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Cladophorella netzahualpillii sp. nov. (Cladophorales, Ulvophyceae), a species reproducing by spores

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A new species of *Cladophorella, C. netzahualpillii* Galicia & Novelo, is described from the walls of some tanks of brackish water near Texcoco Lake, Mexico. The characteristic features of this cladophoroid filamentous alga are its reproduction by spores, the absence of a system of prostrate filaments with only a few rhizoids and the simultaneous germination of series of akinetes to form a pseudoparenchymatous basal system. The thallus forms tufts 4–5 mm high, with mainly spherical intercalary and terminal akinetes, which have a lamellated wall and a thick sheath. The sporangia are mainly terminal and cylindrical or triangular in shape with spherical or oblong spores.

INTRODUCTION

The genus *Cladophorella* F.E. Fritsch (1944) currently includes three species and one variety (of the type species, *C. calcicola* F.E. Fritsch). Fritsch described only one species, the other two, *C. fritschii* and *C. sundarbanensis,* being added by Islam (1964). All of the species were found either subaerially, associated with soil, or in brackish water. In 1956, Chapman described a littoral species from New Zealand as *Cladophorella marina,* but this closely resembles *Wittrockiella salina,* which Chapman had described in 1949 (van den Hoek *et al.* 1984; Adams 1994). Although the description of *Cladophorella marina* by Chapman (1956) was very brief, the study by van den Hoek *et al.* (1984) indicated that it should be assigned to *Wittrockiella* Wille. Van den Hoek *et al.* (1984) showed that *Wittrockiella* has affinities with *Cladophora* but is distinct from it, and they also maintained *Cladophoropsis* as an independent genus. *Wittrockiella* forms spheres and tufts in mangrove-dominated areas of Australia and the Caribbean, but it also occurs less conspicuously in algal mats in Europe and North America (van den Hoek *et al.* 1984).

All species of *Cladophorella* are probably of tropical origin. The type species of the genus *C. calcicola* F.E. Fritsch was collected as growths on limestone in heated greenhouses at Cambridge, England (these were absent from nearby outdoor sites), and the others were described from Bangladesh (formerly East Pakistan); in all cases their affinity for calcareous or brackish-water environments was evident. Starmach (1972) records it in the flora of Poland.

The species described here grows on the walls of tanks of brackish water derived from wells of the National Commission of Water (Comisión Nacional del Agua) at Texcoco Lake, Mexico. In this place, a programme is being developed to restore the ancient lake that covered a portion of the Valley of Mexico during the pre-Columbian era. The water used in this program is partially extracted from groundwater via wells.

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MATERIAL AND METHODS

The observations, drawings and photographs are of living material and material preserved in 3% formaldehyde from collections made during April and July 1996 (dry and rainy seasons) from five deposits in water with a salinity ranging from 40–70%. For the observation of cell structure, diluted Lugol's solution (IKI) and Ehrlich's haematoxylin (Johansen 1940; Brown & Bold 1964) were used. Specimens were photographed using Nikon Optiphot and Reichert Zetopan microscopes with differential interference contrast (DIC) optics. Drawings were made using a camera lucida.

The material has been deposited in the herbarium of the School of Sciences of the National Autonomous University of Mexico (FCME).

RESULTS

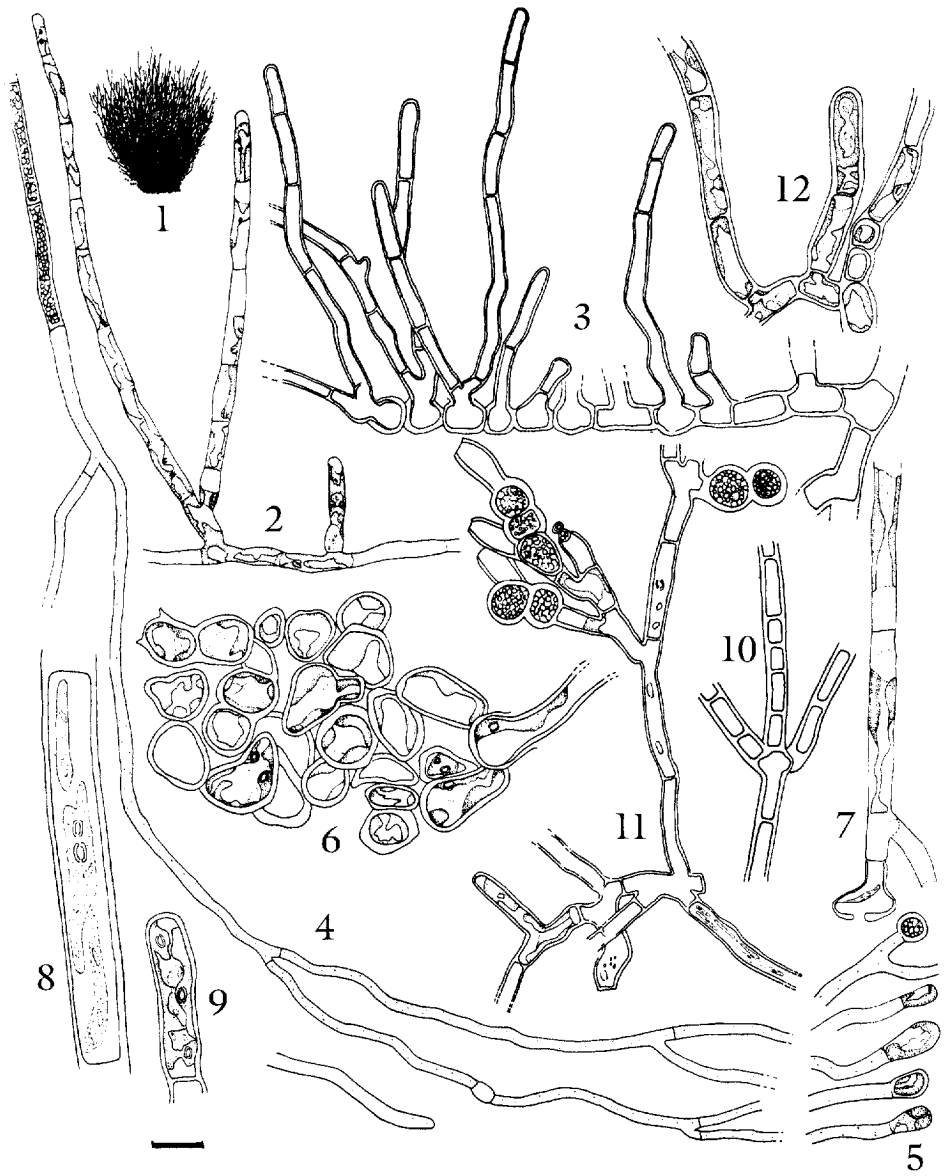
Cladophorella netzahualpillii Galicia & Novelo, *sp. nov.*

Figs 1–46

Thallus filamentosus, ramosus, caespites ad 4–5 mm longos formans; axes principales et ramificationes equaliter conformati. Systema pronum vix evolutum, paucirhizoidale. Filamenta praecipue a serie akinetorum surgentes. Cellulae (6–)8–10 μm latae, (20–)32–96(–105) μm longae. Parietes cellularum undulati. Akineta intercalaria et terminalia cylindrica vel polyedrica, 13–18 μm crassa. Sporangia cylindrica vel triangularia, 18–25 μm lata, 10–12 μm crassa. Species in aquis subsalsugineis habitans.

HOLOTYPE: FCME TEX1 (Figs 1–30). School of Sciences Herbarium, National Autonomous University of Mexico.

ETYMOLOGY: The species is named in honor of Netzahualpillii



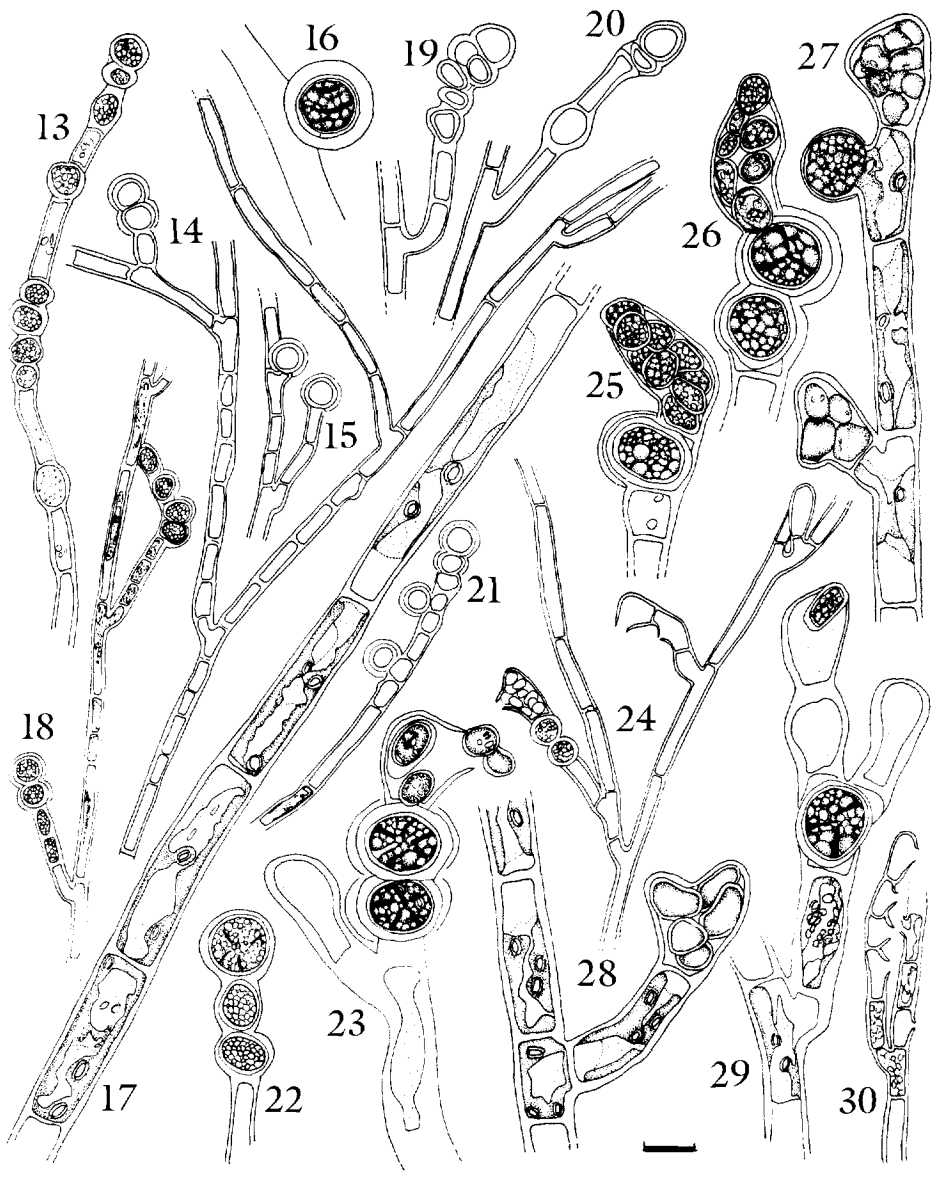
Figs 1–12. *Cladophorella netzahualpillii.* Scale bar = 2 mm (Fig. 1), 10 μm (Figs 6, 8, 9), or 20 μm (Figs 2–5, 7, 10–12).
Fig. 1. Overall appearance of thallus.
Fig. 2. Branched main axis.
Fig. 3. Prostrate filament with terminal akinetes, which have germinated to give a row of erect filaments. Some cells bear the scars of filament fragmentation.
Fig. 4. Basal rhizoid-like septate branches.
Fig. 5. Terminal cells and akinetes in rhizoid-like filaments.
Fig. 6. Basal system of polygonal cells.
Fig. 7. Large oblong cell in germinated filament.
Figs 8, 9. Detail of vegetative cells containing chloroplasts and pyrenoids.
Fig. 10. Opposite lateral with evection in basal part of thallus.
Fig. 11. Typical ramification pattern in terminal branches with laterals, evection and akinetes. Note empty walls above akinetes.
Fig. 12. Main axis with two type of branches; one with an active division zone.

(1465–1516), king of Texcoco, admired by his contemporaries as a wise man, constructor, prudent judge and astrologer.

TYPE LOCALITY: Walls of tanks at the brackish-water extraction wells for El Caracol, Lake Texcoco Management, National Commission of Water (Comisión Nacional del Agua), State of Mexico, Mexico (99°01′30″W, 19°29′15″N), April–July 1996.

HABITAT: Brackish water, salinity 40–70%.

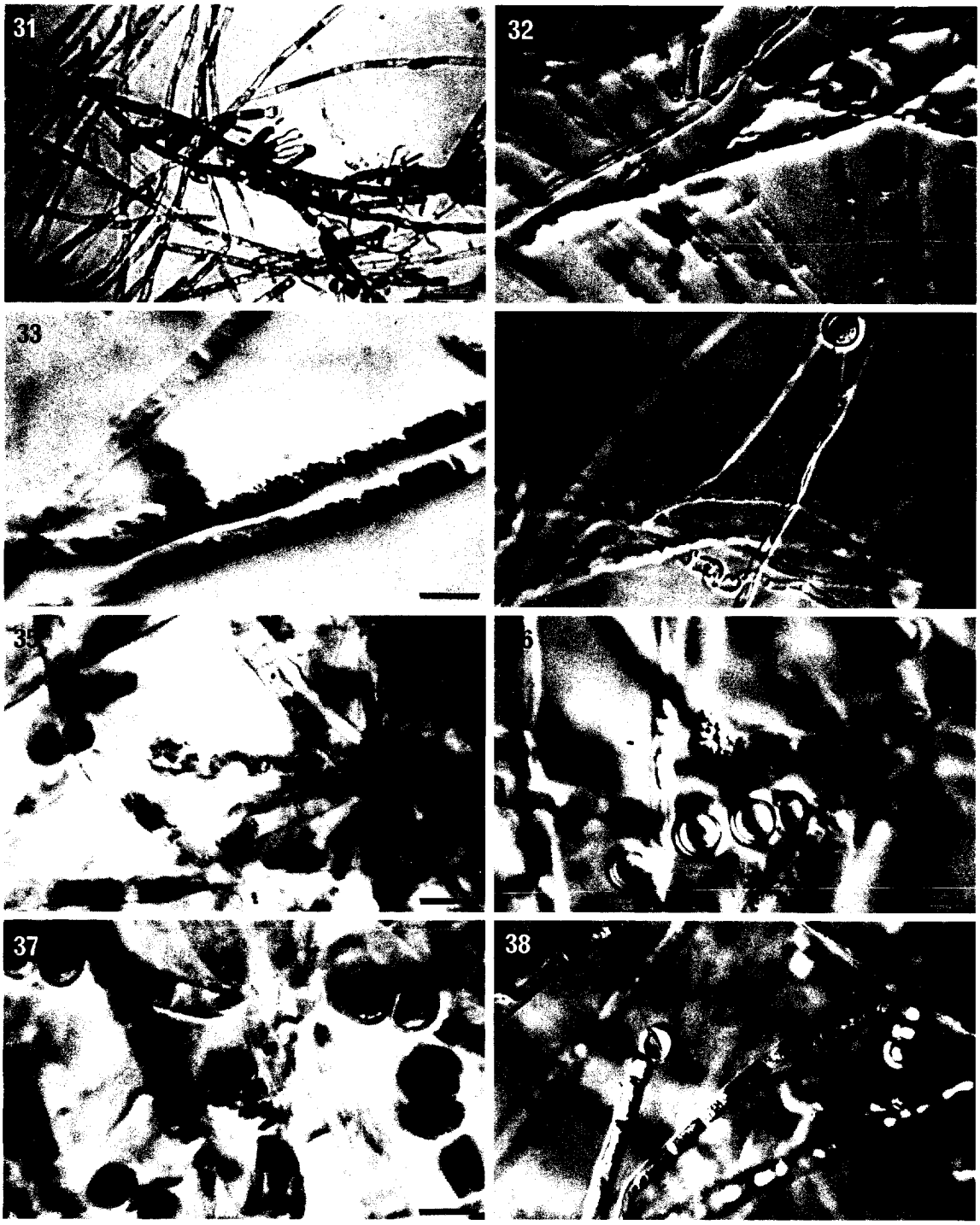
THALLUS MORPHOLOGY: The thallus is filamentous, branched and erect, forming tufts 4–5 mm high, in which all the branches are approximately of the same length (Fig. 1), without evident differentiation between the main axis and the branches (Figs 2, 12, 14, 31, 32). Erect branches grow out from a series of akinetes, which germinate more or less simultaneously and remain attached to the branches from which they originate (Fig. 3). A system of well-developed horizontal prostrate branches does not exist. In specimens



Figs 13–30. Reproduction and structure of *Cladophorella netzahualpillii.* Scale bar = 10 μm (Figs 16, 17, 23, 25–29) or 20 μm (Figs 13–15, 18–22, 24, 30).

Fig. 13. Terminal series of akinetes.
Fig. 14. Vegetative axis with a branch forming akinetes.
Fig. 15. Terminal and intercalary akinetes.
Fig. 16. 'Sessile' akinete on main axis.
Fig. 17. Details of vegetative cells.
Fig. 18. Akinetes with mucilaginous sheath.
Fig. 19. Series of akinetes formed by transverse and longitudinal divisions.
Fig. 20. Terminal akinetes with irregular division.
Fig. 21. Terminal and lateral akinetes.
Fig. 22. Inner thickenings in terminal akinete.
Fig. 23. Akinetes, a mature sporangium and partially liberated spores.
Fig. 24. Sporangial branch with akinetes and empty sporangia.
Figs 25–28. Mature sporangia.
Fig. 29. Empty sporangium and akinete with thick wall.
Fig. 30. A series of empty sporangia.

collected in April, some basal filaments were observed that were septate and branched. These were composed of more delicate cells than in the branches; the cells were elongate (8–9 μm diameter, 27–57 μm long) and contained scarcely any cytoplasm (Figs 4, 34). The terminal cells were differentiated, being short and clavate, and there were also terminal akinetes (Fig. 5). These filaments may be interpreted as rhizoids, but they were rare. In addition, short cylindrical, polygonal and



Figs 31–38. Vegetative features, sporangia and akinetes of *Cladophorella netzahualpillii*, DIC optics (except Fig. 31). Scale bars = 200 μm (Fig. 31), 30 μm (Figs 32, 34), 20 μm (Figs 33, 35–38).
Figs 31, 32. Main axis and branches.
Fig. 33. Detail of vegetative cells.
Fig. 34. Solitary akinete with thick wall in prostrate filaments.
Fig. 35. Triangular mature sporangium (left centre).
Figs 36, 37. Sporangia and akinetes in prostrate filaments.
Fig. 38. Empty wall of akinete.

irregularly shaped cells could be observed, which lacked rhizoids and from which erect branches arose (Fig. 6). In specimens collected in July, large oblong to cylindrical cells were observed, from which, again, erect filaments emerged (Figs 7, 46).

Erect branches are formed of cylindrical cells, not constricted at the septa, which are (6–)8–10 μm in diameter and (20–)32–96(–105) μm long. In vegetative branches, where there are practically no akinetes, the cells can be longer, 122–154 μm (Figs 8, 14, 31, 33), and the walls are frequently undulate (Figs 9, 11, 23). Some branches are formed by 20 or more thin (5 μm in diameter) and very long cells, with a terminal akinete (Figs 13, 15). In filaments having a series of akinetes, there are two kinds of cells: those proximal to branching sites are short with dense contents, whereas distal cells are long with little or no cytoplasmic content (Fig. 11).

Growth of the erect filaments is both intercalary and apical. In the lower and middle parts of the branches there are series of short cells with dense cell contents, measuring 9–11 μm in diameter and 12–15(–24) μm long, which give rise distally and basally to elongated cells (Figs 14, 33). Apical cells are 20–58 μm long and have dense cell contents.

Branching is subapical, lateral and basically alternate (Fig. 24); it is generally sparse, although occasionally a cluster of three or four branches emerge close together (Fig. 31). Branches are held at an acute angle to the axis (Figs 14, 31). Evection does occur, although rarely. Towards the base of the thallus, two opposite branches can sometimes be found emerging from the same cell (Fig. 10).

CELL STRUCTURE: Young cells have a parietal, laminar or H-shaped chloroplast (Figs 17, 33). The oldest cells have a reticulate chloroplast, and when they have elongated, the chloroplast appears as a series of longitudinal bands. Two to four very large pyrenoids occur per cell, each with two associated starch grains (Fig. 8). In long cells, there are 14–20 nuclei, which are small and spherical or slightly oblong; there are 7–9 large, elongate nuclei in short cells and 6–13 nuclei in akinetes.

Cell walls are thin (1.1–1.5 μm) and lamellate, as in other cladophoroid algae. The septum may be flat or curved, with the distal end of the cell convex and the proximal end concave. Akinetes and the cells adjacent to them have thickened walls. In akinetes, the wall is smooth and homogeneous, and under the DIC optics, it does not polarize light as do the walls of the other cells. The akinete wall does not have such evident lamellation as in other cells and is 2.4–3.9 μm thick. A mucilaginous, finely lamellate sheath is also present, enclosing the mature akinetes, especially terminal ones (Figs 15, 16, 18–23, 25, 26, 39, 41, 45).

REPRODUCTION: This occurs by fragmentation, spores and akinetes. Fragmentation takes place mainly basally in the longest branches, near the zones of division. Many filaments lacked basal differentiated zones and this, together with scars on irregular basal cells (Fig. 11), suggests that fragmentation occurs with high frequency.

Sporangia are formed mainly in the basal zone. They can be terminal on short branches or intercalary; they sometimes occur next to one or two akinetes and may form series, of up to four sporangia (Figs 23–30, 35–37). Cells adjacent to sporangia and akinetes are extremely short and lack contents. Sporangia are cylindrical, linear or triangular and measure 10–12 μm at their widest point by 18–25.1 μm long. They contain 4–8 large spores or 16–24 smaller spores. In all cases, the spores are spherical and appear to be aplanospores. Spore germination has not been observed.

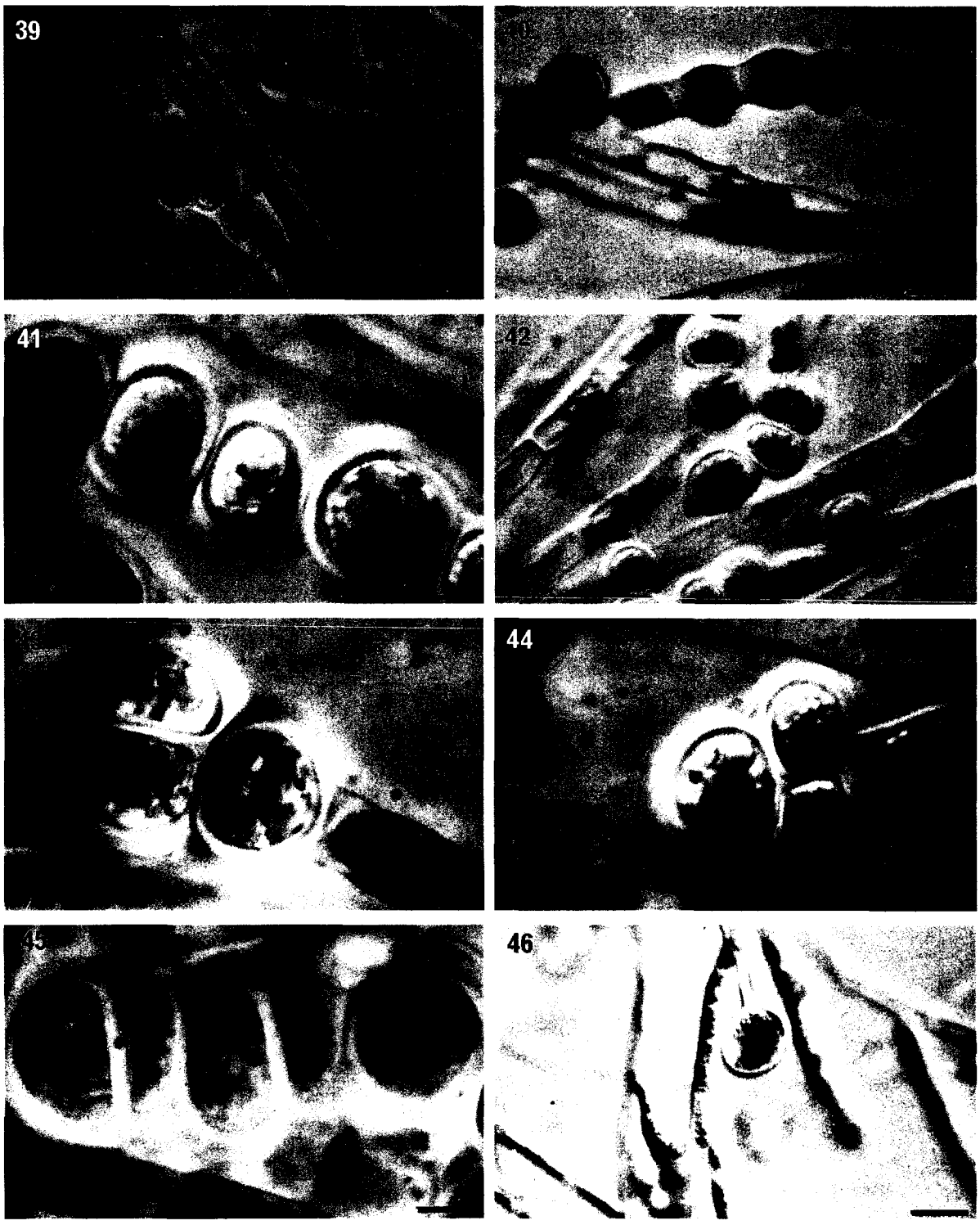
Akinetes are formed in the lower parts of the thallus and are mainly terminal on branches, being always at least as green as other cells but with much denser contents. They can be solitary, but also occur in pairs or form series of three to six. Occasionally they are intercalary or formed directly from cells of the main branches, thus giving the impression of being sessile (Figs 13–16, 18–23, 37–40). Their shape is spherical to subspherical or hemispherical with a diameter of 13.1–17.9 μm; they may be elongated parallel to the branch axis or transversely (Figs 20, 43–45). In the case of terminal series, akinetes can form polygonal clusters (Fig. 19) and may divide at right angles to the longitudinal axis of the branch (Figs 20, 45).

The contents of the akinetes are divided into four or six zones by thickenings that resemble incomplete membranes (Figs 16, 22, 23). The starch content of akinetes is considerably higher than in neighbouring cells. Occasionally it is possible to find an empty wall with a longitudinal rupture at the end of a series of akinetes, thus implying the release of their cellular contents (Fig. 38). It is also noticeable that the akinete sheaths from different branches can fuse, forming groups resembling a palmelloid stage, and they can be so dense in the basal region that they become pseudoparenchymatous (Figs 6, 42, 45). During akinete germination, the first division usually occurs parallel to the substrate (Fig. 3), although diagonal divisions have also been observed.

DISCUSSION

Comparisons with other *Cladophorella* taxa are made in Table 1. *Cladophorella netzahualpillii* appears from this to be closest to *C. calcicola,* but this is mainly because the original description was more comprehensive for *C. calcicola* than for other species. Lamellation of the wall, present in *C. netzahualpillii,* was also noted in *C. fritschii* (Islam 1964), but since this is a feature of cladophoroid algae, it was probably not thought worth mention by other authors. *Cladophorella sundarbanensis* has a similar ratio of cell length to width and also lacks a well-developed system of prostrate filaments, but the akinetes bear longitudinal striations not present in the Mexican species. *Cladophorella netzahualpillii* is more delicate than the other species of the genus, with the cells of the erect branches, akinetes and cellular walls having smaller dimensions. The most noticeable diagnostic feature, however, is the presence of spores, which are not reported in any of the other *Cladophorella* species (Fritsch 1944; Islam 1964; Shyam 1980).

The presence of spores and of very large cells at the base in specimens collected in July can be interpreted as part of the reproductive strategy of this species, including fragmentation of the thallus, germination of solitary akinetes or of a series of basal and intercalary akinetes, release of the contents of terminal akinetes as a large spore, and germination of im-



Figs 39–46. Akinetes and large basal cells of *Cladophorella netzahualpillii,* DIC optics. Scale bars = 20 μm (Figs 39, 40, 42, 46) or 10 μm (Figs 41, 43–45).

Fig. 39. Four-celled series of akinetes.

Fig. 40. Thick-walled akinetes; terminal akinete has divided longitudinally.

Figs 41, 42. Terminal akinetes from two different filaments. A common mucilaginous sheath links them.

Figs 43, 44. Terminal akinetes with longitudinal division.

Fig. 45. Wide mucilaginous sheath around terminal akinetes with irregular division.

Fig. 46. Large basal cells, with filaments arising from them.

Table 1. Comparative features of *Cladophorella* taxa.[1]

	C. calcicola var. *calcicola*	*C. calcicola* var. *orientalis*	*C. fritschii*	*C. sundarbanensis*	*C. netzahualpillii*
Growth form	dense tufts	carpet-like cushions	?	?	cushions, tufts
Thallus colour	bright green	bright green	dark green	green	bright green
Thallus prostrate system	profusely branched	with long rhizoids	with long rhizoids	present, with long rhizoids lacking septa	none evident, but with septate branched rhizoids
Erect filaments	all at the same height, branched	long, branched	long, branched	profusely branched	profusely branched
Thallus height (mm)	3–10	2–6	2–10	2–6	4–5
Type of growth	apical and intercalary	?	?	?	apical, intercalary, zoned
Evection	rare	? (two arm-like projections)	present (from figures)	not evident	rare
Type of ramification	in all directions, lateral in prostrate filaments	in all directions	principally alternate	lateral (from figures)	in all directions
Shape of cells in prostrate system	short cylindrical, irregular	triangular, rectangular or irregular	oblong, rectangular, triangular or irregular	cylindrical, triangular	very elongate cylindrical
Presence of obvious cellular content	mainly towards apex of filaments	near apex	?	?	some prostrate cells, all apical cells, cells near akinetes
Nucleus shape	spherical, oblong	?	?	?	spherical, oblong
Number of nuclei in vegetative cells	8–12	?	?	?	7–16 (20)
Cell wall	thin, smooth, straight	thick, undulate	thick, lamellate	?	thin, slightly undulate, lamellate
Cell wall width (μm)	1.7–2	2–8	3–14	2–4	1.1–1.5
Prostrate cell length (μm)	100–260	96–162	112–126	10–227	27–57
Prostrate cell diameter (μm)	36–51	(32–)96–104	84–98	31–54	8–9
Erect cell diameter (μm)	24–36(–46)	(8–)23–39(–58)	(42–)55–88	25–55	(6–)8–10(–11)
Erect cells, length (μm)	3–7 × as long as broad	3–10(18) × as long as broad; 42–280(–415)	1.5–3.5 × as long as broad; (84–)110–165	2–6(–15) × as long as broad	3–19 × as long a broad; (12)–32–90(–154)
Akinete position	terminally, intercalary rare	terminal, intercalary	terminal intercalary	terminal	terminal, subterminal, intercalary
Akinete nuclei number	up to 18	?	?	?	6–13
Akinete series	solitary or in pairs, 4–5 in erect branches	solitary or in pairs	solitary, in pairs or in triplets	solitary	solitary, in pairs and in series of 3–6
Akinete shape	almost spherical, oblong, cylindrical, subcylindrical, or irregular	round, elliptical, oblong, or irregular	round, oblong, elliptical, or irregular	oblong to subcylindrical	spherical, oblong, elliptical, polygonal, or triangular
Akinete diameter (μm)	oblong: 51–60; spherical: 68–82	(16–)39–52	(77–)106–175	25–42(–60)	(7–)13–21
Akinete length (μm)	76–164	(21–)57–94	(86–)154–247	61–138	19.5–25
Akinete wall thickness (μm)	3.7–5(–12)	5–8	11–28	2–4	2.4–3.9
Longitudinal striations in akinete wall	very evident	present	very evident and anastomosing in old cells	not evident	not evident
Lamellar structure in akinete walls	very evident, with spaces between lamellae	evident (from figures)	evident (from figures)	not evident	evident, very thin
Thallus fragmentation	principally basal	basal (from figures)	basal (from figures)	basal (from figures)	basal and intercalary
Habitat	limestone, subaerial	limestone and calcareous soil, subaerial	terrestrial, subaerial	flooded soils around mangroves, brackish-water	brackish-water

[1] Sources of information: Chapman (1956), Fritsch (1944), Islam (1964), Shyam (1980) and this paper.

mobile spores. The presence of large cells may be interpreted as a product of the last two. Under adverse conditions during drought, the attachment systems are reduced to a mucilaginous sheath in the case of mature akinetes and to entangled filaments with a few rhizoids.

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