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Pediastrum sensu lato (Chlorophyceae) of central Mexico

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Résumé – Cette étude fournit de nouvelles connaissances sur les préférences écologiques *Pediastrum*, plus c´est la première description détaillée des populations naturelles et des cultures au Mexique. Elle fournit des informations sur l'ultrastructure et les conditions environnementales de *Pediastrum sensu lato* dans en endroits dans le centre du pays. Ce genre (Hydrodictyaceae, Chlorophyceae) n'a pas une origine monophylétique plutôt a été divisé en cinq genres : *Lacunastrum, Monactinus, Parapediastrum, Pseudopediastrum* et *Stauridium*. Les spécimens étudiés ont été recueillies entre Octobre 2009 et Novembre 2013. Les microscopes optique et électronique ont révélé la présence de sept espèces. Parmi celles-ci, *Monactinus simplex*, *Pediastrum duplex*, *Pseudopediastrum boryanum* et *Stauridium tetras* ont une distribution dans le monde entier. Au lieu de cela, *Monactinus simplex* var. *echinulatum*, *M. simplex* var. *sturmii* et *Pediastrum simplex* var. *clathratum* ont été enregistrées comme des taxa rares. La distribution des espèces en examinant la littérature de 1841-2015 a signalé l'existence de 23 taxa dans 17 états du Mexique, où les états de Mexique, Veracruz et Michoacan avec plusieurs espèces. L'analyse Canonicale des Correspondances a montré que les relations entre les espèces et les conditions environnementales des taxons rares sont liées à des températures élevées, des sites bien oxygénés et des conditions eutrophes.

Distribution / diversité / Hydrodictyaceae / Sphaeropleales / SEM

Abstract – This study gives new insights into ecological preferences of the *Pediastrum* genus as well as the first detailed description of its natural populations and cultures in Mexico. This artile provides information on the ultrastructure and environmental conditions of *Pediastrum sensu lato* at six locations in the center of Mexico. This genus (Hydrodictyaceae, Chlorophyceae) does not have a monophyletic origin; rather, it has been divided into the following five genera: *Lacunastrum, Monactinus, Parapediastrum, Pseudopediastrum* and *Stauridium*. The study specimens were collected between October 2009 and November 2013. Light and scanning electron microscopy revealed the presence of seven species. Of these, *Monactinus simplex*, *Pediastrum duplex*, *Pseudopediastrum boryanum* and *Stauridium tetras*

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have worldwide distribution. By contrast, *Monactinus simplex* var. *echinulatum*, *M. simplex* var. *sturmii* and *Pediastrum simplex* var. *clathratum* were found to be rare taxa. The species distribution as determined by literature review of articles from 1841 to 2015 registered the presence of 23 *taxa* in 17 Mexican states, namely, Mexico City, Veracruz and Michoacan which were the sites with the largest numbers of species. Canonical correspondence analyses showed that the relationships between the species and the environmental conditions of the rare taxa are related to well-oxygenated, high temperature and eutrophic conditions.

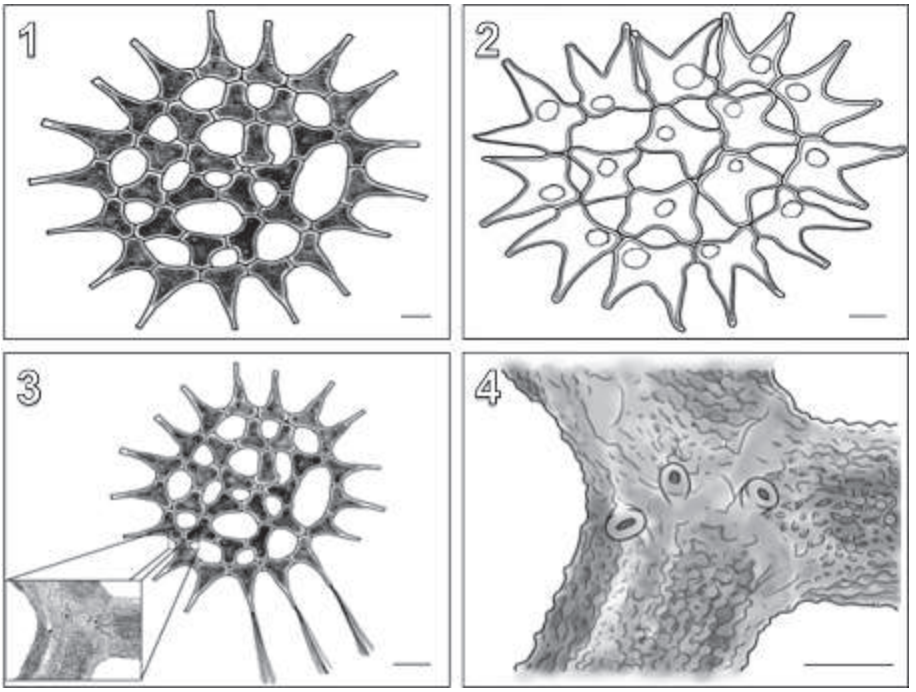
Distribution / diversity / Hydrodictyaceae / Sphaeropleales / SEM

INTRODUCTION

The genus *Pediastrum* Meyen (Sphaeropleales, Hydrodictyaceae) is a microscoscopic green algae with one layer the cells are arranged into flat, dish-shaped, circular o irregular coenobia with radially oriented marginal cells. These cells have processes of various lengths and shapes, and all cells originate from a single maternal cell (Komárek & Jankovská, 2001). Coenobia, which are usually made up of 16-32 cells, are organized concentrically and can touch one another or form holes. Internal cells are different from marginal cells. The cell shapes are primarily polygonal o square, but in some species, the morphology of marginal cells sometimes displays lobes and processes (Fig. 1), or incisions (Fig. 2). The processes have tiny apical pores through which tufts of bristles emerge (Fig. 3). Rosettes occur near the connecting walls between cells (Fig. 4). Cell wall is composed of a thick inner cellulose layer and a thin outer sporopollenin combined with silicon oxide. Different ornamentation is presented on the surface. The seven types of cell-wall sculptures together with morphological differences in the coenobia forms are diagnostic features used to distinguish among different species (Lenarczyk, 2014).

Sulek (1969), Parra (1979) and Komárek & Fott (1983) published monographs of *Pediastrum*, detailing its specific morphological features. Based on the ecology and geographic distribution, Comas (2005) classified its fossils and recent species into the following four groups: 1) those that had a broad distribution during different geological ages and currently occur in cold and moderately acidic environments of temperate zones, 2) those that are present in palynological sediments but are currently distributed in mesotrophic to eutrophic environments, 3) recent species that have only been found in oligotrophic environments and 4) recent species that are found in tropical and subtropical zones.

Some examples of group 1 are *Pediastrum musterii* Tell & Mataloni and *P. patagonicum* Tell & Mataloni. Group 2, includes *Monactinus simplex* (Meyen) Corda, *Pediastrum angulosum* Ehrenberg *ex* Meneghini, *P. duplex* Meyen, *Stauridium tetras* (Ehrenberg) E. Hegewald and *Pseudopediastrum boryanum* (Turpin) E. Hegewald *taxa,* with have broad worldwide distribution (Lenarczyk, 2014). For group 3, only *Pediastrum marvillense* Thérézien & Couté was noted in Marville Lake in the Kerguelen Islands. Finally, group 4 contains species such as *Pediastrum argentinense* Bourrelly & Tell, which has been listed for tropical and subtropical America; *P. tricuspidatum* Conrad, which is known only in Lake Kamande, Zaire; *P. longicornutum* (Gutwinski) A. Comas and *P. orbitale* Komárek from Cuba and *P. asymmetricum* Yamagishi & Hegewald from Japan.



Figs 1-4. Morphological features in *Pediastrum* genus (scale bars = 10 μm).

Lenarczyk (2014, 2015) identified the influence of environmental variables on the occurrence of *Pediastrum* and recognized hot spot sites in lakes in Poland. Likewise, Weckström *et al*. (2010) used multivariate statistical analyses to identify the influence of several environmental variables on the occurrence of *Pediastrum* from Finland.

Within the last few decades, the phylogeny of the genus *Pediastrum sensu lato* has been studied using ultrastructure of the flagellar apparatus and various molecular markers (Krienitz & Bock, 2012). Specifically, Buchheim *et al*. (2005) analyzed the Hydrodictyaceae lineage using sequences from the 26S region and the *rbc*L gene, in addition to cell wall ornamentation features, which were observed using scanning electron microscopy (SEM). Based on this information, those authors indicated that the genus is polyphyletic, and it is proposed to be divided into the following four independent genera: *Monactinus*, *Pseudopediastrum*, *Stauridium* and *Parapediastrum*. Under this classification, *Pediastrum simplex* was transferred to *M. simplex, Pediastrum tetras* to *Stauridium tetras* and *Pediastrum boryanum* to *Pseudopediastrum boryanum*. Similarly, McManus *et al*. (2011) recognized *Pediastrum duplex* var. *gracillimum* as new genus *Lacunastrum*. Jena *et al*. (2014) reported the phylogenetic analysis of 28 strains from three continents (Asia, Africa and Europe) using the SSU (small subunit) and ITS2 (internal transcribed spacer 2) regions, confirming that the *Pediastrum* genus is polyphyletic and has high phenotypic plasticity.

In Mexico, the information available about the *Pediastrum sensu lato* genus is the result of analyzing isolated publications from phycofloristic, limnological and

paleolimnological research that was performed between 1841 and 2015. This analysis indicated that there are 23 *taxa* distributed within 17 states (Ortega, 1984; Flores-Tena & Silva-Briano, 1995; Komárková & Tavera, 2003; Mora-Navarro *et al*., 2004; López-Adrián & Barrientos-Medina, 2005; Comas *et al*., 2007; Flores-Tena, 2008; Hernández-Morales *et al*., 2008; Moreno-Ruiz *et al*., 2008; Tavera & Díez, 2009; Novelo & Tavera, 2011; Campos-Campos *et al*., 2012; Figueroa-Torres *et al*., 2008, 2015; López-Mendoza *et al*., 2015). However, to date, there have been no integral studies with the goal of determining *Pediastrum* species distribution and habitats in Mexico. Therefore, based on the concept of morphospecies, the present study is the first to report on the distribution of the *Pediastrum* genus in different locations within the central region of Mexico as well as the environmental conditions associated to each taxon.

MATERIALS AND METHODS

Bibliographic documentation

A bibliographic search was performed for the *Pediastrum* genus in Mexico for the period 1841-2015 using the following databases: Biological Abstracts and Taxfich (Novelo & Tavera, 2011). With this search we generated an Excel database with 213 entries, which included taxonomic and geographic information as well as citations. Once the database was obtained, the accepted names were updated based on Buchheim *et al*. (2005), McManus *et al*. (2011), Jena *et al*. (2014) and Guiry & Guiry (2016).

Phycological surveys

Sampling was performed in six locations within three federal entities: State of Mexico, Mexico City and Veracruz (Fig. 5). Biological materials were collected using 20 μm mesh plankton net at surface level, between October 2009 and November 2013. A fraction of each sample was preserved using 2% glutaraldehyde, and the rest of the sample was transferred for isolation and culturing (Kowalska & Wolowski, 2010).

Physicochemical parameters

The following parameters were measured at the collection sites: pH, which was measured with a potentiometer (Cole Parmer); temperature, which was measured with a Brannan mercury thermometer; geographic coordinates, which were measured with a Magellan Meridian GPS; alkalinity, total hardness and dissolved oxygen, which were measured using titrimetric methods (APHA, 1989).

Nitrates and orthophosphates were quantified using the Nessler, cadmium reduction and molybdovanadate methods, respectively, with a Hach DR2800 spectrophotometer in the laboratory (APHA, 1989).

Taxonomic classification

Biological materials were observed using a Nikon YS2-T light microscope (LM) equipped with a Sony DSC-P200 8.2 megapixel camera. In parallel, SEM was

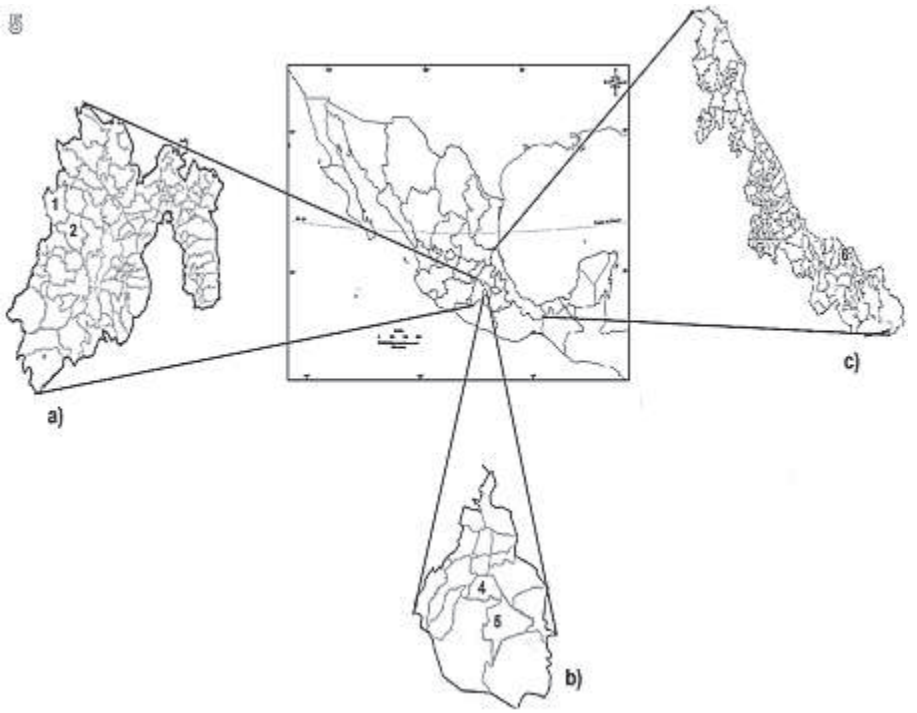


Fig. 5. Sample site localities - a) **State of Mexico**: (1) Villa Victoria Dam, 19°27'22.8" N, 99°59'10.5" W. (2) Santa Cruz del Rincón Lake, 19°40'05" N, 99°06'10" W. (3) Eco Parque Acoatl, Tlalnepantla de Baz, 19°31'53" N, 99°06'43" W. b) **Mexico City**: (4) Cantera Oriente, 19°19'03" N, 99°10'21" W. (5) Xochimilco, Nativitas Jetty, 19°15'50" N, 99°05'23" W. c) **Veracruz**: (6) Catemaco Lake, 18°21'00" N, 95°01'00" W.

performed on a subsample with 2% glutaraldehyde, which was filtered with a 0.45 µm Millipore filter, followed by air-drying. After this procedure, the sample was mounted on double-sided tape on an aluminum base. Finally, the sample was coated with gold for observation using a JEOL model JSM 6380 LV located in the Plant Physiology laboratory at Unidad de Biotecnología y Prototipos (UBIPRO) (Kowalska & Wolowski, 2010). For specific determinations, the works of Parra (1979), Komárek & Fott (1983), Comas (1989, 1996), Cambra-Sánchez *et al*. (1998), Komárek & Jankovská (2001), Hällfors (2004), Pérez *et al*. (2009), Loaiza-Restano (2013) and Lenarczyk (2014) were consulted. Furthermore, 25 organisms were used for the measurements. The terminology and cell wall ornamentation typing followed the criteria of Komárek & Jankovská (2001), while the granular density was measured based on Nielsen (2000). The studied materials were deposited in the phycological collection of the IZTA herbarium (Thiers, 2015).

Culturing conditions

Monospecific cultures were prepared by isolating one cell with a thinned Pasteur pipette and placing it in 3 M Bold's basal medium under a 12:12 photoperiod, using incandescent lamps with a luminous intensity of 90 µmol m^2 s^1 and temperatures between 18 and 22°C (Rojo *et al*., 2009).

Analysis of variables and certain taxa

The relationships between species and physicochemical parameters were analyzed via canonical correspondence analysis (CCA), using the Monte Carlo permutation test (4999 permutations, $\alpha = 0.05$) in the software program CANACO, ver. 4.5 (Ter-Braak & Smilauer, 2009).

RESULTS

Bibliographic documentation

The bibliographic of the period 1841-2015 for the *Pediastrum sensu lato* genus yielded 23 *taxa* for Mexico: *Monactinus simplex* (Meyen) Corda, *M. simplex* var. *echinulatum* (Wittrock) Pérez, Maidana & Comas, *M. simplex* var. *sturmii* (Reinsch) Pérez, Maidana & Comas, *Lacunastrum gracilimum* (West & West) McManus, *Parapediastrum biradiatum* (Meyen) E. Hegewald *in* Buchheim *et al*., *Pediastrum angulosum* Ehrenberg *ex* Meneghini, *P. argentinense* Bourrelly & Tell, *P. biwae* Negoro, *P. boryanum* var. *cornutum* (Raciborski) Sulek, *P. boryanum* var. *longicorne* Reinsch, *P. duplex* Meyen, *P. duplex* var. *cohaerens* (Bohlin) Ergashev, *P. heptactis* (Ehrenberg) Meneghini, *Pediastrum patagonicum* Tell & Mataloni, *P. reticulatum* (Lagerh.) Zacharias, *P. simplex* var. *clathratum* Schröter, *P. tetras* var. *tetraodon* (Corda) Hansgirg, *P. clathratum* var. *baileyanum* Lemmerman, *P. simplex* f. *compactum* Chodat, *Pseudopediastrum boryanum* (Turpin) E. Hegewald *in* Buchheim *et al*. *Pseudopediastrum boryanum* var. *caribeanum* (Comas) Pérez, Comas & Maidana, *Pseudopediastrum integrum* (Nägeli) Jena *et* C. Bock and *Stauridium tetras* (Ehrenberg) E. Hegewald *in* Buchheim *et al*. (2005). For some entries, the original naming was conserved because the reported species have not been currently accepted (Guiry & Guiry, 2016).

These *taxa* have been found in Aguascalientes, Campeche, Mexico City, State of Mexico, Guanajuato, Hidalgo, Jalisco, Michoacan, Morelos, Oaxaca, Puebla, Quintana Roo, San Luis Potosí, Sinaloa, Tabasco, Tlaxcala and Veracruz. Of these 17 states with genus information, those presenting the largest numbers of species were Mexico City, Veracruz and Michoacan, with 12, 10 and 8 species, respectively. In addition, 15 states still require phycological surveys to establish the presence of this genus (Fig. 6).

Physicochemical parameter quantification

Table 1 shows the environmental conditions in which each taxon was identified. The altitude ranged between 334 to 2563 m a. s. l. The pH values were 7.3 to 9.7, indicating that the *Pediastrum* species occurred in neutral to basic environments. Their water temperature varied from 12 to 24°C, as the variation interval was 12°C. The environments in which these species occurred were barely to slightly mineralized. The dissolved oxygen ranged within 4-8.7 mg L^{-1}, indicating that the species grow in well-oxygenated locations. The nitrate values ranged from 0.2 to 1.2 mg L^{-1} and the orthophosphate values ranged from 0.1 to 2.7 mg L^{-1}.

Fig. 6. Distribution of records about *Pediastrum* genus in 17 Mexican states, during 1841-2015 period. The arabic numbers indicate the amount of cited species. States in white have no records.

Taxonomic description

Based on the taxonomic system for *Pediastrum sensu lato* genus in the present study follows Guiry & Guiry (2016), listed according to the valid name and presenting some synonyms, a morphological description, the studied material, the location, LM and SEM micrographs.

Monactinus simplex (Meyen) Corda 1839 **Figs 7-14**

Synonym: *Pediastrum simplex* Meyen 1829

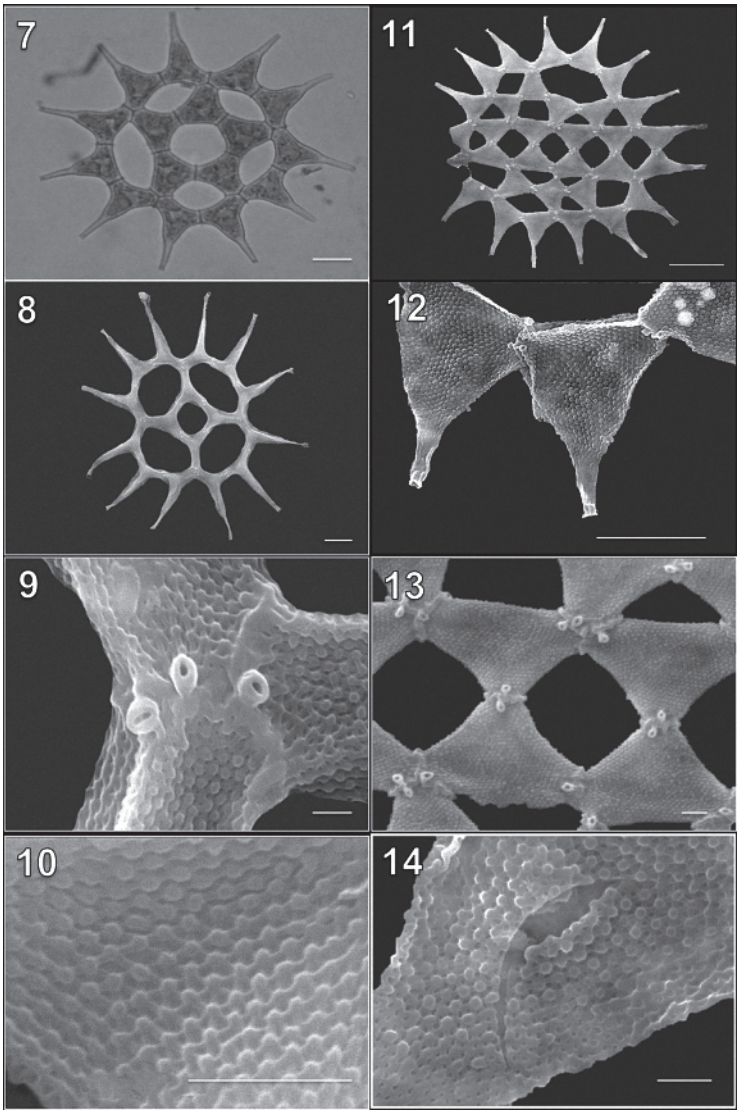
Description: coenobium with 16-34 concentrically arranged cells, more or less circular and with a diameter of 85-129 µm, with perforations (Figs 7, 11); marginal cells 25-38 µm long, 11-14 µm wide, trapezoid or triangle-shaped, always only with one conically narrowed lobe, terminating with long, narrow, cylindrical processes (Figs 8, 12); inner cells 12-20 µm long, 9-20 µm wide, with 3-5 polygonal, ovoid or spheroidal sides and with ample spacing between cells. SEM papillose cell wall is type 7 (Figs 10, 14), with 2-4 lateral rosettes larger than those from the cell surface (Figs 9, 13). Specimens of Villa Victoria had 16 cells and 3 rosettes, while Catemaco Lake were 34 and 4 respectively.

Studied locations: Villa Victoria dam, State of Mexico. April 2012, IZTA-1845. Catemaco Lake, Veracruz, October 2009, April 2011, January 2011, IZTA-1881.

Distribution in Mexico: Aguascalientes: Niagara and Abelardo L. Rodriguez Dams; Mexico City: Chapultepec Lake, Cantera Oriente, Xochimilco; State of Mexico: Villa Victoria Dam; Guanajuato: Ignacio Allende Dam; Hidalgo; Atezcac Lake; Michoacan: Patzcuaro and The Alberca Lakes, Jalisco: Chapala Lake, Oaxaca: Tehuantepec River; Veracruz: Catemaco Lake and San Luis Potosi: Panuco River.

Table 1. Results of physical-chemical parameters from each of the *taxa* habitats

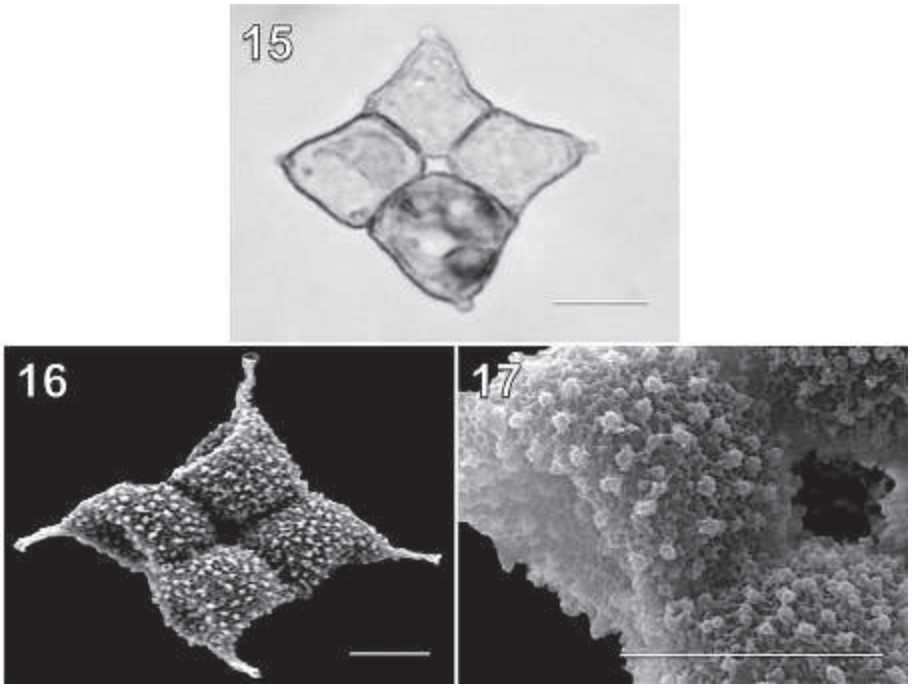
	Altitude (masl)	*pH*	*Water Temperature (°C)*	*Alcalinity (mg L-1 $CaCO_3$)*	*Total Hardness (mg L^{-1} $CaCO_3$)*	*Disolved Oxygen (mg L^{-1})*	*Nitrates (mg L^{-1})*	*Ortophosphates (mg L^{-1})*
Monactinus simplex (Meyen) Corda	334 - 2563	7.8 - 8.1	18 - 24	51 - 57	66 - 79	6 - 8.7	0.2 - 0.7	0.1 - 2.7
M. simplex var. *echinulatum* (Wittrock) Pérez, Maidana & Comas	334	8	24	52	66	8.7	0.7	2.7
M. simplex var. *sturmii* (Reinsch) Pérez, Maidana & Comas	334	8	24	52	66	8.7	0.7	2.7
Pediastrum duplex Meyen	2240 - 2563	7.3 - 8.4	18 - 24	51 - 189	68 - 205	4 - 7.6	0.2 - 1.2	0.1 - 2.3
P. simplex var. *clathratum* Schröter	334 - 2563	7.8 - 8.1	24	52 - 57	66 - 79	6 - 8.7	0.2 - 0.7	0.8 - 2.7
Pseudopediastrum boryanum (Turpin) E. Hegewald	334 - 2300	7.3 - 9.7	12 - 24	52 - 189	35 - 218	4 - 8.7	0.3 - 1.2	0.4 - 2.7
Stauridium tetras (Ehrenberg) E. Hegewald	334 - 2500	7.3 - 8.1	18 - 24	51 - 137	66 - 178	4 - 8.7	0.5 - 1.2	0.1 - 2.7



Figs 7-14. *Monactinus simplex* (Meyen) Corda, wild population - (Figs 7-10) Villa Victoria Dam, State of Mexico and (Figs. 11-14) Catemaco Lake, Veracruz (LM and SEM micrographs) (Scale bars = 10 µm (Figs 7, 8, 11, 12) and scales bars = 2 µm (Figs 9, 10, 13, 14)).

Monactinus simplex* var. *echinulatum (Wittrock) Pérez, Maidana & Comas 2009 **Figs 15-17**

Synonym: *Pediastrum simplex* var. *echinulatum* Wittrock 1883
Description: Coenobium with 4 cells, 24-40 µm in diameter with small perforations (Fig. 15); 18-20 µm long, 12 µm wide. Each marginal cells with one lobe, thinned and gradually sloping, extending to form a single thinned processes of 7 µm long (Fig. 16). SEM revealed type 6 cell walls with irregularly disposed spines (Fig. 17).



Figs 15-17. *Monactinus simplex* var. *echinulatum* (Wittrock) Pérez, Maidana & Comas, wild population from Catemaco Lake, Veracruz (LM and SEM micrographs) (Scales bars = 10 µm).

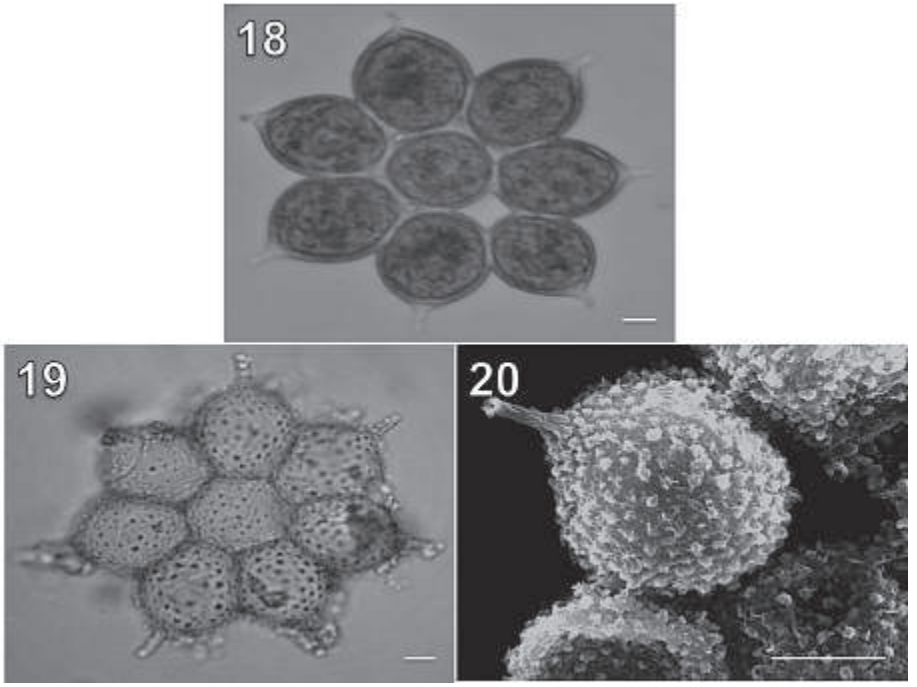
Studied locations: Catemaco Lake, Veracruz. April 2010, January 2011, IZTA-1877.
Distribution in Mexico: Veracruz: Catemaco Lake; Zapotitlan Lake, Jalisco (Romo-Barajas *et al*., 2006).

Monactinus simplex* var. *sturmii (Reinsch) Pérez, Maidana & Comas 2009 **Figs 18-20**

Synonyms: *Pediastrum sturmii* Reinsch 1867, *Pediastrum simplex* var. *sturmii* (Reinsch) Wolle 1887
Description: Coenobium with 8 cells, 57-85 µm in diameter, circular and with small perforations; marginal cells of 23-34 µm long, 22-26 µm wide, with a lobe leading to a thinned processes; marginal cells on mature coenobium with lobes that possess convex lateral sides; inner cells of 23 µm long, 25 µm wide, with 3-5 polygonal, ovoid or spherical sides (Figs 18-19). SEM revealed type 6 cell walls with long warts (Fig. 20).
Studied locations: Catemaco Lake, Veracruz. April 2010, January 2011, IZTA-1878.
Distribution in Mexico: Veracruz: Catemaco Lake.

Pediastrum duplex Meyen 1829 **Figs 21-32**

Synonyms: *Pediastrum napoleonis* (Turpin) Meneghini 1840, *Pediastrum pertusum* Kützing 1845, *Pediastrum selenaea* Kützing 1845, *Pediastrum duplex* var. *reticulatum* Lagerheim 1882, *Pediastrum duplex* var. *clathratum* (A. Braun) Lagerheim 1882.
Description: Coenobium circular or irregular with 8-32 concentrically arranged cells, 43-108 µm in diameter, with small perforations; marginal cells with a deep



Figs 18-20. *M. simplex* var. *sturmii* (Reinsch) Pérez, Maidana & Comas, wild population from Catemaco Lake, Veracruz (LM and SEM micrographs) (Scales bars = 10 µm).

V-shaped incision, 15-18 µm long, 12-15 µm wide, with two long, conical lobes, in the same plane as the coenobium and with two short cylindrical processes on the ends (Figs 21, 24, 27); inner cells 4.5-5 µm long, 8.5-9.0 µm wide, H-shaped with concave sides, joined only at the corners (Figs 22, 25, 28). SEM showed the cell wall forming a net with warts, types 3 and 4, as well as rosettes (Figs 23, 26, 29, 32). The materials in culture showed greater size (Fig. 24) or anomalies in the morphology of the coenobium (Figs 30, 31).

Studied locations: Santa Cruz del Rincon Lake, State of Mexico. April 2012, IZTA-1838. Xochimilco, Nativitas Jetty. Mexico City. July 2012, IZTA-1839.

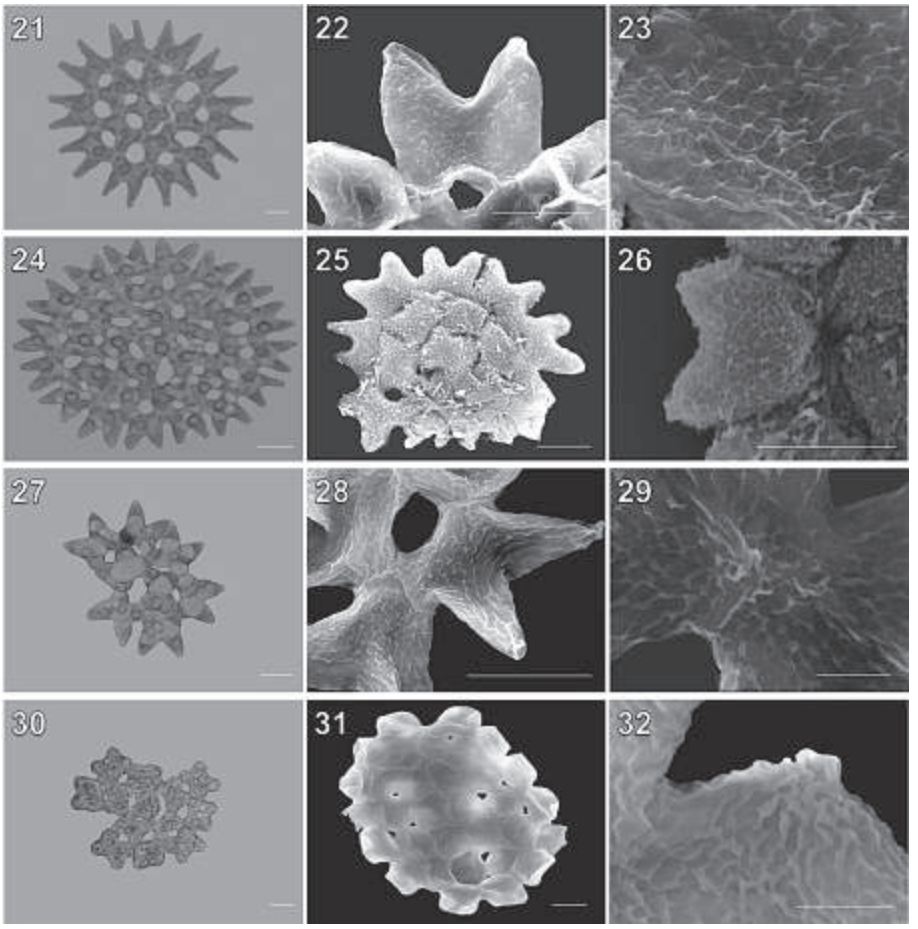
Distribution in Mexico: Aguascalientes: La Codorniz, El Saucillo and Media Luna Dams; Campeche: Edzna; Mexico City. Xochimilco; State of Mexico: El Sol Lake; Hidalgo; Jalisco: Chapala Lake; Michoacan: La Alberca, Patzcuaro and Zirahuen Lakes; Morelos: Amacuzac River; Quintana Roo: Coba; Tlaxcala; Veracruz: Catemaco Lake.

Pediastrum simplex* var. *clathratum Schröter 1883 **Figs 33-36**

Description: Coenobium with 24-32 cells, 130 µm in diameter, with a circular to irregular shape; always with perforation larger than cell diameter (Figs 33, 35); marginal cells 20-30 µm long, 12-15 µm wide, with only one projection, a very thin lobe and processes; inner cells 17-20 µm long, 8-20 µm wide. SEM revealed type 7 cell walls, with 2-5 lateral rosettes (Figs 34, 36).

Studied locations: Villa Victoria Dam, State of Mexico. IZTA 1846. Catemaco Lake, Veracruz, April 2012, IZTA-1846.

Distribution in Mexico: Veracruz: Catemaco Lake.

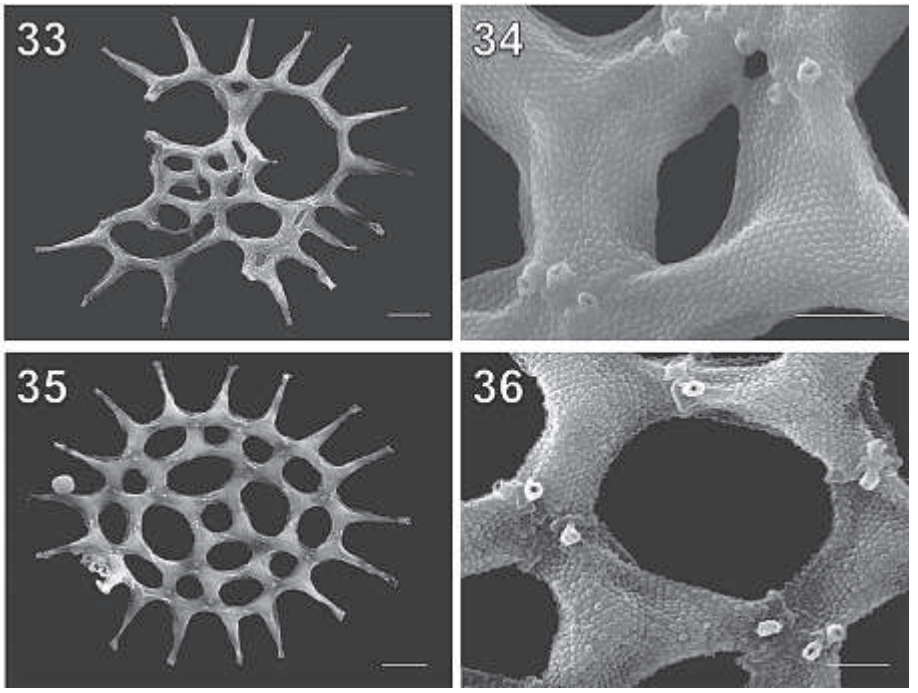


Figs 21-32. *Pediastrum duplex* Meyen. Samples from Santa Cruz del Rincon Lake, State of Mexico wild population (Figs 21-23) and cultured material (Figs 24-26). Samples from Xochimilco, Nativitas Jetty, Mexico City wild population (Figs 27-29) and cultured material (Figs 30-32) (Scales bars = 10 μm, except Figs 23, 26, 29 and 32 = 2 μm).

Pseudopediastrum boryanum (Turpin) E. Hegewald *in* Buchheim *et al*., 2005
Figs 37-42

Synonyms: *Helierella boryana* Turpin 1828, *Pediastrum boryanum* (Turpin) Meneghini 1840

Description: Coenobium with 16 cells of 37-110 μm in diameter, circular and flat, without perforations (Figs 37, 40), arranged concentrically. Marginal cells with irregular or polygonal shape, 7-23 μm long, 7-22 μm wide, each cell with two lobes on plane of coenobium, the processes 2-3 μm long, hyaline, cylindrical, and with truncated ends; one deep U- or V-shaped incision (Figs 38, 41). Inner cells 5-9 μm long, 4-6 μm wide, polygonal. SEM showed the cell wall forming a net-like shape with warts, types 4 and 5, with three lateral rosettes. Granular density (GD) of 16 to 20 in 10 μm (Figs 39, 42).



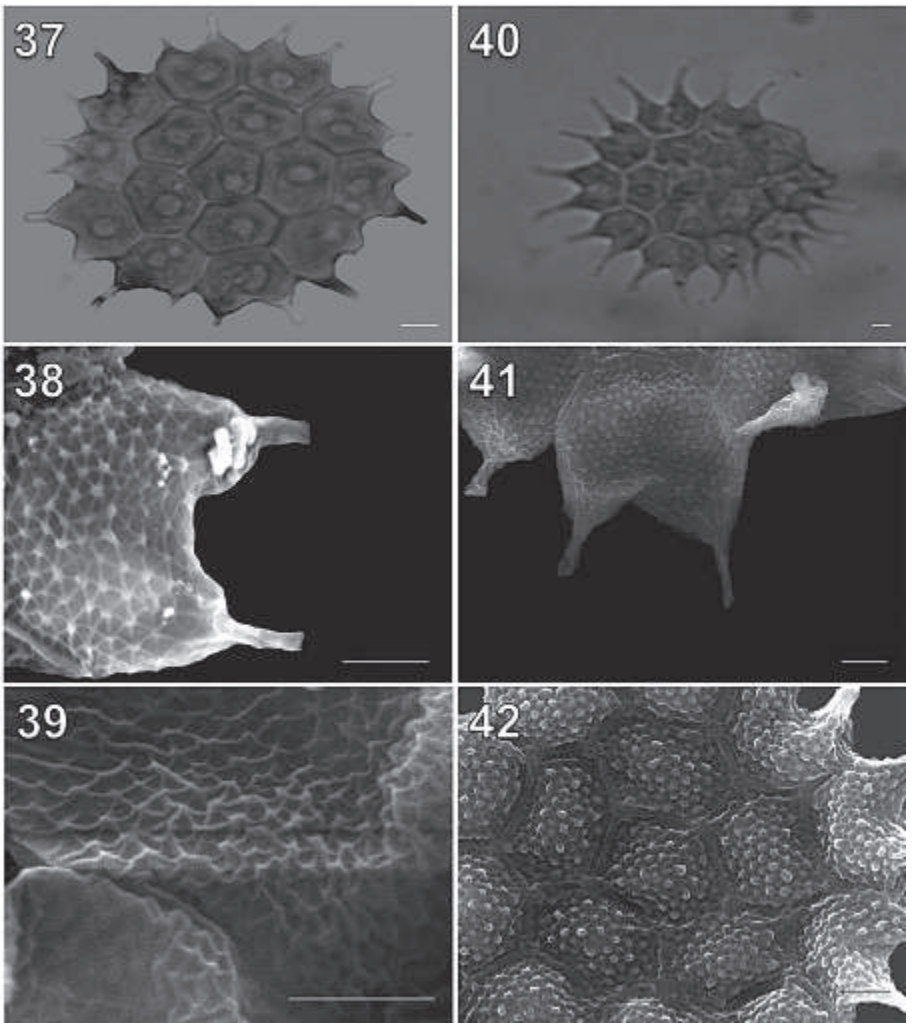
Figs 33-36. *Pediastrum simplex* var. *clathratum* Schröter, wild population from Villa Victoria Dam, State of Mexico (Figs 33-34) and wild population from Catemaco Lake, Veracruz (Figs 35-36) (Scale bars = 10 µm (Figs 33, 35), = 2 µm (Figs 34, 36)).

Studied locations: Eco Parque Acoatl, Tlalnepantla de Baz, State of Mexico. March 2012, IZTA 1840. Catemaco lake, Veracruz. October 2009, April 2010, January 2011, IZTA-1883. Cantera Oriente, Mexico City. May 2012, IZTA-1841.
Distribution in Mexico: Aguascalientes: Presidente Calles Dam; Mexico City: Xochimilco, Cantera Oriente; State of Mexico: El Sol, Chalco and Texcoco Lakes; Guanajuato: Ignacio Allende Dam; Hidalgo: Moctezuma River; Jalisco: Chapala Lake; Michoacan: Patzcuaro and Zirahuen Lakes; Morelos: Fish farming; Oaxaca: Ejutla and Atoyac Rivers; Puebla: Tehuacan Valley; Veracruz: Catemaco Lake.

Stauridium tetras (Ehrenberg) E. Hegewald *in* Buchheim *et al*., 2005

Figs 43-46

Synonyms: *Micrasterias tetras* Ehrenberg 1838, *Pediastrum tetras* (Ehrenberg) Ralfs 1845, *Helierella renicarpa* Turpin 1828, *Stauridium bicuspidatum* Corda 1835, *Euastrum hexagonum* Corda 1835, *Euastrum ehrenbergii* A.K.J. Corda 1839, *Stauridium obtusangulum* Corda 1839.
Description: Coenobium with concentrically arranged cells, with 4-8 cells, not perforated, 11-20 µm in diameter (Figs 43, 45); marginal cells of 6-8 µm long, 5-8 µm wide, trapezoid-shaped, with a U-shaped incision and with cylindrical lobes and two short processes on both sides of the incision (Fig. 46); hexagonal inner cells of 6-8 µm long, 5-8 µm wide, with four angles, whose sides are straight and connect all along with neighboring cells, with a central incision similar to the marginal cells. SEM showed net-like, warty and undulating type 2 cell walls (Fig. 44).

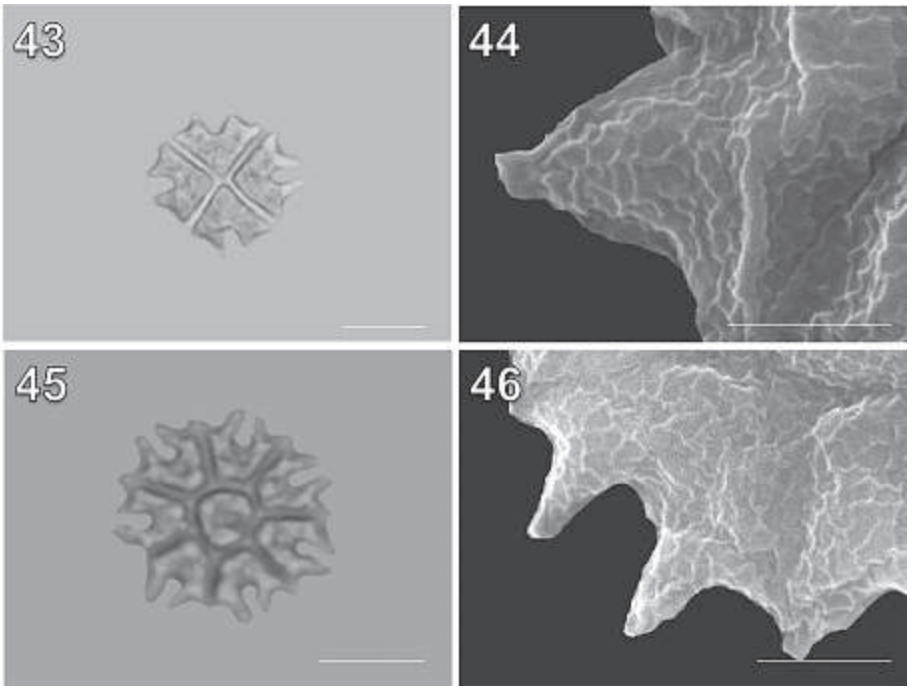


Figs 37-42. *Pseudopediastrum boryanum* (Turpin) E. Hegewald, from Eco Parque Acoatl, Tlalnepantla de Baz, State of Mexico wild population (Figs 37-39) and cultured material (Figs 40-42) (Scale bars = 2 μm, except (Fig 37 and 40) 10 μm).

Studied locations: Santa Cruz del Rincon Lake, State of Mexico. April 2012, IZTA-1844. Catemaco Lake, Veracruz. October 2009, April 2010, January 2011, IZTA-1882.
Distribution in Mexico: Mexico City: Xochimilco, Tezozomoc; State of Mexico: Santiago Tilapa Lake; Morelos: Zempoala Lake; Oaxaca: Ejutla River; Puebla: San Felipe Lake, Ixtaccihuatl, Tehuacan Valley; Tlaxcala; Veracruz: Catemaco Lake.

Analysis of variables and determined taxa

Diversity of the *Pediastrum* was investigated in relation to environmental factors. According to CCA (F = 2.93 P = 0.001). *P. duplex* was placed in quadrant I



Figs 43-46. *Stauridium tetras* (Ehrenberg) E. Hegewald, wild population from Santa Cruz del Rincon Lake, State of Mexico wild population (Figs 43-44) and culture material (Figs 45-46) (Scale bars = 10 µm (Figs 43, 45) and 2 µm (Figs 44, 46)).

with relation to altitude, hardness, alkalinity and ammonia. *Monactinus simplex* and *S. tetras* were placed in quadrant II, showing no affinity to the assayed parameters. *M. simplex* var. *echinulatum, M. simplex* var. *sturmii* and *P. simplex* var. *clathratum* were placed in quadrant III with relation to environments with good oxygenation, phosphates concentration and temperature. Finally, *Pseudopediastrum boryanum* was grouped in quadrant IV with relation to pH (Fig. 47).

DISCUSSION

The phycofloristic research in Mexico cited between 1841 and 2015 lists 23 *taxa* for the *Pediastrum sensu lato* in 17 states within the country, suggesting that there are many other unexplored regions in the national territory. The states in which the largest numbers of species were found were Mexico City, with 12, followed by Veracruz with 10 species, and Michoacan, with eight species.

Thirteen of the *taxa* that were previously recorded in the Mexican literature were not confirmed in the present study; it is crucial to study the taxonomical status. They are *Pediastrum clathratum* var. *baileyanum* Lemmerman from Patzcuaro Lake, Michoacan (Osorio-Tafall, 1941); *P. dimorphus,* as cited at the El Niagara Dam in Aguascalientes (Flores-Tena, 2008); *P. heptactis* (Ehrenberg) Meneghini (Ralfs,

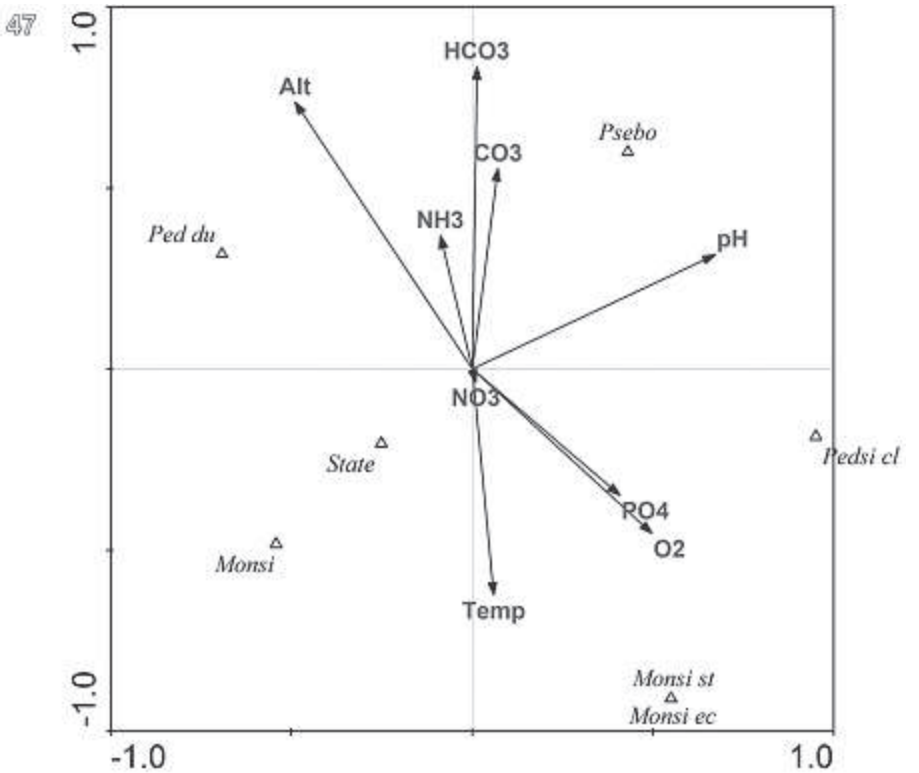


Fig. 47. CCA of physical-chemical parameters and *Pediastrum sensu lato* species. (Δ) *taxa*, (↑) vectors. O_2.- Dissolved oxygen, NH_3 ammonium, NO_3.- Nitrates, PO_4.- Orthophosphates, HCO_3.- Total Alcalinity, CO_3.- Total hardness, Temp.-Water Temperature, Alt.- Altitude and pH.- pH; Monsi.- *Monactinus simplex*, Monsi ec.- *M. simplex* var. *echinulatum*, Monsi st.- *M. simplex* var. *sturmii,* Ped du.- *Pediastrum duplex*, Pedsi cl.- *P. simplex* var. *chlathratum*, Psebo.- *Pseudopediastrum boryanum*, State.- *Stauridium tetras*.

1848; Ehrenberg, 1854; Mendoza-González, 1973) from Hidalgo and State of Mexico environments; *P. senarium* (Ehrenberg) Ortega from the Moctezuma River, Hidalgo (Ehrenberg, 1841); *P. simplex* f. *compactum* Chodat from the Valley of Mexico (Sámano-Bishop & Sokoloff, 1931); *P. tetras* var. *tetraodon* (Corda) Hansgirg from fish farms in Morelos (Quiroz-Castelán *et al*., 1999) and the Xochimilco Channels (Figueroa-Torres *et al*., 2015).

The listings for *Pediastrum biradiatum* that were mentioned by Ponce de León (1909) for Sinaloa and Rioja (1942) in Puebla have not been confirmed since their initial descriptions, which makes it important to find this taxon in neighboring regions to corroborate its occurrence in Mexican environments. In addition, *P. angulosum* and *P. argentinense* have only been mentioned in Quintana Roo (López-Adrián & Barrientos-Mediana, 2005). Moreover, *Pediastrum reticulatum* (Lagerh.) Zacharias, *Lacunastrum gracilimum* (West & West) McManus, *Pediastrum patagonicum* Tell & Mataloni, and *Pseudopediastrum boryanum* var. *caribeanum* (Comas) Pérez, Comas & Maidana were found in the Xochimilco Channels (Figueroa-Torres *et al*., 2015; López-Mendoza *et al*., 2015).

During this study period, seven species of *Pediastrum sensu lato* were identified; the species number in the central region of the country was smaller, in comparison with the findings of Komarék & Jankovská (2001), who presented 24 species in the most recent *Pediastrum* monograph. In addition, Comas (1996) found 14 species in Cuba and Parra *et al*. (1983) 14 species in Chile. In 50 localities in the northern, central and southern regions of Poland, Lenarczyk (2014) observed 19 species. Dillard (1989) found 16 species *Pediastrum* in water bodies of the Southeastern United States, and John *et al*. (2002) found 13 species in Britain. Loaiza-Restano (2013) reported the presence of 15 species in Brazil.

In this work, the information gathered through SEM allowed for the characterization of cell wall ornamentation in wild and cultivated species. This ornamentation showed changes that can be explained by the environmental conditions, the age of the coenobium and the morphological variability. These factors assist in distinguishing among the changes in the ultrastructure patterns of the cell walls that have been previously reported for this genus (Sulek, 1969; Parra, 1979; Millington *et al*., 1981; Nielsen, 2000; Lenarcyk, 2014).

According to Komárek & Fott (1983), Komárek & Jankovská (2001), *M. simplex* had a type 3 granular cell wall. Lenarczyk (2014), found *M. simplex* specimens in Poland that possess a reticulate cell wall, and the Mexican populations showed papillose ornamentation type 7.

Monactinus simplex var. *echinulatum* and *M. simplex* var. *sturmii* were designated on the basis of a new combination proposed by Pérez *et al*. (2009) and in reference to the names accepted by Guiry & Guiry (2016). However, Jena *et al*. (2014) indicated that *M. simplex* var. *sturmii* should be transferred to *M. sturmii* due to the observed differences in helix III of the ITS2 secondary structure, which must be confirmed for the Mexican populations. The sizes given for coenobium from Mexico and Brazil were smaller (50-85 µm in diameter) than those found for Poland, which are over 248 µm in diameter (Loaiza-Restano, 2013; Lenarczyk, 2014).

Pediastrum duplex has been described as having different types of cell wall ornamentation. Komárek & Fott (1983) denoted the cell wall ornamentation as smooth and type 1, while Komárek & Jankovská (2001) reported type 2. The analyzed Mexican populations showed type 3 and 4 ornamentations in wild and cultivated populations, respectively. Our finding confirms the evident morphological modifications that occur in a cultivated coenobium. For example, in Fig. 27, the coenobium resembles that of *P. willei* but forms small processes, which differs from the properties of the cited species (Comas *et al*., 2006). The same phenomenon occurs in cultures from the Xochimilco populations, in which abnormalities in the coenobium are observed (Figs. 30 and 31).

Pediastrum simplex var. *clathratum* is a preliminary entry and has not been subject to full verification (Guiry & Guiry, 2016).

For *Pseudopediastrum boryanum*, the presence of rosettes as indicated by Millington *et al*. (1981) was confirmed, as was the ornamentation of the cell wall, which ranges from smooth to net-like with warts.

Finally, *S. tetras* had smaller diameters than those recorded in Poland and Brazil at 11-20 µm, in comparison to the diameters of 18 to 74 µm that were indicated by (Fott & Komárek, 1983; Komárek & Jankovská, 2001; Loaiza-Restano, 2013; Lenarczyk, 2014). Moreover, Lenarczyk (2014) observed a smooth cell wall. By contrast, our specimens displayed ornamentation type 2.

Analyses of the physicochemical and nutrient parameters indicated that *Pediastrum sensu lato* grows under various environmental conditions; thus, these species are eurythermal and have a broad distribution.

Regarding the quantification of nutrients, the collection environments correspond to distrophic environments, such as that of the Xochimilco Channels (Figueroa-Torres *et al*., 2008; Tavera & Diez, 2009; López-Mendoza *et al.,* 2015), or enviroments, as in Catemaco Lake (Komárkova & Tavera, 2003). Under these locations, we confirmed the growth of species that are indicative of distrophic-eutrophic environments, such as *Monactinus simplex, P. duplex, P. boryanum* and *S. tetras,* which have been previously registered in other regions of the world under these conditions (Parra, 1979; Nielsen, 2000; Prasertsin & Peerapornpisal, 2012; Lenarczyk, 2014, 2015). Similarly, the previous records by Comas *et al*. (2007) verified that these *taxa* grow in eutrophic environments. In particular, Flores-Tena & Silva-Briano (1995) recognized *M. simplex* in this trophic level in Aguascalientes.

Pediastrum duplex was also found in the Amacuzac River in Morelos, where the ecological conditions included temperatures between 21.5 and 29°C, pH values between 6.9 and 8.1, and oxygen values between 5.3 and 7.8 mg L^{-1} (García-Rodríguez *et al*., 2011). Furthermore, this species was linked to the high temperature and alkalinity of environments of the Yucatan Peninsula (López-Adrián & Barrientos-Medina, 2005), which is consistent with the CCA results in this study. For this species, in the locations studied in this work, the temperature range was 18-24°C; the pH and dissolved oxygen values were similar; and the phosphate concentrations were 0.1-2.7 mg L^{-1}. This finding confirms that this taxon is eurythermal, with good growth in eutrophic environments and worldwide distribution (Komárek & Jankovská, 2001; McManus & Lewis, 2011; Lenarczyk, 2014, López-Mendoza *et al*., 2015).

World distribution

The world distribution the seven species from the present study is show in Table 2. Four of them are found worldwide in oligotrophic to hypereutrophic water bodies (*Monactinus simplex, Pediastrum duplex, Pseudopediastrum boryanum* and *Stauridium tetras*). For *M. simplex*, Komárek & Jankovská (2001) and Lenarczyk (2014) found this species in meso-eutrophic water bodies, at water temperatures 18.1-26.3°C, pH 7.9-9.2, conductivity 181-1588 µS/cm, nitrates $\leq$ 5 mg L^{-1} and orthophosphates $\leq$ 0.15 mg L^{-1}, this limnological conditions similar to the those analyzed Mexican material, except for orthophosphates at 0.8 mg L^{-1}. *Pseudopediastrum boryanum* is common over a more or less wide range of eutrophic habitats and is usually found in slightly alkaline freshwaters containing lake plankton. It is also found in mesohaline bays of Baltic Sea (Komárek & Jankovská, 2001) and central Europe (Lenarczyk, 2015).

Three taxa were rare at the world scale and discontinuos: *Monactinus simplex* var. *echinulatum*, *M. simplex* var. *sturmii* and *Pediastrum simplex* var. *clathratum* (Comas *et al*., 2006; Lenarczyk, 2014, 2015; Guiry & Guriy, 2016).

Multivariate statistic methods (CANOCO) and CCA test have been successfully used to evaluate data from a 15-year phytoplankton monitoring study from the Rimov Reservoir where a group of species are associated with high concentrations of sodium and high temperature, while a different group dominated under low temperatures and higher water volumes (Komárkova *et al.*, 2003). According to our data, *S. tetras* and *M. simplex* show no relation to the analyzed variables, which explains why these *taxa* are widely distributed throughout the world. Instead, *P. boryanum* grows in alkaline pH, as well as the conditions described in Lenarczyk (2014), in which this species has been registered with pH values from 7.4 to 9.6.

Table 2. Summary of the distribution of the *taxa* studies by different authors

	M. simplex	*M. simplex var. echinulatum*	*M. simplex var. sturmii*	*Pediastrum duplex*	*P. simplex var. clathratum*	*P. boryanum*	*S. tetras*
Andorra*				X		X	X
Argentina*	X			X		X	X
Australia [4]	X		X	X		X	X
Bolivia*						X	
Brazil [11, 13, *]	X	X	X	X	X	X	X
Britain [6]	X			X		X	X
Chile [1]	X			X		X	X
China [7, 8, *]	X	X		X		X	X
Colombia [12, 15]	X	X		X		X	X
Cuba [3]	X	X	X	X	X	X	X
Czech R.& Slovakia							X
Egypt*	X			X			X
Ellesmere Island*						X	X
Germany*	X			X		X	X
Ghana*	X			X			X
India							X
Iran*						X	X
Iraq*	X		X	X	X	X	X
Israel [5]		X					
Korea [10, *]	X	X		X		X	X
Nepal*						X	
New Zealand*	X			X		X	X
Pakistan*	X						
Poland [14]	X	X	X	X	X	X	X
Portugal*	X			X		X	
Romania*	X	X	X	X		X	X
Russia*	X			X		X	X
Singapore*	X			X		X	X
Spain [9*]	X	X	X	X		X	X
Sudan*	X		X	X		X	X
Tasmania*	X			X			X
Turkey*	X			X		X	X
USA[2]	X	X	X	X		X	X

[1] Parra *et al*. (1983), [2] Dillar, (1989), [3] Comas (1986), [4] Ling & Tyler, 2000), [5] Komárek & Jankovská (2001), [6] John *et al.* (2002), [7] Cao *et al.* (2005), [8] Hu & Wei (2006), [9] Pérez *et al*. (2009), [10] Kim & Kim (2012), [11] Loaiza-Restano (2013), [12] Echenique *et al.* (2013), [13] Loaiza-Restano & Bicudo (2014), [14] Lenarczyk (2014), [15] Alba *et al.* (2011), * Guiry & Guiry (2016).

On and other hand, *Monactinus simplex* var. *sturmii*, *M. simplex* var. *equinulatum* and *P. simplex* var. *clathratum* are found in well-oxygenated, high-temperature and eutrophic environments. However, Lenarczyk (2014) found that in Poland, *M. simplex* var. *equinulatum* grew in environments with low nitrates and phosphates under mesotrophic conditions.

This is the first study to address the diversity of *Pediastrum sensu lato* in Mexico and include wild and cultivated populations with LM and SEM observations as well as the environmental properties of the tropical regions in which the populations are distributed.

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