently in ponds in central China [9]. However, some authors consider it to be primarily a marine species [3,6,11,12,15].

An apparent cause of this discrepancy is misidentification: lack of focus on key morphological features such as thecal plate tabulation in some works addressing more cytological and/or ecological aspects have caused some authors [3,12] to relate the presence of *D. baltica* in freshwater to different look-alike taxa, limiting its reported ecological distribution primarily to marine systems. Therefore, detailed studies of both morphology and ecological aspects of this species are needed to clarify its habitat diversity and distribution, and to shed light on the problematic systematics of the group. In the present study, we report the occurrence of a nearly monoalgal *D. baltica* dinoflagellate bloom in a freshwater channel of the Xochimilco district in central Mexico. We provide documentation of the systematically relevant aspects of thecal tabulation and cytology of this taxon and report observation of field-bloom-occurring morphologies that may correspond to *in situ* life cycle stages.

2. MATERIALS AND METHODS

2.1 Study Site

The study sites were located at the freshwater channel system of the Xochimilco farming region (19°16′30″N 99°08′20″W) in the south of Mexico City at an altitude of 2240 meters above sea level. This water system was originally part of the great lake area where the Aztecs settled in 1215 and now exists as a small lake/channel system that receives rainwater and municipally treated water inputs. This system is considered to be a freshwater environment according to the registration of less than 2% average salinity [16]. The climate is temperate with maximum rainfall and humidity from May to September. In 2011 the average annual rainfall was 67.5 mm and the average annual temperature was 16°C with extreme values of 9° and 33°C [17]. The Xochimilco area is composed of small, shallow

(average depth of ~3 m) lakes and channels that form an extensive network of 522 km^2. The region is heavily farmed and fertilized, and received treated municipal water to compensate for volume loss during droughts. These practices have resulted in a eutrophic to hypereutrophic aquatic system [18]. According to Tavera & Díez [19], *D. baltica* has been observed in the same lake area in La Virgen lagoon and El Bordo channel (Fig. 1) in addition to El Japón channel (Fig. 1). The primary sites investigated here (Fig. 1) were the El Japón channel (19° 16' 56.10" N; 99° 04' 15.57" W), where *D. baltica* was observed to exhibit recurrent blooms in the phytoplankton during spring (87% of the total phytoplankton) and the Tláhuac channel (19° 16' 06.70" N; 99° 00' 27.72" W).

2.2 Environmental Sampling and Monitoring

Sampling at El Japón and Tláhuac channels was conducted monthly from September 2010 to January 2011 and fortnightly from February to August 2011. Cell abundance was determined at the surface water (250 mL) collected from a fixed location beside a pier. Duplicate samples were preserved with 0.25% (final) Lugol's solution (for cell counts) or 2% formalin (for herbarium material deposited at the Faculty of Sciences, FCME, UNAM). *Durinskia baltica* cells and the rest of the phytoplankton community were counted from Lugol's-fixed samples using a Sedgwick-Rafter counting chamber [20]. To determine cyst abundance in sediments at each site, surface sediment cores (10 cm^2 surface area) were collected in triplicate from randomized spots over an established area of 2 m^2. Only the core surfaces (0.6 cm^2 depth) of each sample were processed to determined cyst abundance. *D. baltica* cyst identification was based on described morphology [21,22]. *D. baltica* cysts were counted using a Sedgwick-Rafter counting chamber. *In situ* environmental parameters were recorded at each site. Water temperature, pH, conductivity, and dissolved oxygen were measured with calibrated field sensors (Conductronic PC18™, Puebla, Pue., México). For nutrient determination, 300 mL of surface water was collected at each site using water analysis methods approved by USEPA [23]. Briefly, total phosphorus was determined by acid digestion; total dissolved inorganic nitrogen (DIN) as nitrate (NO_3–N) by cadmium reduction, nitrite (NO_2–N) using the diazotization method, and ammonium (NH_4–N) by the salicylate method. These methods were carried out with a DR2010 Portable Datalogging Spectrophotometer ™ (Hach™, Loveland, CO, USA). Local precipitation and temperature data were obtained through the National Meteorological System [17]. Chemical analyses were carried out with dry methods following the procedures described in APHA [24] for inland surface waters. Briefly, bicarbonates were measured by acid titration to pH 4.6 using a methyl red and bromocresol green mixture as the indicator, and carbonates were measured by titration with phenolphthalein. Sulphate was determined with turbidimetry. Calcium and magnesium concentrations were obtained by complexometric titration with EDTA. Chloride was determined with an ion-selective electrode by adding 5M of a $NaNO_3$ solution as an ionic strength adjuster. Sodium and potassium were measured by atomic emission spectroscopy.

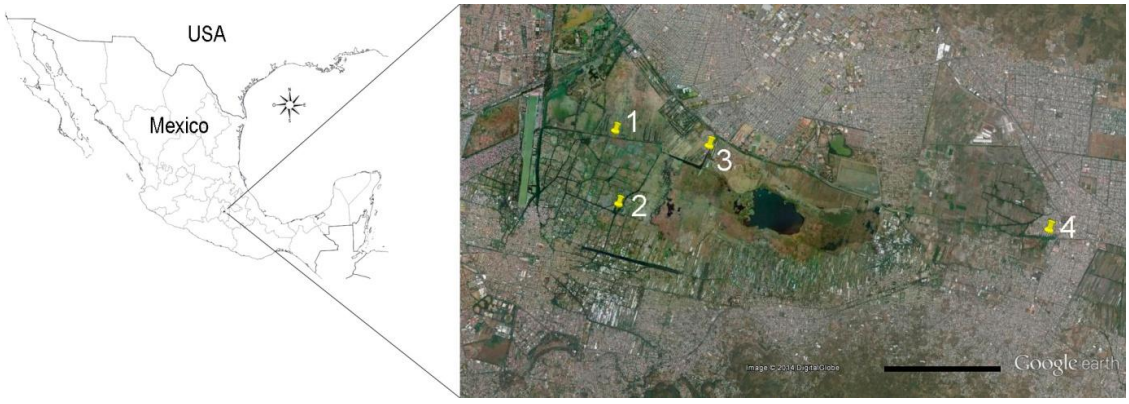

Fig. 1. Freshwater sites in the region of Xochimilco, Mexico, where *Durinskia baltica* has been observed

1 = El Bordo channel; 2 = La Virgen lagoon; 3 = El Japón channel; 4 = Tláhuac channel. Source: Google Earth (images date, August 4, 2014). Bar = 2 km

2.3 Morphology and Cytology

Specimens collected from El Japón channel were identified according to literature descriptions [2,14]. For light microscopic determination of thecal tabulation and general morphology, cells were bleached with sodium hypochlorite [25] and treated with chloral hydrate (25%), or trypan blue (1:1) and calcofluor white stain/evans blue (1:0.5) (Fluka™, Buchs, Wdg., Switzerland). Individual specimens were observed and photographed using light microscopy in ventral, dorsal, apical and antapical views with a Nikon Optiphot microscope™ equipped with a digital camera (Nikon Coolpix S10™, Melvile, NY, USA).

For scanning electron microscopy (SEM), samples (250 mL) from the study sites were prefiltered through Millipore Nylon mesh (20 µm mesh size) to remove larger particles. Cells were concentrated on nitrocellulose membrane (0.22 µm GSWP) filters by gravity filtration (Millipore™, Billerica, MA., USA). Filters with cells were fixed for two hours at 23°C with 2% (final) glutaraldehyde (GTA) in 20 mL sodium cacodylate buffer (NaCac) 0.1 M, pH 7.2.

Specimens were then dehydrated through an ascending ethanol series (50%, 70%, 95% and 100% for 15 minutes at each concentration) and transferred to a Petri dish with amyl acetate for 2-3 hours, after which the amyl acetate was replaced with CO_2 to critical point dry (Tousimis Samdri-700). Filter specimens were mounted on SEM stubs, coated with 20 nm Au-Pd, and observed and photographed using a Jeol 5410 LV SEM (Jeol JFC1100™ ionizer, Peabody, MA, USA).

For transmission electron microscopy (TEM), samples (250 mL) from El Japón channel were first concentrated by centrifugation. Pelleted material (ca. 100 µl) was then preserved as for SEM and washed 3 x 10 min with 0.1 M NaCac buffer, pH 7.2. A secondary fixation was performed with OsO_4 (2% final conc.) in NaCac buffer for 1 h at 23°C and the sample was then dehydrated in an ascending ethanol series (70%, 85%, 95%, 100%) for 15 min at each concentration. Samples were embedded in LR-White resin (Sigma–Aldrich™, St. Louis, MO, USA) and the resulting block sectioned (40 nm). Sections were treated for 60 min with 2% uranyl acetate in aqueous solution for one hour and observed with a Jeol 1200EXII TEM.

2.4 Data Analysis

SPSS Statistics 19.0 (IBM Corp. Armonk, NY, USA) was used to conduct descriptive statistics and correlations between variables. By scaling some of the variables (SPSS visual binning), a Categorical Principal Components Analysis (CATPCA) was performed with transformation for the data. Selected variables were chosen based on the statistically significant Pearson correlation values at P = .01 (two-tailed), or P = .05 (two-tailed); environmental variables were also previously discriminated according to their normal distribution using a statistical measure of asymmetry. The reliability of the constructed model was based on the internal consistency coefficient (Cronbach's Alpha) for each specified dimension (2 dimensions) and the combination of both dimensions established the total eigenvalue.

3. RESULTS

3.1 General Morphology and Cytology

Vegetative cells were spherical to oval in shape, slightly flattened dorsiventrally, and measured 24.0–34.0 µm length x 18.0–22.0 µm width (n = 226). Typically the epitheca and the hypotheca were similar in size (Fig. 2a), although the epitheca was slightly longer (3.0–5.0 µm) in some specimens. The cingulum was oriented as planozone-descending; and the sulcus was wide and extended to the antapex. The dinokaryon (nucleus) showed permanently condensed chromosomes and was generally located in the cell center and exhibited an elongated shape, measuring 16.0–24.0 µm length x 10.0–12.0 µm width (n = 197) (Figs. 2a, 3a–b). A prominent red stigma (eyespot) composed of two parts was found within the sulcal region of the hyposome (Fig. 2a, Figs. 3a, c). Red accumulation bodies were also typically observed in the episome and numerous golden-brown discoid to irregular chloroplasts were scattered throughout the cell (Fig. 2a). A nuclear structure of symbiotic origin was observed to be generally located in the margin of the cells (Figs. 2a, 3a); this structure shared a common membrane with chloroplasts (Fig. 3a). TEM observations demonstrated a detailed arrangement of membranes and various structures showing in particular the stigma complex (Fig. 3a–c). Temporary cysts (i.e. when the cell loses mobility and emerges from the theca) were observed occasionally. In this structure the stigma was not observed and the cell darkened in color and took a circular shape,

losing the cingulum girdle (Fig. 2b). Such temporary cysts measured 27–35 µm in diameter (n = 100). Presumed planozygotes and hypnozygotes (resting cysts) of *D. baltica* were observed; with hypnozygotes present mainly in the sediment (Fig. 2c, d). The size ranged from 30–50 µm length x 20–30 µm width (n = 56). Hypnozygotes showed an elongated shape, with a thick, smooth wall, and a mild depression in lateral view; they presented homogeneous cell content and a brown color that was darker than the vegetative cells (Fig. 2d).

3.2 Thecal Plate Structure

The observed plate pattern of all specimens exhibited the formula: Po, x, 4 ', 2a, 6'', 5c, 4s, 5''', 2'''' (Figs 4a–d, 5a–d, 6a–d). Plates were smooth with no ornamentations and slight pores distributed near the suture lines (Fig. 6c). The apical pore (Po) was circular and situated above a small rectangular plate *x* (Figs. 4d, 5c, 6b, c). The epitheca was asymmetric, plate 1' was rhombic and had two adjacent intercalary plates. The first plate (1a) was smaller and five sided (Figs. 5b, 6b, d); the second plate (2a) was very large, hexagonal and located just above plates 3'' and 4'' (Figs. 4b–d, 5b, c).

Precingular plates were five sided; only 4'' and 6'' were four sided (Figs. 4a–b, 5a). The cingulum was composed of five plates. Postcingular plates were symmetrical and four sided except plate 3''' that was five sided (Figs. 4b, 5d). The sulcus presented four plates (Figs. 4a–b, 5a–b); the anterior sulcal plate (Sa) was smaller and located just below plate 1'; the left sulcal plate (Sd) was larger and formed a fold that partially covered the right sulcal plate (Si). The posterior sulcal plate (Sp) was the largest, rhombic in shape, and reached the antapical region just within the suture of plates 1'''' and 2'''' (Fig. 6a-b).

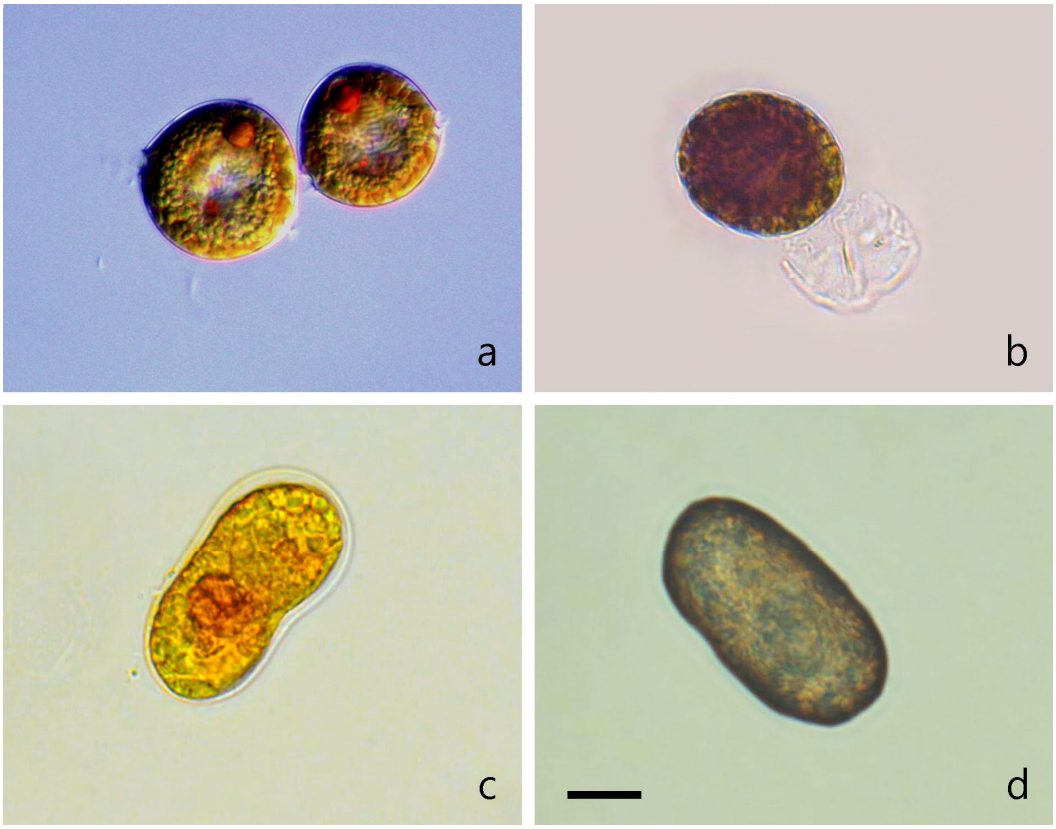

Fig. 2. LM photographs of living cells of *D. baltica* from El Japón channel

a) Characteristic chloroplasts and dinokaryotic nucleus in the center of the cell; bi-partite stigma located in the hypotheca and reddish accumulation bodies; b) D. baltica´s temporary cysts. c) Planozygote; d) Hypnozygote or resting cyst. Bar = 10 µm

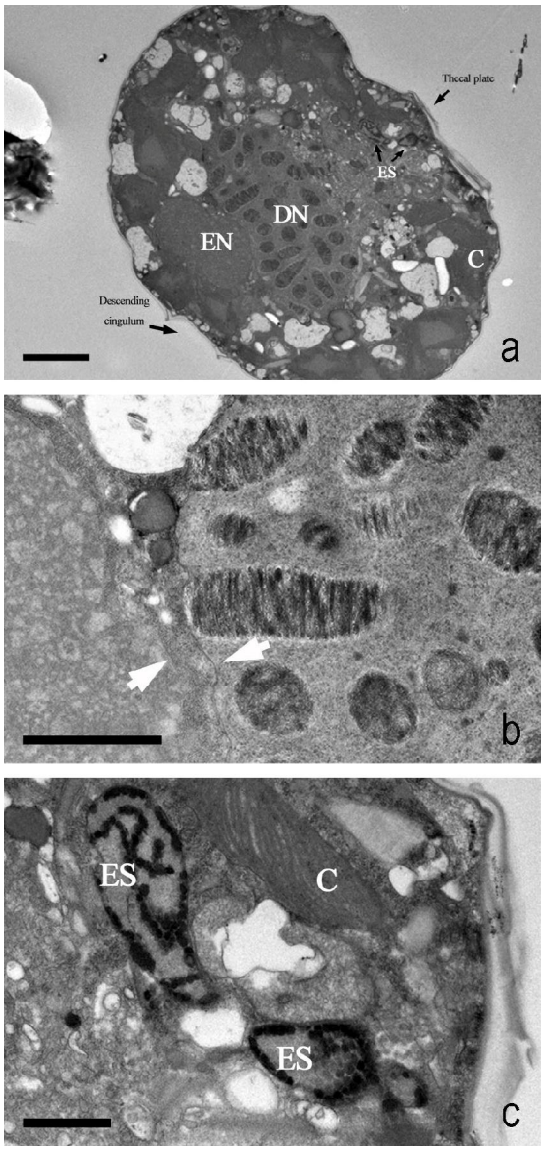

Fig. 3. TEM uranyl acetate stained sections of *D. baltica* from El Japón channel

a) Cell showing the dinokaryotic nucleous (DN), endosymbiont nucleus (EN) and chloroplasts (C). The thecal plates at the edge of the section, Bar = 5 µm; b) Separate membranes of dinoflagellate and endosymbiont nuclear compartments (white arrows), Bar = 1 µm; c) Enlargement of figure a: The bipartite eyespot (ES) and chloroplasts (C). Bar = 1 µm

3.3 Ecology of *Durinskia baltica* in El Japón Channel, Variability of Environmental Parameters and Statistical Correlations

Durinskia baltica was observed continuously in both El Japón and Tláhuac channels and only sporadically elsewhere in the channel system (Fig. 1). Vegetative cell and cyst densities showed significant variability during the study period. Dense bloom formation was observed only at the El Japón channel in 2011 with significant abundance in spring (96.74 x 10^5 cells l^{-1}). This density was higher than the one observed during summer (35.1 x 10^5 cells l^{-1}), and preceded the highest density of cysts which occurred in early summer (Fig. 7). Maximum density of *D. baltica* in Tláhuac channel occurred in spring (119 x 10^3 cells l^{-1}), and the highest summer density was 32 x 10^3 cells l^{-1}. Density fluctuations between cells (water column) and cysts (sediment) generally showed an inverse relationship (Fig. 7). Cell density in the water column was correlated with temperature, while cysts abundance in the sediments was correlated with precipitation (Fig. 8). No clear relationship was observed between nutrients and bloom dynamics (Fig. 9). According to Lira (2012), in spring 2011 *D. baltica* cell density at El Japón channel comprised 87% of the total phytoplankton community in terms of abundance with 38 phytoplankton species recorded, whereas in summer 2011 *D. baltica* represented 72% of the total phytoplankton community with 36 phytoplankton species recorded. In contrast *D. baltica* density in the spring at the Tláhuac channel was only 1% of the total phytoplankton community that consisted of 37 species.

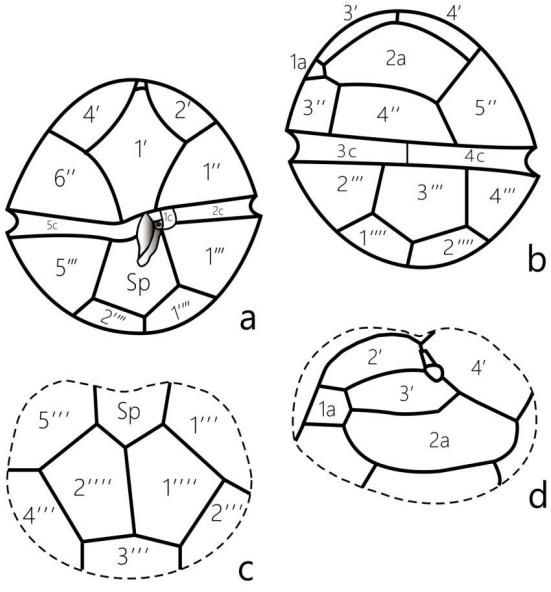

Fig. 4. Diagrammatic plate arrangement of *Durinskia baltica*

a) Ventral view; b) Dorsal view; c) Antapical view; d) Apical view

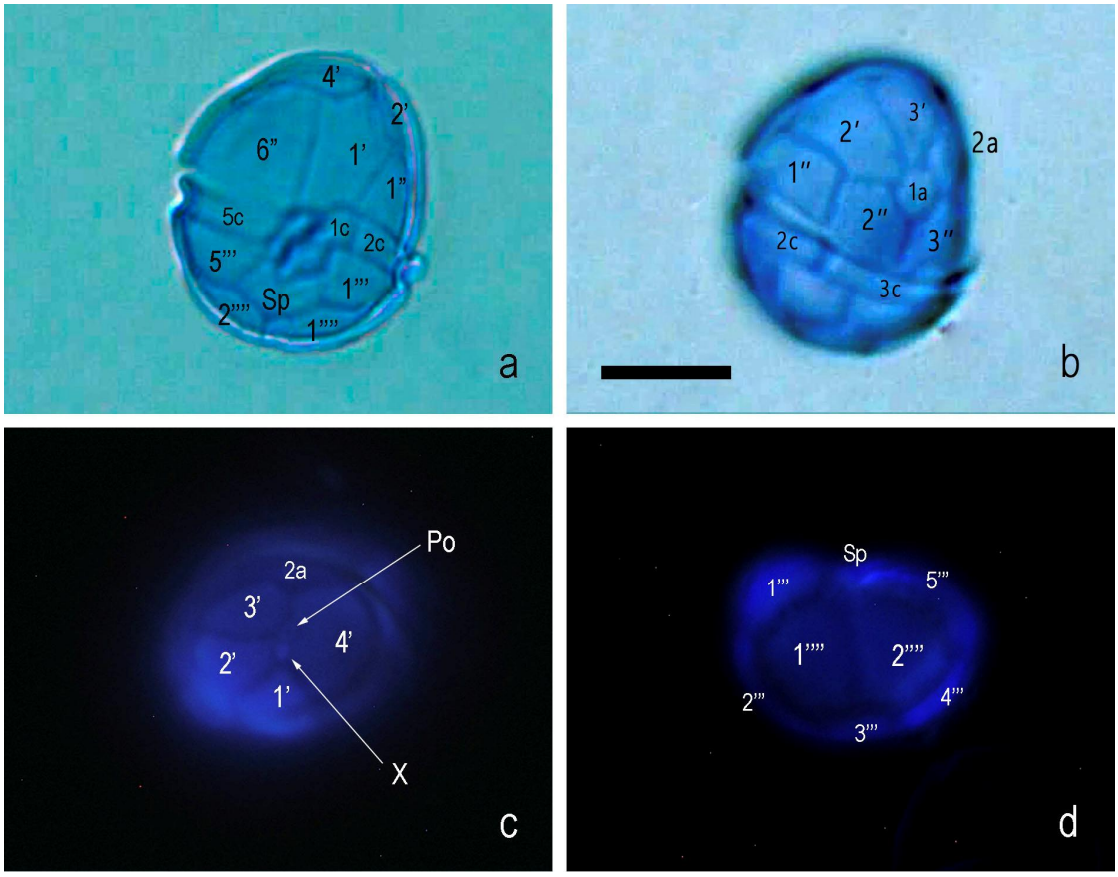

Fig. 5. LM and fluorescence microscopy of *D. baltica* thecal plates from El Japón channel

a) Ventral view; b) Semilateral view; c) Apical view (from inside), arrows point out the pore (Po) and Plate x; d) Antapical view. Images a+b = Trypan blue (Sigma); c+d = Calcofluor White Stain (Fluka). Bar = 10 µm

Pearson correlations confirmed a statistically significant relationship between *D. baltica* flagellate cells and environmental temperature (Airtemp, r = 0.526, *P* = .02) and pH (r = -0.637, *P* = .003) (Table 1). Cysts were correlated with annual rainfall (r = .772, *P* = .001). No statistically significant correlation between Ptot (total phosphorus) and cell density was observed (Table 2, r = -.315, *P* > .05).

However, CATPCA showed that there were associations not displayed through the single correlation. Maximum densities of vegetative cells were associated with the warm season (spring-summer); when a neutral pH and a mesotrophic status prevailed according to the minimum concentration of total phosphorus registered (60 mg L^{-1}) (Table 3, Fig. 10). The percentage of total variance explained by the CATPCA (> 76%, Table 4a) showed a strong association between the maximum and minimum cell densities and ambient conditions (trophic status, pH) (Table 4b, Fig. 10). Cyst density was closely related to the precipitation level and also behaved consistently with the cell density based on the two-dimensional model. When total cell density presented its maximum, cysts had their minimal density (dimension 1) and the opposite behavior was represented in dimension 2.

3.4 El Japón Channel Hydrochemistry

Water from El Japón channel has a bicarbonate sodium character according to the following ratio, HCO_3^- [5.3 meq L^{-1}]> Na^+ [4.5 meq L^{-1}]> Mg^{2+} [2.8 meq L^{-1}] > Cl^- [2.3 meq L^{-1}]> SO_4 [2.2 meq L^{-1}]. This character was determined based on the proportional concentration of cations and anions, calculated in milliequivalents per liter. Total alkalinity was 253.5 mg L^{-1}.

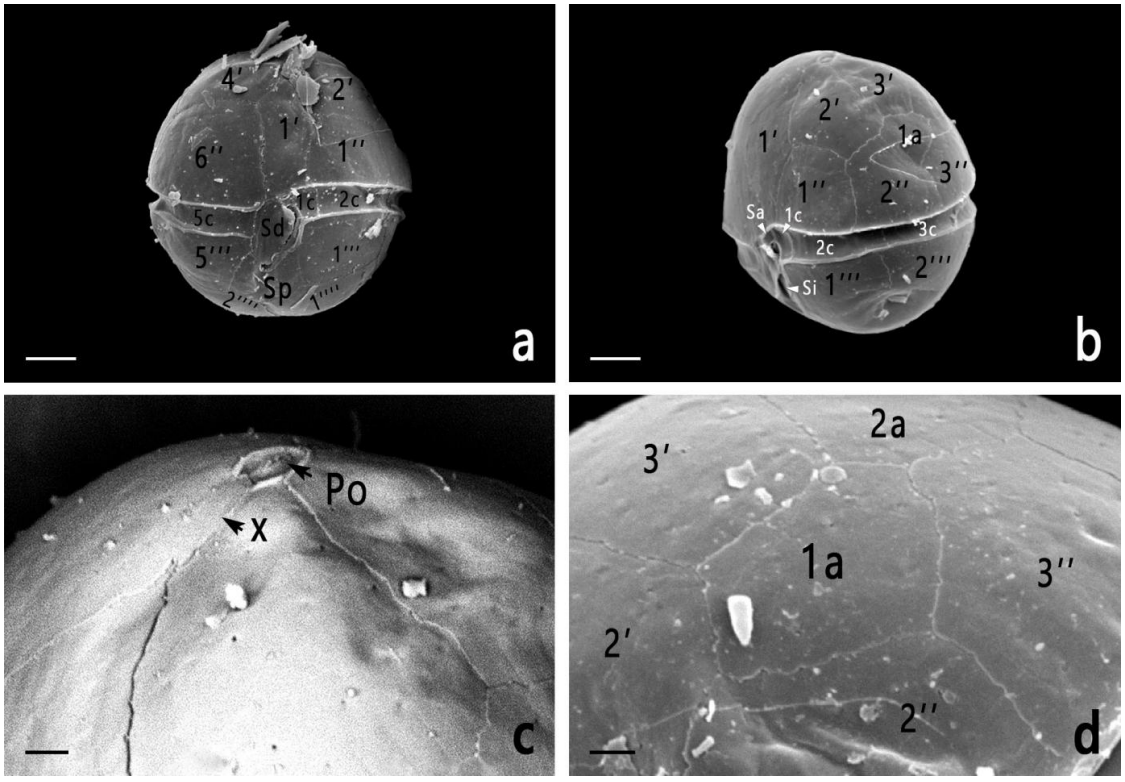

Fig. 6. SEM photographs of *D. baltica* from El Japón channel

a) Ventral view with great Sp-plate and Sd-plate hiding the sulcal plates Sa and Si; b) Dorsal view with apical pore, and the plate 1a (typically pentagonal), Sa and Si plates are distinguished under the Sd plate; c) Greater magnification view of the pore (Po) and the channel plate (x) and joints of the theca plates, with few small granules along the sutures; d) The plate 1a in greater magnification. a, b and d, method of secondary electrons; c, backscattered electron method. Scale bars in a and b = 5 µm with 10 kV accelerating voltage; scale bars in c and d = 1 µm with 10 kV accelerating voltage

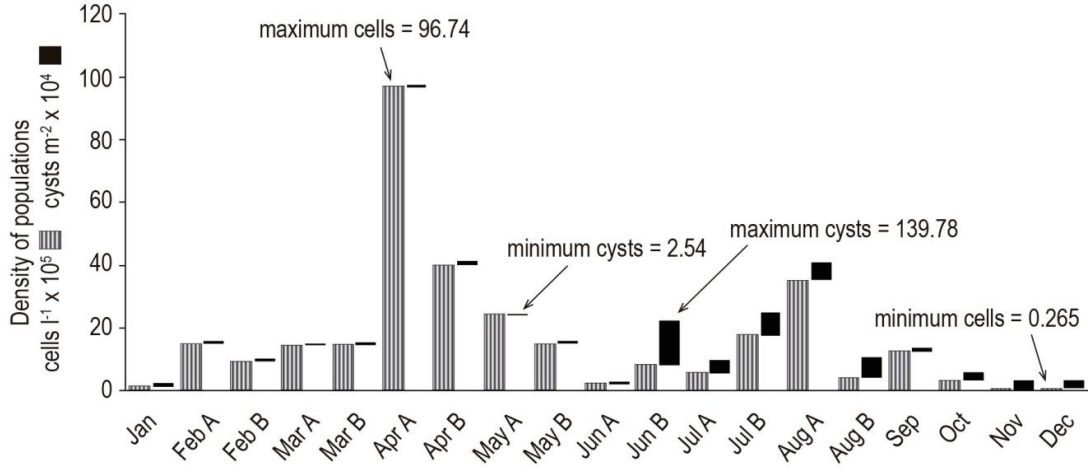

Fig. 7. Observed densities of vegetative cells and cysts of *D. baltica* in El Japón channel

Data from January and September to December correspond to 2010 and data from February to August to 2011

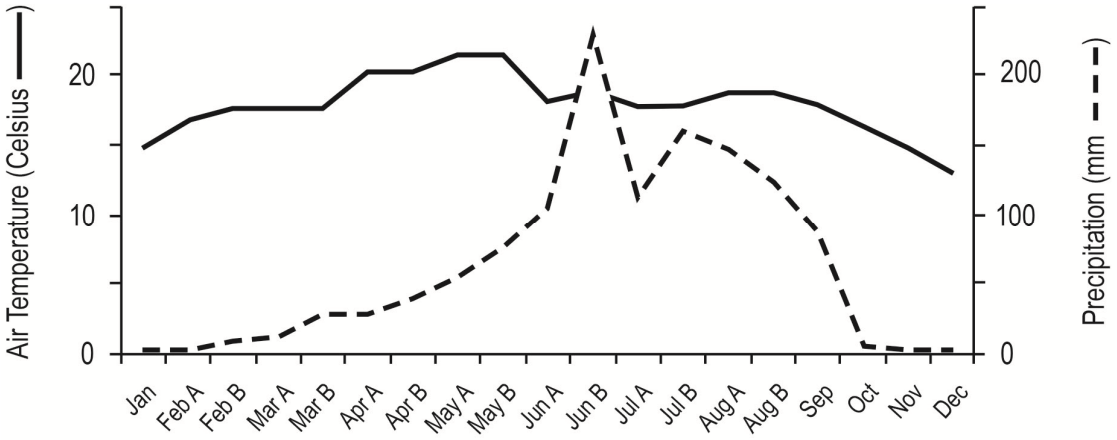

Fig. 8. Relationship between values recorded in El Japón for climatic temperature (solid line) and rainfall (dotted line)

Data from September to January were collected in 2010 and data from February to August were collected in 2011

Table 1. Pearson correlations for the monitored variables cells, cysts, pH, temperature (Temp.), dissolved oxygen (D.O.), conductivity (Cond.), dissolved inorganic nitrogen (DIN), Total phosphorus (Ptot), rainfall and air temperature (Air Temp.)

	Cells	Cysts	pH	Temp.	D.O.	Cond.	DIN	Ptot	Rainfall	Air Temp.
Cells										
Pearson	1									
Cysts										
Pearson	-.179	1								
Sig.	.463									
pH										
Pearson	-.637**	.011	1							
Sig.	.003	.965								
Temp.										
Pearson	.212	.312	-.561*	1						
Sig.	.383	.193	.012							
D.O.										
Pearson	-.040	-.186	.463*	-.834**	1					
Sig.	.871	.446	.046	.000						
Cond.										
Pearson	.040	.404	-.114	-.053	.163	1				
Sig.	.872	.086	.642	.831	.504					
DIN										
Pearson	-.249	-.061	.308	-.267	.131	.057	1			
Sig.	.304	.803	.200	.268	.594	.818				
Ptot										
Pearson	-.315	.404	.349	-.390	.336	.241	-.091	1		
Sig.	.190	.086	.143	.099	.159	.320	.710			
Rainfall										
Pearson	-.135	.772**	-.120	.628**	-.561*	.186	-.096	-.077	1	
Sig.	.583	.000	.624	.004	.012	.446	.694	.755		
Air Temp.										
Pearson	.526*	.051	-.756**	.746**	-.587**	-.096	-.452	-.468*	.315	1
Sig.	.021	.837	.000	.000	.008	.697	.052	.043	.189	

*Those that were statistically significant (two-tailed) are specified for the level of P = .01** and P = .05**

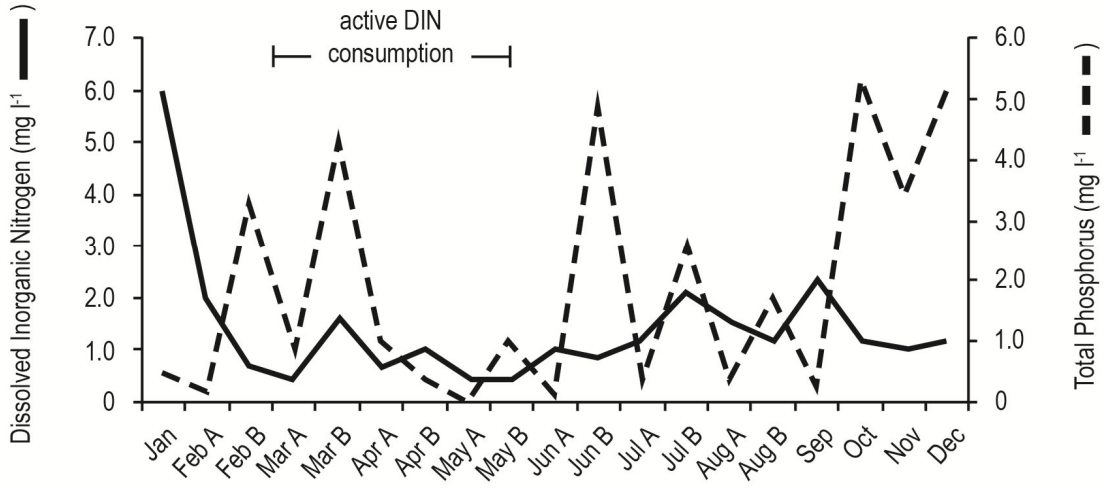

Fig. 9. Relationship between values recorded in El Japón for dissolved inorganic nitrogen and total phosphorus

Data from September to January were collected in 2010 and data from February to August were collected in 2011

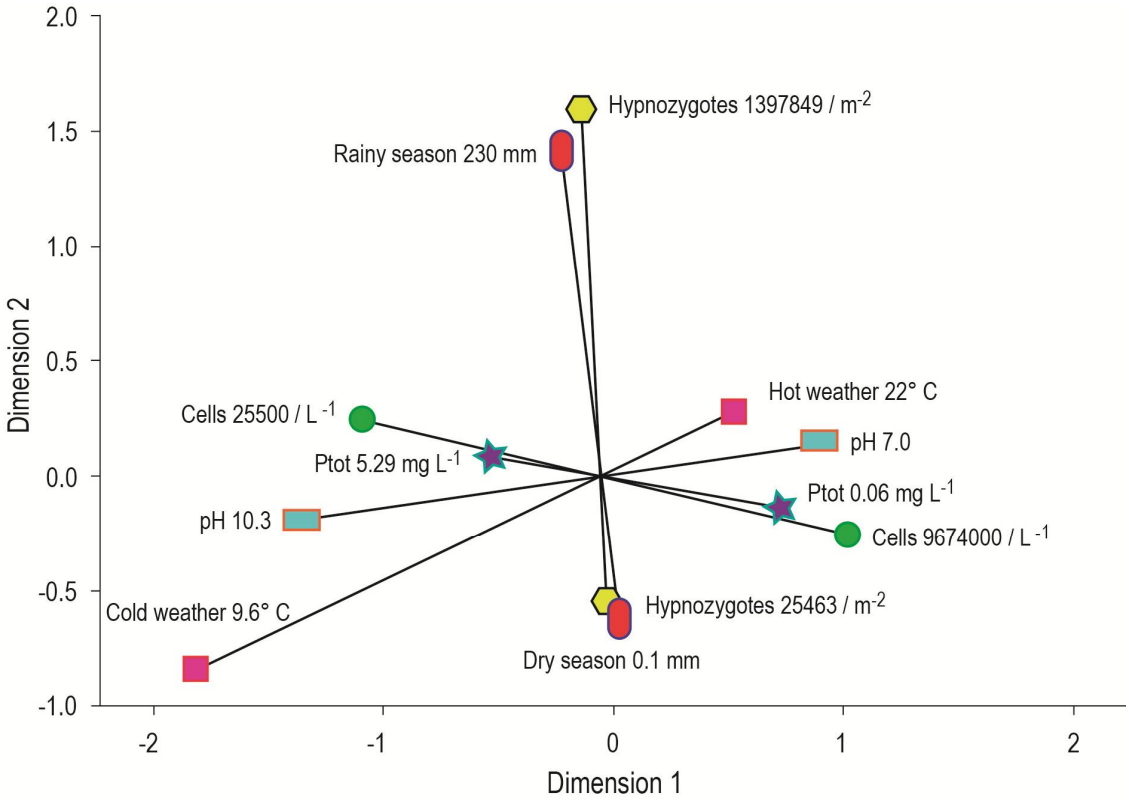

Fig. 10. Structure of the variables categorized according to the two-dimensional model CATPCA

The distances and lengths of vectors showed consistency with the positive and negative saturation values and set the interactions and the maximum and minimum values for each category

Table 2. Asymmetry statistic

Variable	Asymmetry	Standard error
pH	0.719	0.524
TempWater	-0.807	0.524
Dissolved oxygen	1.086	0.524
Conductivity	1.590	0.524
DIN	2.906	0.524
Ptot	0.753	0.524
Rainfall	0.973	0.524
Airtemp	-0.427	0.524

Those in bold are normally distributed according to twice the value of the standard error. N = 19

4. DISCUSSION

In this study we have described for the first time a dinoflagellate bloom of *Durinskia baltica* in the Xochimilco channel system of Mexico and its correlation with environmental parameters. This dinoflagellate causing blooms in El Japón channel and La Virgen lagoon located within Xochimilco channel system was previously reported as the freshwater species *Peridiniopsis occulatum* [19], mostly because the morphology of the theca is quite similar in both species. Indeed, *P. occulatum* is currently considered as *Durinskia occulata* (Stein) Gert Flaim et Hansen, a basionym which has been reported as the only freshwater species for the genus *Durinskia* [12]. However, first observations [19] did not provide any information regarding the thecal tabulation for their species. Moreover, their study was based on a phylogenetic DGGE analysis of Xochimilco algae in which the observed dinoflagellate species clustered very close to *Peridiniopsis penardii* (Lemmermann) Bourrelly and *P. kevei* (both freshwater species). According to Zhang & Hu [9] *D. baltica* has a close genetic affinity with *Peridiniopsis penardii*, *P. kevei* and *P. niei* Liu, Pei et Hu. These are apparently common freshwater bloom forming species in China, and suggests a close phylogenetic relatedness between species that incorporated a diatom endosymbiont. Although all species from the *Durinskia* genus (reported in marine, brackish or freshwater habitats) are thought to harbor an endosymbiont diatom [6,8,26], this had not been previously verified for *D. occulata*, whose inclusion in this genus was based on gross morphology [12]. If *D. baltica* is able to live in fresh water along with *D. occulata* and both are morphologically very similar, an important element in the potential distinction between them is the presence of the endosymbiont nucleus and stigma characteristics. The stigma of *D. baltica* is long or groove-shaped according to [12]; consists of two parts and is surrounded by three membranes [6]. This organization was not entirely clear in our TEM micrographs (Fig. 3c), however, apparently the presence of the 3 membrane surrounded stigma is common to all dinoflagellates possessing a diatom endosymbiont, such as *Gaelidium rugatum* Tamura, Shimada et Horiguchi, *Peridiniopsis penardii* (Lemmermann) Bourrelly and *P. kevei* Grigorszky [7]. Xochimilco populations share this apparent synapomorphy and therefore are identified as *D. baltica* rather than *D. occulata*. Additionally, [12] noted that in *D. occulata* the anterior sulcal plate seems absent and plate 1a was four sided (rhomboid). In contrast, our observed Xochimilco dinoflagellates had the anterior sulcal plate (Sa) and the plate 1a was five-sided (Fig. 6b, d). These authors also mentioned that *D. occulata* exhibits a more spherical shape compared to the dorsiventrally flattened vegetative cell of *D. baltica*; the degree of dorsiventrally flattening is a character that has been variably interpreted [2,6]. The overall morphology observed with light microscopy, SEM and TEM of *D. baltica* populations from Xochimilco was very consistent and supports its taxonomic assignment to *D. baltica*. Ecological study of *D. baltica* in Xochimilco indicates a relation between the flagellate (vegetative) cell peak abundance in spring, and subsequent cyst peak abundance in summer (Fig. 7). These observations suggest (as for other freshwater Dinophyta) that resting cyst formation occurs as a population-level event upon bloom termination [27,28]. The apparent increase of planozygotes observed during the bloom may be related with the resistant cyst formation (Figs. 2c-d), although sex may not always be required for resistant cyst formation [21]. It is apparent from our results (Fig. 10) that the behavior of vegetative cells and cysts seemed to be regulated by different environmental factors. Although both cell types share climate influence (Table 4b), vectors (none was orthogonal) representing these interactions are separately oriented in the two different model dimensions 1 and 2 (Fig. 10). The hydrochemistry of the Xochimilco system seemed to provide high buffering capacity (based on measured alkalinity), suggesting that the drastic observed changes of pH may have been related to anthropogenic activity impact. Saturation values above 0.3 (Table 4b) suggested that *D. baltica* vegetative cell abundance was closely associated with pH, Ptot and weather; relationship with pH and Ptot was negative, suggesting that the density decreases in an extreme basic pH condition and a situation of hypertrophy. It was noted that *D. baltica*

dominated the phytoplankton community in El Japón channel with 87% of the total phytoplankton community in spring (38 species total), 72% of the total phytoplankton community in summer (36 species total). This behavior may suggest that *D. baltica* is able to grow in wide ecological environmental conditions in freshwater. If so, it is not surprising that a species capable of growing in a wide range of salinity has not been correlated with the conductivity (Table 1). Resting cyst formation in *D. baltica* was correlated with the change from dry to rainy season, and the observed fluctuation in population density matched nutrient concentrations, especially at the highest precipitation period (Figs. 8-9). However, the statistical analyses did not confirm any relationship between cysts and other parameters except rainfall. The CATPCA model suggested with a different weight on each model dimension an alternation between cysts and flagellate cells densities (Fig. 10), which may indicate that rain could couple phases of reproduction-encystment-germination (Figs. 7-8). A similar situation was also observed with dinoflagellate species in marine environments [29]. Bloom formation in both marine and freshwater dinoflagellates, including *D. baltica*, are often related to nutrient variations and temperature [3,4,9]. Eutrophic to hypereutrophic conditions also seem to favor dinoflagellate blooms with densities reaching over 13 000 000 cel l^{-1} in environmental conditions similar to those recorded at Xochimilco (e.g. high temperatures in shallow, eutrophic and stratified water columns) [30,31,32,33]. This condition suggests that *D. baltica* responds to changes in temperature and nutrient concentrations, but with modalities influenced by rainfall coupled to land use (basin agricultural management). The observed relationship between precipitation and the bloom may be caused by an amplification of the channel nutrient input during the rainy season. The documentation of species of Dinophyta in inland waters is smaller than in marine environments. In freshwater environments with eutrophication problems, the study of this group is important in the aspect of resource inventory,

Table 3. Scaling of variables based on the visual binning from SPSS

Categories list	Density of cells (org L^{-1})	Density of cysts (unit m^{-2})	pH (units)	Total phosphorus (mg L^{-1})	Air temperature (Celsius)	Rainfall (Millimeters)
First	26500 –818000	25463–93385	7.0 – 7.5	0.06 – 0.37	13.0 – 14.9	0.1 – 28.0
Cut point =	819001	94000	7.5	0.4	15.0	28.0
Second	934000 – 1764000	101873–326854	7.7 – 8.4	0.46 – 0.87	16.5 – 18.6	39.8 – 93.7
Cut point =	2000000	326854	< 8.5	0.90	18.6	94.0
Third	2423000 – 9674000	430108–1797849	8.6 – 10.3	1.05 – 5.29	18.8 – 21.0	101.9 – 230.0
Cut point =	> 2000000	> 420854	> 8.5	1.0	> 18.6	> 94.0

Cut points on the original values of each variable are indicated. The categories were built as equal wide intervals

Table 4. a) 76.7% (percentage accounted) of the variance was explained with two dimensions and showed a high internal consistency coefficient (Cronbach's alpha> 0.8). b) Association of variables in each individual component suggest associations with aquatic or environmental parameters

a. CATPCA model summary

Dimension	Cronbach's alpha	Total (Eigenvalue)	Variance accounted (Percentage)
1	0.731	2.557	42.615
2	0.612	2.042	34,041
Total	0.939	4.599	76.656

b. Saturation values above 0.3 in each component

	Dimension 1	Dimension 2
Cells	.911	
Hypnozygotes (Cysts)		.958
pH	-.901	
Ptot	-.551	
Weather	.772	.373
Rainfall		.955

because eutrophic conditions promote its flowering and because the possible adaptive function that would be the production of toxins of these organisms [34]. This links the management of aquatic ecosystems in which algal blooms occur with the economic and health areas [13].

5. CONCLUSIONS

In Xochimilco it will be necessary to study the *D. baltica* life cycle to discern how the bloom may be influenced by each life stage as well as the dormancy period, and the origin and age of the cysts accumulated in the sediments [21,35]. Nevertheless, the current results suggest that *D. baltica* is indeed a species that inhabits freshwater environments, where it can achieve significant blooms. Also implied is that this species is able to thrive in relatively high temperature in shallow, eutrophic columns. Finally, as we have seen a seasonal effect, and hence an association with temperature, we can conclude that water bodies in urban-influenced tropical latitudes may be well suited environments for the developing of *D. baltica* blooms.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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