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Morphological and ecological characterization of Charales (Chlorophyta) from calcareous tropical streams in Mexico

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Abstract – Charales populations from calcareous tropical streams in central Mexico were investigated through the sampling of six stream segments during the dry season, the most favourable growth period. Five taxa were found: *Chara haitensis, C. vulgaris* var. *nitelloides, C. zeylanica* var. *diaphana, Nitella furcata* var. *sieberi* and *N. tenuissima* var. *tenuissima. Chara haitensis* and *N. tenuissima* are reported for the second time in central streams and described in detail for the first time in the freshwater green algal flora from Mexico. Physical and chemical water quality data were also recorded in the investigated environments. All taxa were found at high temperatures (22-28 ºC), neutral to moderately alkaline waters (pH 7-8) with high ionic content (total dissolved solids 593-1884 mg l^{-1}, specific conductance 826-1952 µS cm^{-1}), with sulfate-bicarbonate/calcium as dominant ions. The populations were associated with slow current velocity (≤ 30 cm s^{-1}), shallow water depth (≤ 41 cm) and silt to sand substratum with high organic matter content. The taxa were found in a wide range of incident radiation (340-2234 µmol photons m^{-2} s^{-1}), except for *N. furcata* var. *sieberi* which was found in shady sites (24 µmol photons m^{-2} s^{-1}). All the populations investigated were found in mesotrophic to eutrophic conditions.

Charophyceae / Calcareous Streams / *Chara* / Distribution / Ecology / Mexico / *Nitella* / Taxonomy / Tropical

Résumé – Morphologie et caractères écologiques de quelques espèces de Charales (Chlorophytes) des cours d'eau calcaires tropicaux du Mexique. Les populations de Charales des cours d'eaux calcaires du Mexique central ont été étudiées en échantillonnant six segments de rivières pendant la saison sèche, la période la plus favorable à la croissance. Cinq taxons ont été observés: *Chara haitensis, C. vulgaris* var. *nitelloides, C. zeylanica* var. *diaphana, Nitella furcata* var. *sieberi* et *N. tenuissima* var. *tenuissima*. Ils sont décrits et les données relatives à la qualité physico-chimique des eaux où ils croissent sont précisées. *Chara haitensis* et *N. tenuissima* sont signalés pour la deuxième fois de rivières de la région centrale; notre travail constitue la première description détaillée de ces taxons pour la flore des algues vertes du Mexique. Tous ces taxons ont été trouvés à des températures élevées (22-28°C), dans des eaux neutres à modérément alcalines (pH 7-8), bien minéralisées (sels dissous 593-1884 mg l^{-1}, conductivité 826-1952 µS cm^{-1}), avec les sulfates-bicarbonates-calcium comme ions dominants. Ces populations étaient associées à des courants lents (< 30 cm s^{-1}), de faible profondeur (< 41 cm) et des substrats argileux ou sableux à contenu

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organique important. Elles ont été trouvées dans une large gamme de radiations incidentes (340-2234 µmol photons/m^{-2} s^{-1}) sauf pour *N. furcata* var. *sieberi* qui a été observé dans des sites ombragés (24 µmol photons m^{-2} s^{-1}). Toutes les populations croissaient dans des conditions mésotrophes à eutrophes.

Charophycées / *Chara* / Distribution / Ecologie / Mexique / *Nitella* / Régions Tropicales / Rivières Calcaires / Taxinomie

INTRODUCTION

The Charales are macroscopic green algae, which can be very common in freshwater lakes, ponds, streams and occasionally in acidic to brackish waters of tropical and temperate regions of the world (Vaidya, 1966; Khoja & Hussain, 1990; Andrews *et al.*, 1984; Blazencic *et al*., 1990; Bornette *et al*., 1996; Bornette & Arens, 2002; García & Chivas, 2004). Several factors have been hypothesized or shown to limit their growth, including stream temperature (Zaneveld, 1940; Vieira & Necchi, 2002a; García & Chivas, 2006), salinity and depth (García, 1999; García & Chivas, 2006), ammonium, phosphorous and alkalinity (Bornette *et al.*, 1996), fine sediments as substrate, low current velocity and high irradiance levels (Vieira & Necchi, 2002a, 2002b; Vieira *et al*., 2003). However, data on environmental preferences and tolerance ranges is still scarce for most species of Charales.

The family Characeae of the order Charales is composed of the tribes Chareae (genera *Chara*, *Lamprothamnium*, *Nitellopsis* and *Lychnothamnus*) and Nitelleae (genera *Nitella*, *Tolypella* and *Sphaerochara*). *Chara* and *Nitella* are the two genera with the highest number of known species (Zaneveld, 1940; García & Casanova, 2003; Sakayama *et al.,* 2004). The genus *Chara* includes plants with undivided branchlets, coronula of the oogonium formed by five cells, stipulodes absent or in one or two rows, spines absent to well developed, main axis corticated or ecorticated, branchlets formed by corticated/ecorticated segments, which have whorls of bract cells and bracteoles. The genus *Nitella* is characterized by furcate branchlets, coronula of the oogonium formed by ten cells in two rows; it is always ecorticated without stipulodes, bract-cells, spines and bracteoles (Zaneveld, 1940; Wood & Imahori, 1965). Species identification, which can be problematic owing to the degree of morphological variability within populations, requires detailed analyses of plant and oospore (García & Chivas, 2006). Wood & Imahori (1965) considered characters such as monoecy/dioecy to be of intra-specific value, leading to he lumping of taxa which were previously considered as different species (Braun & Nordstedt, 1882; Zaneveld, 1940). In the last decades, combined analyses of external morphology of the oospore wall and molecular phylogeny have proved useful for delimiting species within the genera *Chara* (Cáceres, 1978; Mandal & Ray, 1999; Ray *et al*., 2000) and *Nitella* (Cáceres & García, 1989; Mandal *et al.*, 1995; Sakayama *et al.,* 2004, 2005).

In Mexico, 18 taxa of *Chara* and 9 taxa of *Nitella* have been reported (Ortega, 1984; Valadez *et al*., 1996; Montejano *et al*., 2000), of which 4 in central Mexico, 3 in the lower Panuco River basin (*Nitella tenuissima* (Desvaux) Kütz., *Chara globularis* Thuillier and *Chara vulgaris* L.) and 1 in the Balsas River basin (*Chara canescens* Desvaux *et* Loiseleur *in* Loiseleur-Deslongchamps). However, in the literature there is scant morphological and environmental information on Characeae in Mexico, which is the aim of the present investigation.

MATERIAL AND METHODS

Fieldwork was carried out at the end of the rainy season (November 2004-June 2005), which is the most favorable growth period for *Chara* and *Nitella* in the region (Cantoral & Montejano, 1993). Each sampling location consisted of randomly chosen stream segments, 10 m in length (Text-Fig. 1). Temperature, pH and specific conductance (K_{25}) were measured in each river segment with a Conductronic PC-18 conductivity meter. For water chemistry, 1000 ml (preserved under dark and cold conditions) and 500 ml (preserved in nitric acid at pH 2-3) sub-samples were used. Dissolved nutrients (filtered *in situ* with 0.45 and 0.22 µm pore diameter membranes, preserved with chloroform and then frozen) were measured in the laboratory with a multichannel analyzer (Table 1, APHA *et al*., 1989). Microhabitat variables such as depth, substratum, current velocity and irradiance were measured *in situ* at the center of each sampling area as close as possible to the algal growth using a Swoffer 2100 current velocity meter and a Li-Cor LI-1000 quantum meter with a flat subaquatic sensor of photosynthetic active radiation (PAR). In the laboratory, each 50 g portion of sediment was dried 24 hr at 105 ºC, weighed and wet sieved through a 0.06-0.5 mm sieve. The silt and clay were analyzed for pipette analysis (Folk, 1974). One part of sedimentary portion was used for loss on ignition method (550º C for 4 h, Heiri *et al.*, 2001).

Five to ten thalli were collected and preserved in 2.5 % glutaraldehyde for subsequent examination in the laboratory. Morphological features considered

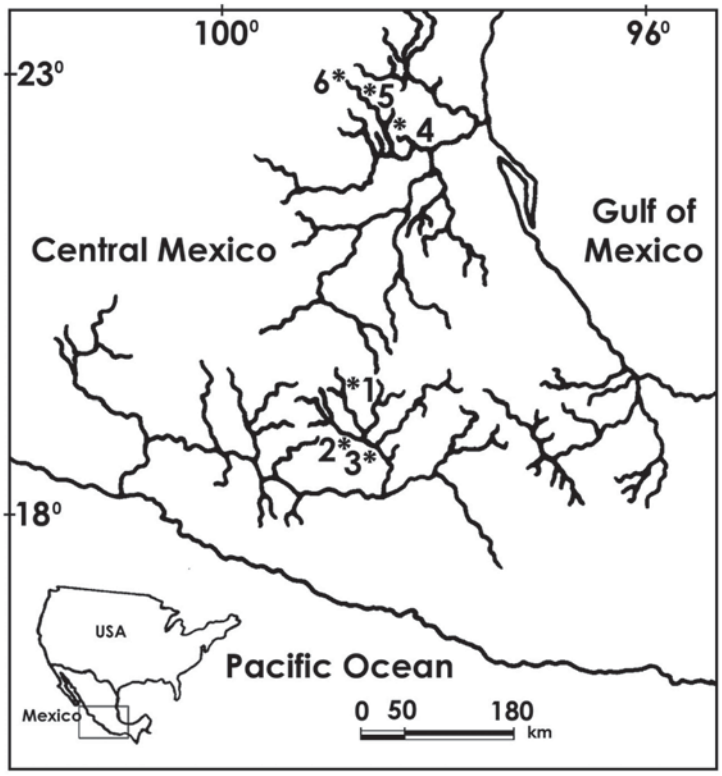


Fig. 1. Location of the study region in central Mexico, showing the investigated the sites with populations of Charales (*). Localities are numbered as in Table 1.

Table 1. Physical and chemicals characteristics of the investigated streams, numbered as in Text-fig.1. Abbreviations: DIN = Dissolved Inorganic Nitrogen; Mz = Average Sediment Size; LOI = Loss of Ignition Method; SRP = Soluble Reactive Phosphorus. All concentrations are in mg l^{-1}, except where indicated.

	1.Itzamatitlán	*2.Los Manantiales*	*3.Salado river*	*4.Micos*	*5.El Meco*	*6.El Salto*
Taxa[a]	*Ch*	*Cv, Cz*	*Ch*	*Nf, Ch*	*Nt, Cv, Ch*	*Ch*
Date	24.iv.2005	18.x.2001 08.xii.2005	05.vi.2005	20.v.2005	20.v.2005	20.v.2005
Temperature (°C)	22	28	26	23-24	22-26	28
pH	8	8	8	7-8	8	8
Specific Conductance (K_{25})	1952	1742	1849	826-834	1350-1602	1265
Mz (Φ)	–	3.0	1.3	1.5	4.4	3.4
LOI (%)	17	13	13	8-20	16	37
Disolved Oxygen	6.0	7.6	4.0	1.7	6.8	5.3
Total disolved Solids	1884	1602	1619	593	1082	972
Total Alcalinity (mg $CaCO_3$ l^{-1})	1884	1602	1619	235	168	134
HCO_3^-	232	602	249	286	205	164
CO_3^{2-}	34	24	5	–	–	–
Cl^-	72	15	12	7	11	10
$SO_4^=$	824	882	1049	196	551	464
Total Hardness (mg $CaCO_3$ l^{-1})	1301	1105	212	461	782	702
Ca^{++} Hardness (mg $CaCO_3$ l^{-1})	880	821	903	361	581	511
Mg^{++} Hardness (mg $CaCO_3$ l^{-1})	821	284	284	100	200	190
Ca^{++}	353	329	362	145	233	205
Mg^{++}	102	69	69	24	49	46
Na^+	101	50	46	10	17	15
K^+	10	17	15	1	2	2
Si^+	2.2	1.2	47	28	1	1.4
Ionic Dominance (meq l^{-1})	$SO_4^=$>HCO_3^->Cl^- Ca^{++}>Mg^{++}_Na^+>K^+	$SO_4^=$>HCO_3^->Cl^- Ca^{++}>Mg^{++}_Na^{++}>K^+	$SO_4^=$>HCO_3^->Cl^- Ca^{++}>Mg^{++}_Na^+>K^+	HCO_3^->$SO_4^=$>Cl^- Ca^{++}>Mg^{++}_Na^+>K^+	$SO_4^=$>HCO_3^-> Cl^- Ca^{++}>Mg^{++}_Na^+>K^+	$SO_4^=$_HCO_3^->Cl^- Ca^{++}>Mg^{++}>Na^+>K^+
Ionic concentration (meq l^{-1})	53.4	53.3	52.4	18.6	31.6	27.3
P-$PO_4^=$	3	0.03	0.3	0.01	0.009	0.01
N-NO_3^-	52	237	45	73	171	71
N-NO_2^-	0.002	0.005	0.1	0.027	0.017	0.00084
NH_4^+	41	0.5	5	0.061	0.038	0.064
DIN	93	237	50	73	171	71
DIN/SRP	31	791	167	7308	19006	7106

[a] *Ch= Chara haitensis; Cv= C. vulgaris; Cz= C. zeylanica var. diaphana; Nf= Nitella furcata var. sieberi; Nt= N. tenuissima var. tenuissima.*

to be of taxonomic importance at the generic and specific levels were measured in each thallus (according toWood, 1963; Griffin and Proctor, 1964; Wood & Imahori, 1965; Proctor *et al*., 1971; Cáceres 1975, 1978; Hussain *et al*., 1990; Soulié-Märsche, 1999; Mandal *et al.*, 1995 and Ray *et al.,* 2000). For the delimitation of sub-genera and sections we used the works of Proctor *et al*. (1971) and Cáceres, 1975, 1978. The tallus length was measured in five plants, while the other morphometric features were measured in thirty replicates. The discrimination based on the shape of the oospore was expressed by isopolarity index (ISI, length: width ratio oospore per 100; Soulié-Märsche, 1999). For microscopic analyses and photographic documentation of cytological characters, we used an Olympus BX51 microscope, with an SC35 photomicrography system. For examining the external morphology of the oospore wall a scanning electron microscope (SEM) Jeol model JSM-5310LV was used. Kruskall-Wallis and Mann Whitney tests (*U*) on SPSS 12 statistics software were used to assess statistically significant differences ($p< 0.05$) of morphometric characters between populations.

RESULTS AND DISCUSSION

Morphological analysis

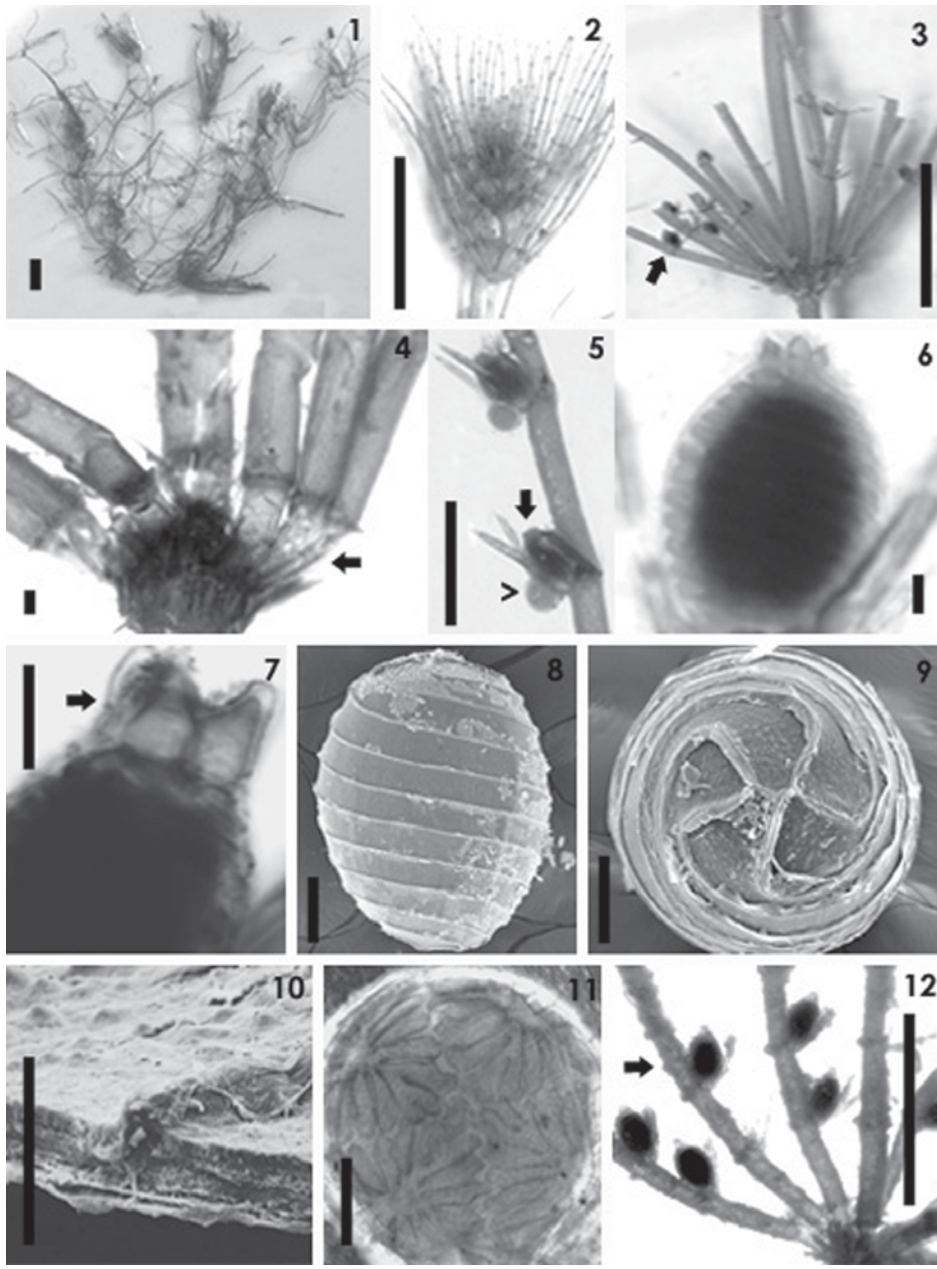
Twelve populations of Charales living in calcareous tropical streams in Mexico were analyzed, and five species identified.

Genus *CHARA* L.

Chara haitensis Turpin **Figs 1-12**

Monoecious, 8-37 cm in length, slightly to heavily incrusted. Axes moderately slender to stout, 400-5900 µm in length, 500-2600 µm in diameter. Cortex triplostichous and isostichous. Stipulodes in two rows, opposite to the basal segment branchlet; apex acute-acuminate, upper 393-1415 µm in length, 73-169 µm in diameter; lower 148-1,036 µm in length, 70-190 µm in diameter. Spine-cells solitary, cylindrical or triangular, apex acuminate, 57-1925 µm in length, 26-129 µm in diameter. Branchlets 6-18, 0.8-4.3 cm long, segments 6-13; lowermost and apical segment are ecorticated 371-2000 µm in length, 312-940 µm in diameter, end segment formed by 1-3 ecorticated cells, 152-1065 µm in length, 62-223 µm in diameter, apex of end cell acuminate. Posterior bract-cells 3-11 occasionally reduced, 70-1465 µm in length, 40-234 µm in diameter. Anterior bract-cell 2 often well developed, 478-1312 µm in length, 73-211 µm in diameter. Two bracteoles by node, 1055-2725 µm in length, 90-232 µm in diameter. End segment with 4-5 verticilate bract-cells, small, acuminate 79-486 µm in length, 41-117 µm in diameter. Nodes fertiles second to fourth. Gametangia conjoined or sejunted. Oogonia 255-1000 µm in length, 100-781 µm in diameter. Coronula 35-226 µm length, 82-394 µm diameter, cells pyramidal, with rounded edges. Antheridia 284-558 µm in diameter.

Oospore morphology. The oospores are dark brown, ellipsoidal with rounded apical and basal poles in lateral view and have 8-10 spiral ridges-usually striate is used; 606-748 µm long, 336-550 µm wide, ISI 130-190 and 38-69 µm across the fossa. The oospore wall exhibits a papillate ornamentation, papillae



Figs 1-12. Morphological features of *Chara haitensis*. **1.** Habit of algae. **2.** Detail of apical whorl. **3** and **12**. Fertile branchlet (arrow). **4.** Ecorticated basal segment (arrow). **5.** Gametangia conjoined, upper side oogonia (arrow) and low side antheridia (arrow head). **6.** Mature oogonia. **7.** Coronula cells with curved and divergent edges (arrow). **8.** Oospore. **9.** Basal view oospore. **10.** Ornamentation of oospore wall. **11.** Antheridia. **12.** Sejoined oogonia. Scale bars: 1 cm for Figs, 1, 2, 3, 5, 12; 100 μm for Figs 4, 6, 7, 8, 9, 11; 50 μm for Fig. 10.

0.83-1.53 µm long. *C. zeylanica* and *C. haitensis* are two species of the section Willdenowia. The main differences between them are the number and shape of antheridia shield cell, the fertility of the lowest branchlet nodes and the oospores characters (Proctor *et al*, 1971; Soulié-Märsche, 1999). In the Mexican populations, the fertility of the lowest branchlet nodes and the antheridia cell triangular were of prime importance. The populations of *C. haitensis* and *C. zeylanica* var. *diaphana* could be separated according to ISI, which coincided with that described by Griffin & Proctor (1964). However, it was not possible to examine the oospore ornamentation and the number of antheridia shield cells.

Distribution in Mexico. Examined specimens: 1) El Meco, San Luis Potosí, Ciudad Maíz, 6.xi.2004, coll. *M. Cartajena* and *J. Carmona*, PA4275 (FCME); 2) El Meco, San Luis Potosí, Ciudad Maíz, 20.v.2005, coll. *M. Cartajena* and *J. Carmona* PA4277 (FCME); 3) Micos, 20.v.2005 coll. *M. Cartajena* and *J. Carmona* PA4278 (FCME); 4) El Salto, 20.v.2005, coll. *M. Cartajena* PA4279 (FCME); 5) Yautepec, Itzamatitlán, 24.iv.2005 coll. *M. Cartajena* and *J. Carmona* BALE4276 (FCME); 6) Morelos, Amacuzac, Salado river, 5.vi.2005, coll. *M. Cartajena* BALE4282 (FCME).

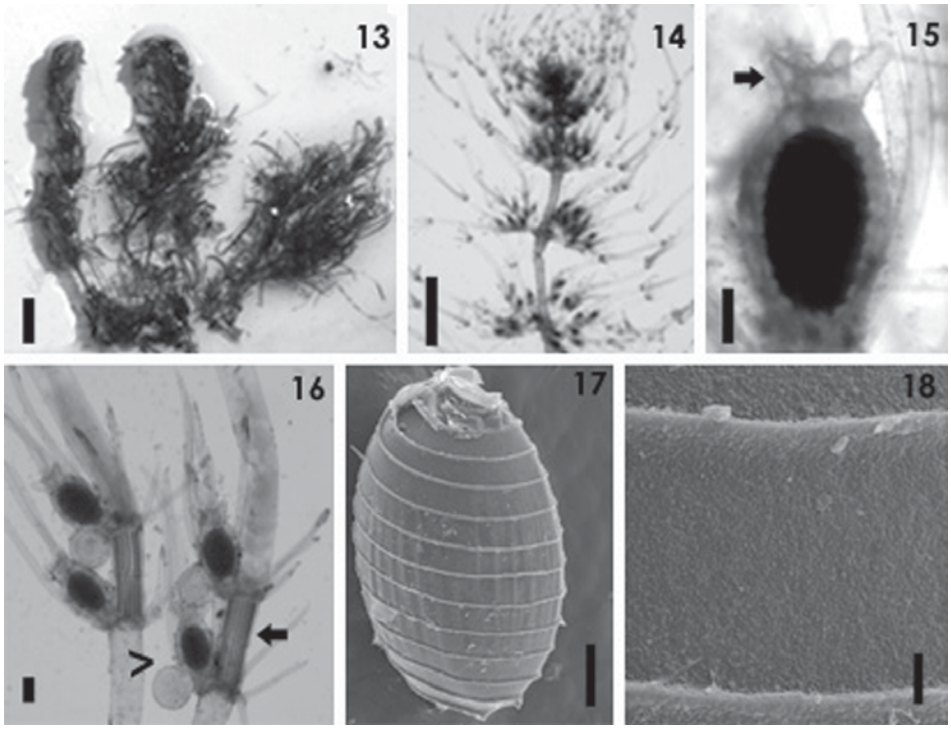
Associated taxa. *Nitella tenuissima* var. *tenuissima, N. furcata* subsp. *furcata* var. *sieberi, C. vulgaris* var. *nitelloides*, *Chroodactylon ornatum* (Thwaites) Hansgirg, *Spyrogira* sp., *Compsopogon coeruleus* (C. Agardh) Montagne, *Vaucheria* sp. *Cladophora* sp., *Thorea hispida* (Thore) Desvaux, *Batrachospermum globosporum* Israelson, *Terpsinoë musica* Ehrenberg.

Taxonomic remarks. This species has been reported in the Americas (as *Chara zeylanica* var. *zeylanica* f. *michauxii* Wood & Imahori, 1965; Proctor *et al.,* 1971; Proctor, 1990; Soulié-Märsche, 1999). However its variability has not been described, which we do here for the first time from Mexican material. *Chara haitensis* can be distinguished from the other characean taxa in this study by the length of tallus ($U = 1$, $p = 0.003$), number of branches in a whorl ($U = 61\text{-}187$; $p < 0.05$), number of segments by branchlet ($U = 8\text{-}125$; $p < 0.05$), length of lowest segment of the branchlet ($U = 26\text{-}215$, $p < 0.05$), gametangia in second and four nodes, oospore length ($U = 5$, $p < 0.05$), oospore diameter ($U = 17\text{-}406.5$, $p < 0.05$), fossa length ($U = 26$, $p < 0.05$) and the ISI ($U = 26\text{-}323$, $p < 0.05$).

Chara zeylanica var. ***diaphana*** (Meyen) R.D. Wood **Figs 13-18**

Monoecious 5-8 cm in length, heavely incrusted. Axes 400-5900 µm in length, 300-700 µm in diameter. Cortex triplostichous and isostichous. Stipulodes in two rows, opposite to the basal segment branchlet, apex acuminate, upper row, 364-1282 µm in length 58-100 µm in diameter; stipulodes of lower row, 366-845 µm in length, 60-100 µm in diameter. Spine-cells solitary, cylindrical or triangular, 70-1255 µm in length, 36-89 µm in diameter. Branchlets 7-13, 0.8-1.9 cm long, segments 5-8; all the segments are ecorticated, except the second one, basal segment ecorticated 757-4361 µm in length, 277-546 µm in diameter. End branchlet segment formed by 1-3 ecorticated cells, 85-316 µm in length, 34-97 µm in diameter, apex of end acuminate. Posterior bract-cells 4 occasionally reduced, 85-316 µm in length, 34-97 µm in diameter. Two anterior bract-cells two, often well developed, 1139-1727 µm in length, 54-83 µm in diameter. Two bracteoles by node, 1139-1727 µm in length, 54-83 µm in diameter. Fertiles nodes: first and second. Gametangia conjoined. Oogonia 555-812 µm in length, 390-550 µm in diameter. Coronula 136-184 µm in length, 165-192 µm in diameter, cells divergent composed of triangular cells with redounded edges. Antheridia 210-320 µm in diameter.

Oospore morphology. The oospores are black in colour, cylindrical in face view and have 11-13 spiral ridges; they are 494-620 µm long, 251-350 µm wide and 20-62 µm across the fossa, ISI 150-200. It was not possible to examine the fossa wall oospore.



Figs. 13-18. Morphological features of *Chara zeylanica* var. *diaphana.* **13.** Habit. **14.** Overlapped whorl. **15.** Divergent cells coronula (arrow). **16.** Segment of corticated branchlet (arrow) and fertile nodes branches (arrow head). **17.** Oospore. **18.** Ornamentation of oospore wall. Scale bars. 1 cm for Figs 13, 14; 100 µm for Figs 15, 16, 17; 10 µm for Fig. 18.

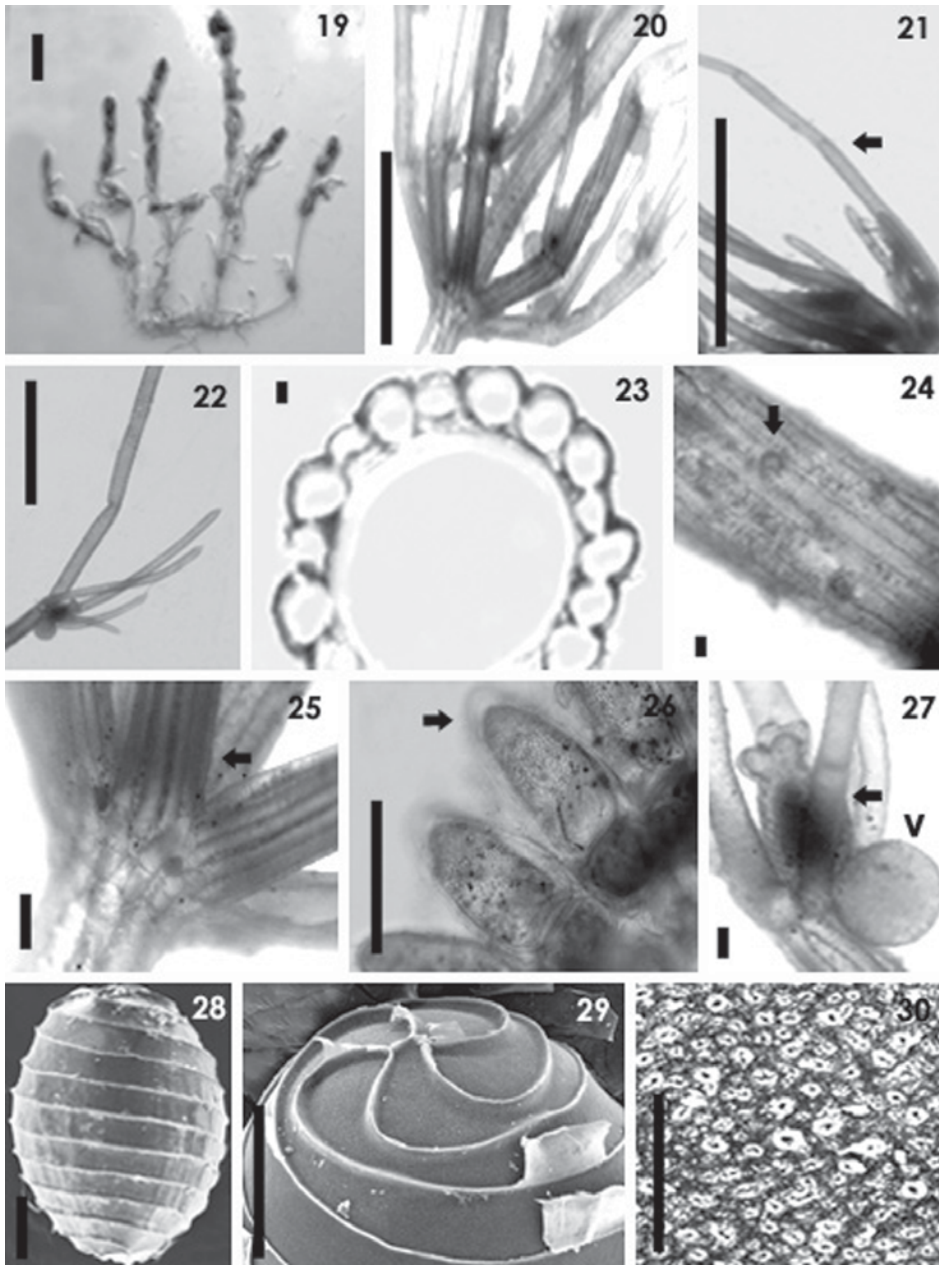
Distribution in Mexico. Examined specimens: 7) Morelos, Tlalquiltenalgo, Los Manantiales, 8.xi.2005, coll. *J. Carmona*, BALE4283 (FCME).

Associated taxa. *Chara vulgaris* var. *nitelloides*, *Blennothrix ganeshii* Watanabe *et* Komárek, *Terpsinoë musica* and *Vaucheria* sp.

Taxonomic remarks. The spine length and stipulodes length (upper and lower) in the Mexican populations are greater than previously reported (Wood & Imahori, 1965). The value of oospore ISI coincides with that described by Griffin & Proctor (1964). *Chara zeylanica* var. *diaphana* is distinguished from other taxa of *Chara* in Mexico by its imbricate whorls, acuminate apex, divergent coronula and corticated second segment of the branch.

Chara vulgaris var. ***nitelloides*** (A. Braun) R.D. Wood **Figs 19-30**

Monoecious, 6-14 cm in length, generally heavily incrusted. Axes moderately slender 600-2100 µm in length, 400-689 µm diameter. Cortex diplostichous and isostichous. Stipulodes in two rows, opposite to the basal segment branchlet, allantoid, apex redounded, upper row 110-307 µm in length 79-132 µm in diameter; lower 56-234 µm in length 53-128 µm in diameter. Spine cells solitary, spherical, 42-111 µm in length, 40-117 µm in diameter. Branchlets



Figs 19-30. Morphological features of *Chara vulgaris* var. *nitelloides*. **19.** Habit of algae. **20.** Fertile branchlets whorl. **21.** Branchlet (arrow). **22.** Length anterior bract cell (arrow). **23.** Cross section of internodes which shows the number of cortical cells. **24.** Spines cells (arrow). **25.** Corticated basal segment (arrow). **26.** Upper row stipulodes (arrow). **27.** Conjoined gametangia oogonia (arrow), antheridia (arrow head). **28.** Oospore. **29.** Oospore basal view. **30.** Ornamentation wall oospore. Scale bars: 1 cm for Figs 19-22, 100 μm for Figs 25-29; 10 μm for Figs 23, 24; 5 μm for Fig. 30.

per whorl 6-9, 1.2-3.2 cm long; segments 1-2, with corticated and ecorticated segment, basal segment corticate 502-1977 µm in length, 226-546 µm in diameter. End branchlet segment with 3-4 ecorticated cells without bract cells; 152-3363 µm in length, 77-287 µm in diameter. Posterior bract-cell absent. Anterior bract-cells 2 often well developed, 1766-3357 µm in length, 119-310 µm in diameter. Two bracteoles by node, 410-1805 µm in length, 91-279 µm in diameter; first and second nodes fertiles. Gametangia conjoined. Oogonia 205-945 µm in length, 91-279 µm in diameter. Coronula 70-192 µm in length, 90-289 µm in diameter. Antheridia 251-493 µm in diameter.

Oospore morphology. The oospores are dark-brown in colour, oval in face view and have 11-12 spiral ridges; they are 491-627 µm long, 351-429 µm wide and 48-61 µm across the fossa, ISI 125-150. The oospore wall is pustular with pores 0.53-1.12 µm in diameter.

Distribution in Mexico. Examined specimens: 8) Morelos, Tlalquiltenango, Los Manantiales, 18.xi.2001, coll. *J. Carmona,* BALE41 (FCME). 9) El Meco, San Luis Potosí, Ciudad Maíz, 20.v.2005, coll. *M. Cartajena* and *J. Carmona* PA4280 (FCME).

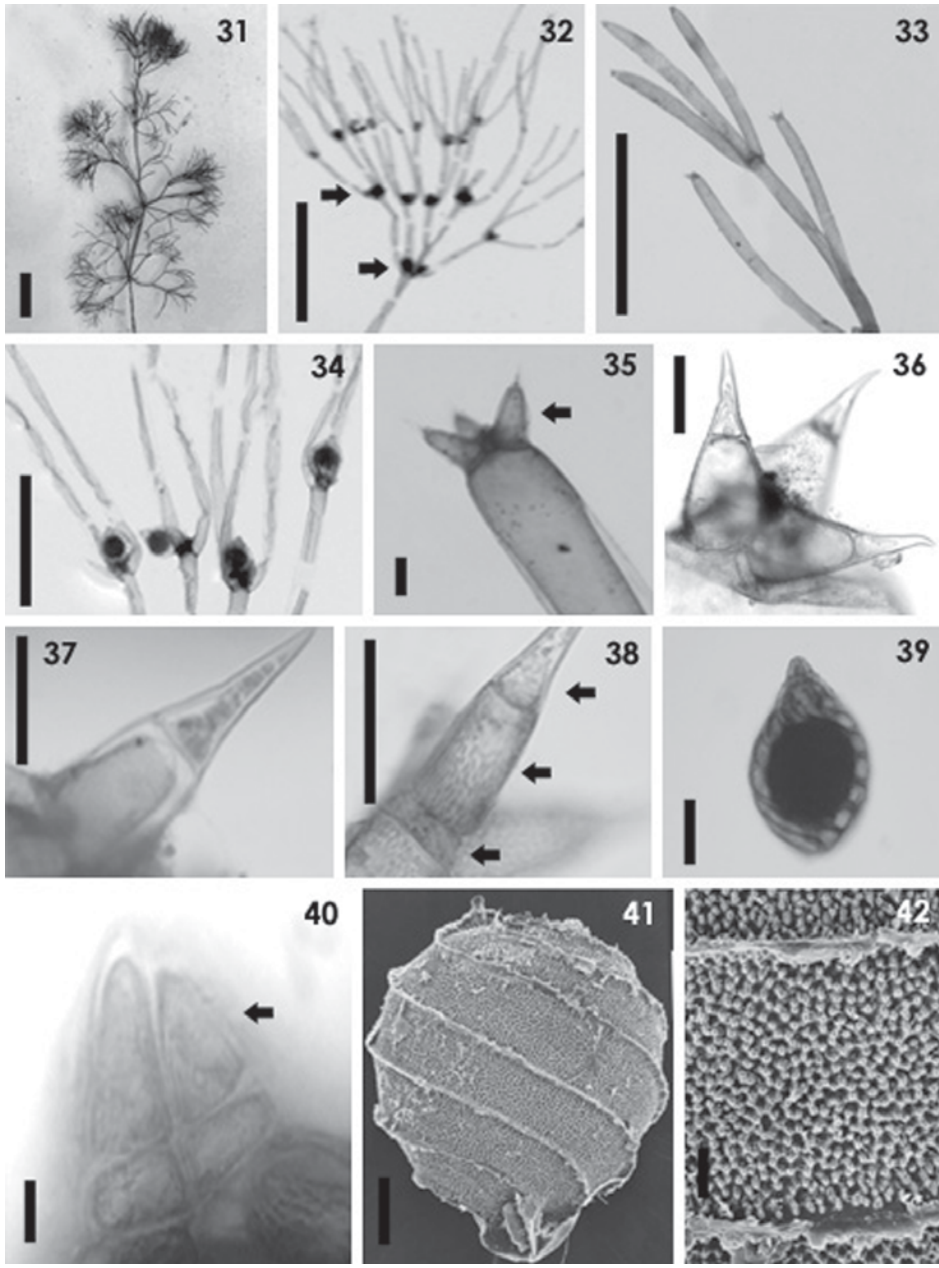
Associated taxa. *Chara haitensis*, *N. tenuissima* var. *tenuissima, Blennothrix ganeshii*, *Terpsinoë musica, Vaucheria* sp., *Chroodactylon ornatum.*

Taxonomic remarks. Wood & Imahori (1965) mentioned that the ecorticated segments of the branch are elongated, but the length was not reported. The length of the internode in the Mexican populations is greater (≤ 2100 µm) than reported in populations from Bolivia (≤ 1500 µm, Wood & Imahori, 1965). However, the branched irregular cortication, the globular spine and the ornamentation composed of pustules with pores are taxonomically significant for this species (Wood & Imahori, 1965; Mandal & Ray, 1999). The morphometric characteristics taxonomically significant for the Mexican populations of *Chara vulgaris* var. *nitelloides* are the position of the fertile node, the number of branchlets per whorl (U= 61-187, p <0.05), the number of segment in the branchlet (U= 0-12.5, p < 0.05), the stipulodes number (U= 0-4, p < 0.05), the number of cortical cells (U= 0, p < 0.05) and the length of bracteole cells (U= 0-805, p < 0.05).

Nitella furcata (Roxb. *ex* Bruz) Ag. *emend*. R.D. Wood
subsp. *furcata* var. ***sieberi*** (A.Br) Wood **Figs 31-42**

Monoecious, 8-22 cm in length, without incrustation. Whorls 1.2-2.7 cm in length, 0.9-3.6 cm in diameter, without mucilage. Branchlets 3-7, homoeoclemous 1.3-2.7 cm in length, 3-furcated. Axes slender to stout, internode 1300-4900 µm in length, 660-1086 µm in diameter. Fertile branchlets 3-6. Sterile dactyls similar to fertile dactyls; three dactyls, abbreviate; usually bi-celled, but occasionally subtended by a short nodal cell. Reduced dactyls 127-442 µm in length, 64-128 µm in diameter, basal cell 72-258 µm in length, 64-128 µm in diameter, end cell 23-106 µm in length, 21-66 µm in diameter, wide base, narrowly conical or elongated. Gametangia conjoined or sejoined at the 2-4 lowest furcations; oogonia 1-3 at each node, antheridia 1-2. Oogonia 87-356 µm in length, 86-333 µm in diameter. Coronula 27-68 µm in length, 45-77 µm in diameter, cells of upper tier 2.8 times longer than lower tier. Antheridia 140-279 µm in diameter, terminal or between two oogonia.

Oospore morphology. The oospores are yellowish-brown in color, orbicular in lateral view and have 6-7 spiral ridges; they are 215-260 µm long, 184-242 µm wide and 35-60 µm across the fossa, ISI 101-124. The oospore ornamentation is reticulate and regularly beaded; beads 0.82-1.4 µm long.



Figs 31-42. Morphological features of *Nitella furcata* var. *sieberi*. **31.** Habit. **32.** Basal oogonia (arrows) in a whorl. **33.** Sterile branchlet. **34.** Fertile branchlet. **35.** Dactyls (arrow). **36, 37.** 2-celled dactyl. **38.** 3-celled dactyl (arrow). **39.** Oogonia. **40.** Cells coronula (arrow). **41.** Oospore. **42.** Fossa cell ornamentation. Scale bars 1 cm for Figs 31-34; 100 µm for Figs 35-39; 50 for Figs 40, 41; 5 µm for Fig. 42.

Distribution in Mexico. Examined specimens: 10) San Luis Potosí, Ciudad Maíz, Micos, 6.xii.2004, coll. *M. Cartajena* and *J. Carmona* PA4274 (FCME); 11) San Luis Potosí, Ciudad Maíz, Micos, 20.v.2005, coll. *M. Cartajena* and *J. Carmona* PA4281 (FCME).
Associated taxa. *Chara haitensis, Thorea hispida, Batrachospermum globosporum, Cladophora* sp.

Taxonomic remarks. This taxon has a wide range or morphological variation. The vegetative and reproductive characteristics in the Mexican populations are similar to those reported by Wood (1963), Wood & Imahori (1965), Cáceres (1975), Sakayama *et al.* (2005), Mandal *et al.* (1995) and Vieira & Necchi (2002a) for the variety *sieberi*. However, the beaded reticulate ornamentation of the oospore is similar to *Nitella furcata* subsp. *furcata* var. *furcata* (Mandal *et al.,* 1995). The Mexican populations of *N. furcata* and *N. tenuissima* are distiguished by the thallus size (U= 0, $p < 0.005$), the length of the dactyl (U= p <0.05), the ISI of the oospore (U= 19, $p < 0.005$), the length and the fossa wall (U= 0 , $p < 0.005$) and shape, and wall ornamentation type.

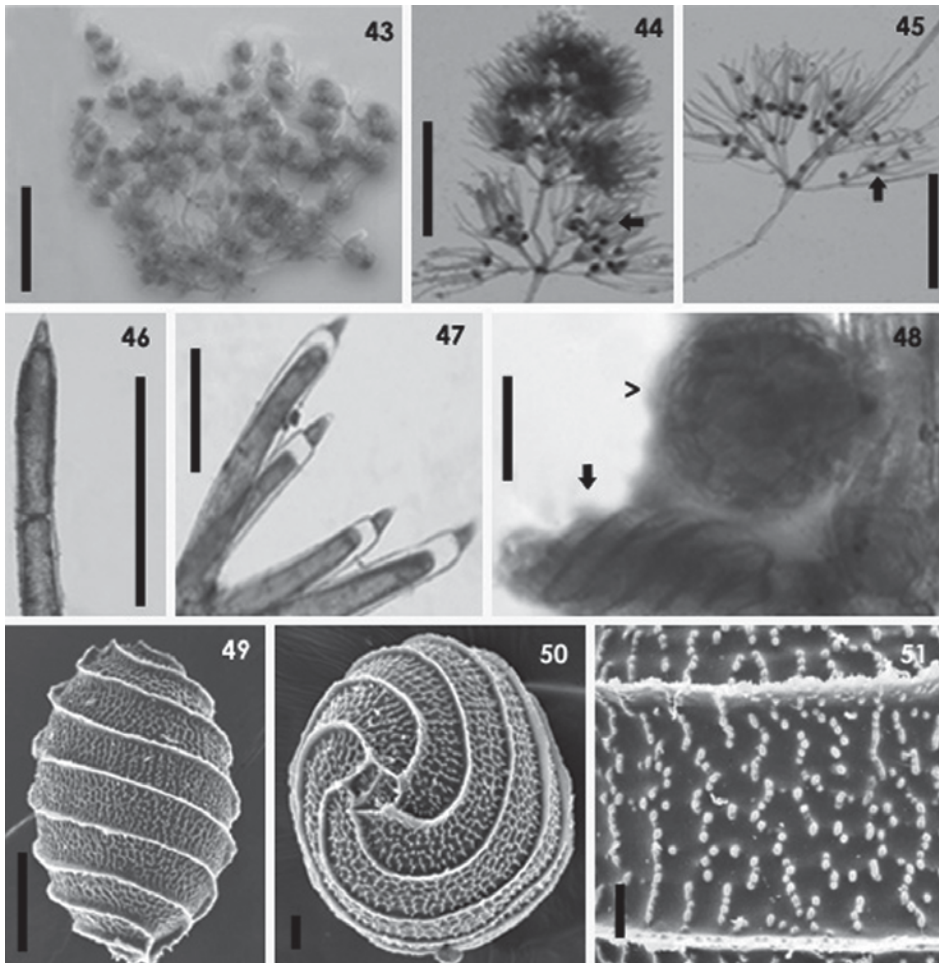
Nitella tenuissima (Desvaux) Kütz. *emend.* R.D. Wood var. ***tenuissima*** **Figs 43-51**

Monoecious, 3-6 cm in length, very heavily incrusted. Whorls 0.3-0.4 cm in length, 0.3-1.2 cm in diameter, without mucilage; 5-7 branchlets per whorl, homoeoclemous 0.2-0.4 in length, 2-3-furcate. Axes slender, 900-1500 µm in length, 124-257 µm in diameter. Fertile branches 3-6. Sterile dactyls similar to fertile ones; 3-4 elongated dactyls; 2- or 3-celled. Dactyls 664-2827 µm in length, 90-150 µmin diameter. Dactyl basal cell 560-2760 µm in length, 90-150 µm in diameter; dactyl end cell 51-110 µm in length, 25-37 µm in diameter. This cell is absent in the oldest branches. Gametangia conjoined or sejoined at the 2-4 lowest ray furcating; 1-3 oogonia per node. Oogonia 107-363 µm in length, 59-268 µm in diameter. Coronula 16-42 µm in length, 35-55 µm in diameter. Antheridia 140-279 µm in diameter.

Oospore morphology. Oospores are yellowish-brown in colour, elliptical in face view and have 6-7 spiral ridges; they are 220-260 µm long, 180-230 µm wide, 6-10 µm across the fossa, ISI 116-151. Oospore ornamentation is a granular regularly beaded; beads 0.82-1.4 µm long. The fossa wall is reticulate and regularly beaded with beads 1.34-1.86 µm long.
Distribution in Mexico. Examined specimen: 12) San Luis Potosí, Ciudad Maíz, El Meco, 6.xii.2004, coll. *M. Cartajena* PA4273 (FCME).
Associated taxa. *Chara haitensis, C. vulgaris* var. *nitelloides* and *Chroodactylon ornatum.*

Ecology and distribution

The investigated populations were found in 6 waterways of first and fifth orders, at altitudes above 800 m a.s.l. and tropical to subtropical climates (Text-Fig. 1, Tables 1 and 2). The ecological variables were similar in all the sites. They were characterized by high temperature (22-28 ºC), neutral to moderately alkaline waters (pH 7-8), high ionic content (total dissolved solids 593-1,884 mg l^{-1}; specific conductance 826-1,952 µS cm^{-1}; total ionic concentration 18.6-53.4 meq l^{-1}), medium mineral content (total water hardness ≥ 212 mg l^{-1} or calcium carbonate ≥ 361 mg l^{-1}), dominance of sulfate-bicarbonate/calcium, substratum formed by fine silt and medium sand as substrate (1.3-4.4 Φ) with high organic matter content (8-37%). Charales populations were collected in pools or meanders with low depth (0-41 cm) and slow flowing waters (0-30 cm s^{-1}). The particularly environmental data reported for Charales in this study suggest a low tolerance to variation in current velocity, depth and substratum type, similar to populations of *C. zeylanica*



Figs 43-51. Morphological features of *Nitella tenuissima* var. *tenuissima.* **43.** Habit. **44. 45.** Fertile whorls (arrow). **46.** 3-celled dactyl. **47.** Dactyls. **48.** Gametangia, oogonia (arrow), antheridia (arrowhead). **49.** Oospore. **50.** Oospore, basal view. **51.** Ornamentation of fossa wall. Scale bars: 1 cm for Figs 43-45; 100 µm for Figs 46-48; 10 µm for Figs 49, 50; 5 µm for Fig. 51.

in Malaysia (Zaneveld, 1940). We found *Nitella furcata* var. *sieberi* growing in a stream with low irradiance values (25 µmol photons m^{-2} s^{-1}), in contrast with the high irradiance values at which the other Mexican Charales populations were found (340-2234 µmol photons m^{-2} s^{-1}). For the six waterways the most favorable growth period was during the dry season or the end of the rainy season. All the Charales populations were found in mesotrophic to eutrophic conditions (Dodds *et al.,* 1997; Angelier, 2000), high dissolved inorganic nitrogen (≥ 50 – 237 mg l^{-1}), low to high soluble reactive phosphorous (0.009-3 mg l^{-1}) and low to medium dissolved oxygen (1.7 – 7.6 mg l^{-1}).

Chara haitensis appears to be more global in its distribution, probably due to its ability to tolerate a wide range of chemical and physical characteristics in

Table 2. Microhabitat characteristics of streams with Charales in calcareous tropical streams in Mexico.

	Itzamatitlán	*Los Manantiales*		*Salado river*	*Micos*		*El Meco*			*El Salto*
Taxa[a]	*Ch*	*Cv*	*Cz*	*Ch*	*Ch*	*Nf*	*Ch*	*Cv*	*Nt*	*Ch*
Current velocity (cm s^{-1}) [b]	5	5	0	1	1	10	30	5	1	0
Irradiance (µmol photons m^{-2} s^{-1}) [b]	1148	340	2029	2029	932	25	702	340	2234	2165
Depth (cm) [b]	41	16	10	0	24	34	18	27	5	8

[a] *Ch= Chara haitensis; Cv= C. vulgaris; Cz= C. zeylanica* var. *diaphana; Nf= Nitella furcata* var. *sieberi; Nt= N. tenuissima* var. *tenuissima.*
[b] average (n = 10)

their stream location, urban pollution included (Proctor *et al.,* 1971; Proctor, 1990; Bornette *et al*., 1996; Soulié-Märsche, 1999; Bornette & Arens, 2002). *Chara vulgaris* var. *nitelloides, C. zeylanica* and *N. furcata* var. *sieberi* are typical tropical species; they have been reported for America, Africa and Asia and grows preferentially in permanent water and reservoirs (Wood, 1963; Choudary & Wood, 1973; Soulié-Märsche, 1999; Mandal *et al*., 1995; Mandal & Ray, 1999; Vieira *et al*., 2002a; Hussain *et al*., 2003). *N. tenuissima* is present in all the continents except Australia but it is infrequent (Groves & Bullock, 1920; Choudary & Wood, 1973; Vieira *et al*., 2002a). The relatively low number of taxa of Charales reported in central Mexico prompts for further studies in other regions with calcareous tropical streams, especially in the southwestern part of Mexico.

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