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PSEUDULVELLA AMERICANA BELONGS TO THE ORDER CHAETOPELTIDALES (CLASS CHLOROPHYCEAE), EVIDENCE FROM ULTRASTRUCTURE AND SSU rDNA SEQUENCE DATA[1]

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The genus *Pseudulvella* Wille 1909 includes epiphytic, freshwater, or marine disk-shaped green microalgae that form quadriflagellate zoospores. No ultrastructural or molecular studies have been conducted on the genus, and its evolutionary relationships remain unclear. The purpose of the present study is to describe the life history, ultrastructural features, and phylogenetic affiliations of *Pseudulvella americana* (Snow) Wille, the type species of the genus. Thalli of this microalga were prostrate and composed of radiating branched filaments that coalesced to form a disk. Vegetative cells had a pyrenoid encircled by starch plates and traversed by one or two convoluted cytoplasmic channels. They had well-defined cell walls without plasmodesmata. Asexual reproduction was by means of tetraflagellate zoospores formed in numbers of two to eight from central cells of the thallus. The flagellar apparatus of zoospores was cruciate, with four basal bodies and four microtubular roots. The paired basal bodies lay directly opposite (DO) one another. The microtubular root system had a 5-2-5-2 alternation pattern, where the "s" roots contained five microtubules in a four-over-one configuration. A tetralobate nonstriated distal fiber connected all four basal bodies. A wedge-shaped proximal sheath subtended each of the basal bodies. The ultrastructural features of the zoospores were those of members of the order Chaetopeltidales. Phylogenetic analyses based on SSU rDNA placed *P. americana* sister to *Chaetopeltis orbicularis* in a well-supported Chaetopeltidales clade. Such a combination of features confirmed that this alga is a member of the order Chaetopeltidales.

Key index words: **Chaetopeltidales; Chlorophyta; life history; phylogeny; *Pseudulvella*; ultrastructure**

Abbreviations: **ML, maximum likelihood; MP, maximum parsimony; NJ, neighbor-joining; TBR, tree-bisection-reconnection**

The genus *Pseudulvella*, established by Wille (1909), includes epiphytic or epizoic, freshwater, or marine green microalgae, which usually produce radiating filaments of uninucleate, pyrenoid-containing cells that coalesce to form a disk, and that reproduce asexually by means of quadriflagellate zoospores. According to the online version of the *Index Nominum Algarum* (*Index Nominum Algarum* 2005) as of October 27, 2005, seven species have been placed in this genus: *Pseudulvella americana* (Snow) Wille (the type, including var. *americana* and var. *indica* Philipose), *P. applanata* Setchell & Gardner, *P. consociata* Setchell & Gardner, *P. heterotricha* Yarish, *P. nadsonii* Rochlina, *P. prostrata* (Gardner) Setchell & Gardner, and *P. rhizoclonii* Setchell.

The genus *Pseudulvella* has a confused taxonomic history. Wille (1909) erected *Pseudulvella* to distinguish *Ulvella americana* Snow, a species with pyrenoid-containing cells, from *U. lens* Crouan & Crouan, the type species of *Ulvella*, which at the time was thought to have cells that lacked pyrenoids (Huber 1892). It was also thought at that time that *Ulvella* species had multinucleate cells. Subsequent research (Dangeard 1931, Papenfuss 1962, Nielsen 1977) showed that pyrenoids are present in cells of *U. lens* and *U. setchellii* Dangeard, and that the cells are uninucleate; the "multinucleate cell" character was contributed to the genus from a species that is now placed in the Cladophorales (Papenfuss 1962).

Snow (1899) described the occasional presence of "evanescent bristles" in her specimens of *P. americana* (as *U. americana*) but did not illustrate them. Most subsequent authors, following Wille (1909), have not

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accepted Snow's report. Collins (1909), however, did accept the presence of bristles, and on this evidence transferred *U. americana* to the genus *Chaetopeltis*.

Papenfuss (1962) could only separate *Ulvella* and *Pseudulvella* on the basis of zoospore flagellar number (four in *Pseudulvella*, two in *Ulvella*), but acknowledged that life history information on *U. lens* was, at that time, incomplete. Nielsen (1977) discovered quadriflagellate zoospores in *U. lens* and *U. setchellii*, and asserted that no characters remained to separate the genera *Ulvella* and *Pseudulvella*, implying that the two should be merged, with *Ulvella* having priority. Nelson's proposal is difficult to assess at present; we know more than Nielsen did about *Ulvella*, but do not know much more about *Pseudulvella*. *Ulvella* is the type of the family Ulvellaceae, and this family has been assigned to the order Ulvales, class Ulvophyceae, on the basis of reproductive morphology, life history, and ultrastructure (O'Kelly and Floyd 1983, 1984a) as well as gene sequence data (O'Kelly et al. 2004a, b). Ultrastructural and gene sequence information is available for none of the *Pseudulvella* species. Very few records of *P. americana* exist, and only two published reports describe its morphology and reproduction (Snow 1899, Philipose 1947). For the remaining species, the principal sources of information are the publications in which they were described. Only *P. heterotricha* (Yarish 1975) and an alga assigned to *P. prostrata* (Chihara 1957) have been studied using modern culture methods. Nielsen (1988) transferred *P. heterotricha* to *Epicladia*, because this species has morphological and reproductive traits similar to those of other Ulvellaceae. However, the known characters of *P. americana*, including freshwater habitat, absence of papillate exit apertures from zoosporangia, release of zoospores in a vesicle, absence of germ tubes from germinating zoospores, and the putative "evanescent bristles," all are inconsistent with placing *P. americana* in the Ulvellaceae and suggest instead that this species belongs to the Chaetopeltidaceae (Chaetopeltidales, Chlorophyceae). If this is correct, then *Ulvella* and *Pseudulvella* are not only distinct at the genus level, but are also distinct at the class level.

We have placed an alga into culture that we believe is a specimen of *P. americana*. We have examined this alga using light and electron microscopy, and reconstructed its phylogeny from gene sequence data, to ascertain the taxonomic and phylogenetic position of *P. americana* and of the genus for which it is the type species.

MATERIAL AND METHODS

Isolation and cultivation. P. americana was collected as an epiphyte on plants of *Rorippa* sp. in June 2000 in the stream El Divisorio (38°19′ S, 61°42′ W), Buenos Aires, Argentina. Thalli of *P. americana* were isolated by picking single thalli and cultured in solid Bold's Basal medium with soil extract, solidified with 1.5% agar (Stein 1973), and kept under controlled conditions of temperature (15° C) and artificial illumination (12:12 LD photoperiod). Zoospore release was stimulated by maintaining subcultures in the dark for 48–96 h. In an attempt to induce sexual reproduction, thalli were incubated in distilled water, or in diluted Knop's medium (Braune et al. 1976).

A voucher specimen of cultured material has been deposited in the Herbarium of the University of California at Berkeley, accession number UC 1819306. In addition, a culture of *P. americana* has been accessioned in UTEX culture collection (UTEX B 2852).

Ultrastructure. Cultured thalli bearing zoosporangia were fixed in 2.5% glutaraldehyde in 0.05 M cacodylate buffer at room temperature, postfixed in 1% osmium tetroxide for 2 h at room temperature, dehydrated through a graded acetone series, and embedded with Spurr's low-viscosity resin (Spurr 1969) by the flat-embedding method (Reymond and Pickett-Heaps 1983). Sections were cut with a diamond knife, mounted on Formvar-coated grids, and then stained with uranyl acetate and lead citrate. The sections were observed with a JEOL 100 CX-II electron microscope (JEOL Ltd., Akishima,Tokyo, Japan) at the Centro Regional de Investigaciones Básicas y Aplicadas de Bahía Blanca (CRIBABB). Flagellar apparatus terms and conventions follow O'Kelly et al. (1994).

DNA extraction and sequencing. Genomic DNA from fresh cultures was extracted using the Nucleon Phytopure DNA extraction kit (Amersham International, Amersham, UK) following the manufacturer's instructions. The nuclear-encoded 18S small subunit (SSU) rDNA gene was amplified using published primers (O'Kelly et al. 2004a). The sequence of 18S rDNA from *P. americana* has been deposited in GenBank (DQ242477). Sequences were aligned using MacClade and the reliable portion of the alignment (1678 nucleotides) was included in the phylogenetic analyses. Accession numbers for the 18S rDNA sequences used in the analyses are given in Table 1.

TABLE 1. Published sequences used in the phylogenetic analyses.

Taxon name	GenBank accession number
Acrochaete repens	AY303592
Acrochaete viridis	AY303594
Acrosiphonia arcta	AY303600
Aphanochaete magna	AF182816
Bolbocoleon piliferum	AY303597
Chaetopeltis orbicularis	U83125
Chaetophora incrassata	U83130
Collinsiella tuberculata	AY198125
Eugomontia sacculata	AY198123
Floydiella terrestris	D86498
Fritschiella tuberosa	U83129
Gloeotilopsis sarcinoidea	Z47998
Gomontia polyrhiza	AY278216
Hormotilopsis gelatinosa	U83126
Hormotilopsis tetravacuolaris	U83124
Ignatius tetrasporus	AB110439
Monostroma grevillei	AF015279
Nephroselmis pyriformis	AB058391
Oltmannsiellopsis viridis	D86495
Phaeophila dendroides	AY454432
Pirula salina	AF124337
Planophila laetevirens	AJ416102
Pseudendocloniopsis botryoides	AJ416103
Pseudulvella americana	This study
Pterosperma cristatum	AJ010407
Pycnococcus provasolii	X91264
Schizomeris leiblenii	AF182820
Stigeoclonium helveticum	U83131
Ulothrix zonata	Z47999
Uronema belkae	AF182821

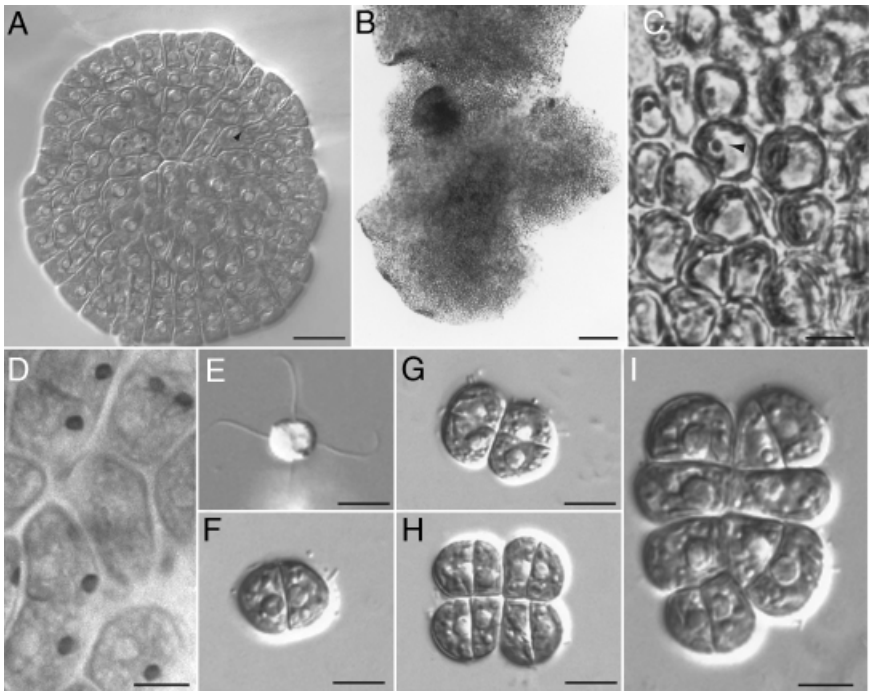


FIG. 1. Light micrographs of *Pseudulvella americana* in culture. Structure of the vegetative cells and asexual reproduction by zoospores. (A) Disk-shaped thallus with monostromatic peripheral and polystromatic central regions. Branched rows of cells radiate from the center (arrowhead). (B) Habit of an irregular thallus that grew detached. (C) Detail of a group of cells showing the parietal chloroplast and the pyrenoid (arrowhead). (D) Detail of mature zoosporangia with two to eight zoospores; prominent eyespots were present before the completion of zoospore differentiation. (E) Quadriflagellate zoospore, polar view. (F) Two-celled germling. (G) Four-celled germling. (H) Eight-celled germling. (I) Young disk-shaped thallus. (A) scale bar, 20 μm, (B) scale bar, 100 μm, (C–I) scale bar, 10 μm.

Phylogenetic analysis. Phylogenetic analyses were performed using PAUP*4b10 (Swofford et al. 2002). For maximum parsimony (MP) analysis, sites were unweighted and a heuristic search with random sequence addition (10 replicates) using the tree-bisection-reconnection (TBR) swapping algorithm was performed. Introduced gaps were treated as missing data. A LogDet distance tree and a neighbor-joining (NJ) tree were generated using a heuristic search algorithm with random addition of taxa, repeated 10 times. For the maximum likelihood (ML) analysis, a model of DNA substitution ($GTR+I+\Gamma$) was chosen using the program Modeltest 3.5 (Posada and Crandall 1998). Parameter estimates were suggested by Modeltest 3.5 and used in the ML heuristic search repeated three times with a different randomly selected addition order. For bootstrapping, a single heuristic search with full branch swapping (TBR) with 100 replicates was used for all analyses.

RESULTS

Characteristics of the thallus. Thalli of *P. americana* from nature and maintained in culture were prostrate when growing firmly attached to a substrate. Thalli were composed of tightly appressed cells, organized as orderly branched rows that radiated from a common center (Fig. 1A). Thallus diameter varied from 100 to 1000 μm. The thalli were monostromatic at the margins but became polystromatic in the center. Unattached thalli lost symmetry and formed an irregular mass of cells (Fig. 1B). At the center of attached thalli, cells were circular or slightly polyhedral, 7–16 μm in diameter, while near the margin they were rectangular, 8–17 μm in length and 5–10 μm in breadth. Cells had a parietal chloroplast with one or two pyrenoids (Fig. 1C, arrowhead).

Life history. Asexual reproduction took place by means of zoospores. Zoosporangia developed from central vegetative cells of the disk that increased in size (Fig. 1A). Two to eight zoospores formed by sequential cleavages. A prominent eyespot appeared before the process of zoospore maturation was complete (Fig. 1D). Zoospores were released by the irregular rupture of the zoosporangial wall, but were together, still surrounded by a vesicle until zoospores broke through the wall. Mature zoospores were pyriform, with four flagella apically inserted into a papillum, and a parietal cup-shaped chloroplast with an eyespot (Fig. 1E). During settlement, zoospores made contact with the substratum by means of their apical papilla. Then, they became rounded, lost their flagella, and secreted a thin hyaline envelope, by which time the cells were firmly fixed to the substratum. Germination was by spherical enlargement, followed by cell divisions that produced, initially, a four-celled disk-shaped thallus (Fig. 1, F and G). Successive perpendicular divisions occurred in each new cell (Fig. 1, H and I), giving rise to the mature multicellular thallus. Sexual reproduction was not observed.

Ultrastructure of vegetative cells and zoospores. Vegetative cells contained a single central nucleus and one parietal chloroplast. The pyrenoids were enclosed by starch plates and traversed by one or two convoluted cytoplasmic channels (Fig. 2, A–C). The cells were bounded by well-defined microfibrillar cell walls that were thick and irregular at the thallus margins (Fig. 2A). Transverse walls were without plasmodesmata (Fig. 2, A and B). Contractile vacuoles were lacking. No centrioles or other cytoskeletal structures were observed.

Zoospores, studied *in situ* within zoosporangia, lacked a cell wall, scales, or other type of external coat (Figs. 2D and 3A). The chloroplast contained a

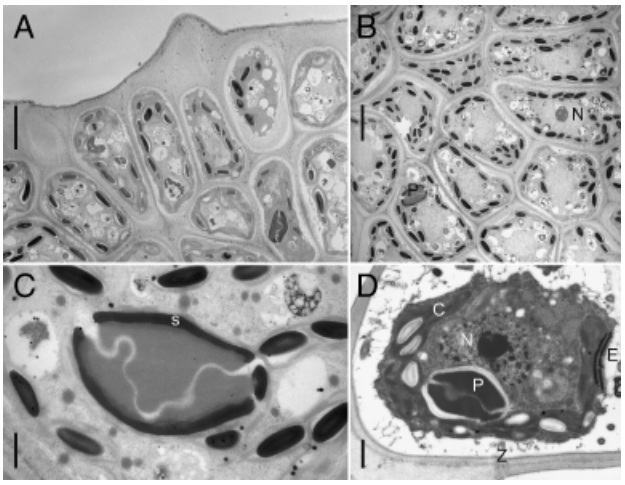


FIG. 2. Fine structure of vegetative cells and zoospores of *Pseudulvella americana*. (A–B). General view of tangential sections through the thallus. (A) Thallus edge showing the thick wall with an irregular margin. (B) Median and central portion of the thallus. (C) Detail of a pyrenoid circled by starch plates and traversed by one convoluted cytoplasmic channel. (D) Tangential longitudinal section of a zoospore within the zoosporangium, showing the nucleus, the chloroplast with one pyrenoid, and the eyespot. C, chloroplast; E, eyespot; N, nucleus; p, pyrenoid; S, starch plate; Z, zoosporangium. (A, B) scale bar, 5 μm. (C, D) scale bar, 1 μm.

pyrenoid with the same structure as in vegetative cells (Fig. 2D) and an eyespot consisting of two rows of globules (Fig. 2D).

The flagellar apparatus of the zoospore was apically inserted, the insertion point forming a small papillum (Fig. 3A). A contractile vacuole subtended the basal bodies (Fig. 3A). The flagellar apparatus consisted of four basal bodies and four microtubular roots (Fig. 3, B and C). The basal bodies were 590–630 nm long, and approximately 220 nm in diameter. In apical view, the upper-pair basal bodies (1 and 2) were directly opposed to one another, with no offset (Fig. 3B), whereas the L-shaped pairs (1 and 4, 2, and 3) formed an angle of approximately 120° (Fig. 3C). In lateral view, the upper-pair basal bodies described an angle of 130–180° (Fig. 3, D–F); the lower-pair basal bodies (3 and 4) showed an angle of 130–150° (Fig. 3, G–I). The microtubular root system had a 5-2-5-2 alternation pattern. The "s" roots contained five microtubules in a four-over-one configuration (Fig. 3J). Striated microtubule-associated components accompanied the five-membered roots (Fig. 3, K and L). The "d" roots contained two microtubules, each with at least one electron-dense component associated with it (Fig. 3, K and L). Roots "d" and "s" formed an angle of 35° and 50°, respectively, with the corresponding basal bodies (Fig. 3, B and C). A tetralobate nonstriated distal fiber interconnected all four basal bodies (Fig. 3, D and I). Each one of the four basal bodies was subtended at its proximal end by a prominent, bordered proximal sheath; the proximal sheaths subtending the upper-pair basal bodies were wedge-shaped (Fig. 3, F, G and M). The inner surface of each upper basal body proximal sheath was in turn connected by an arched, nonstriated proximal fiber (Fig. 3, A and F). Striated bands connected proximally the lateral sides of upper and lower basal bodies (Fig. 3N).

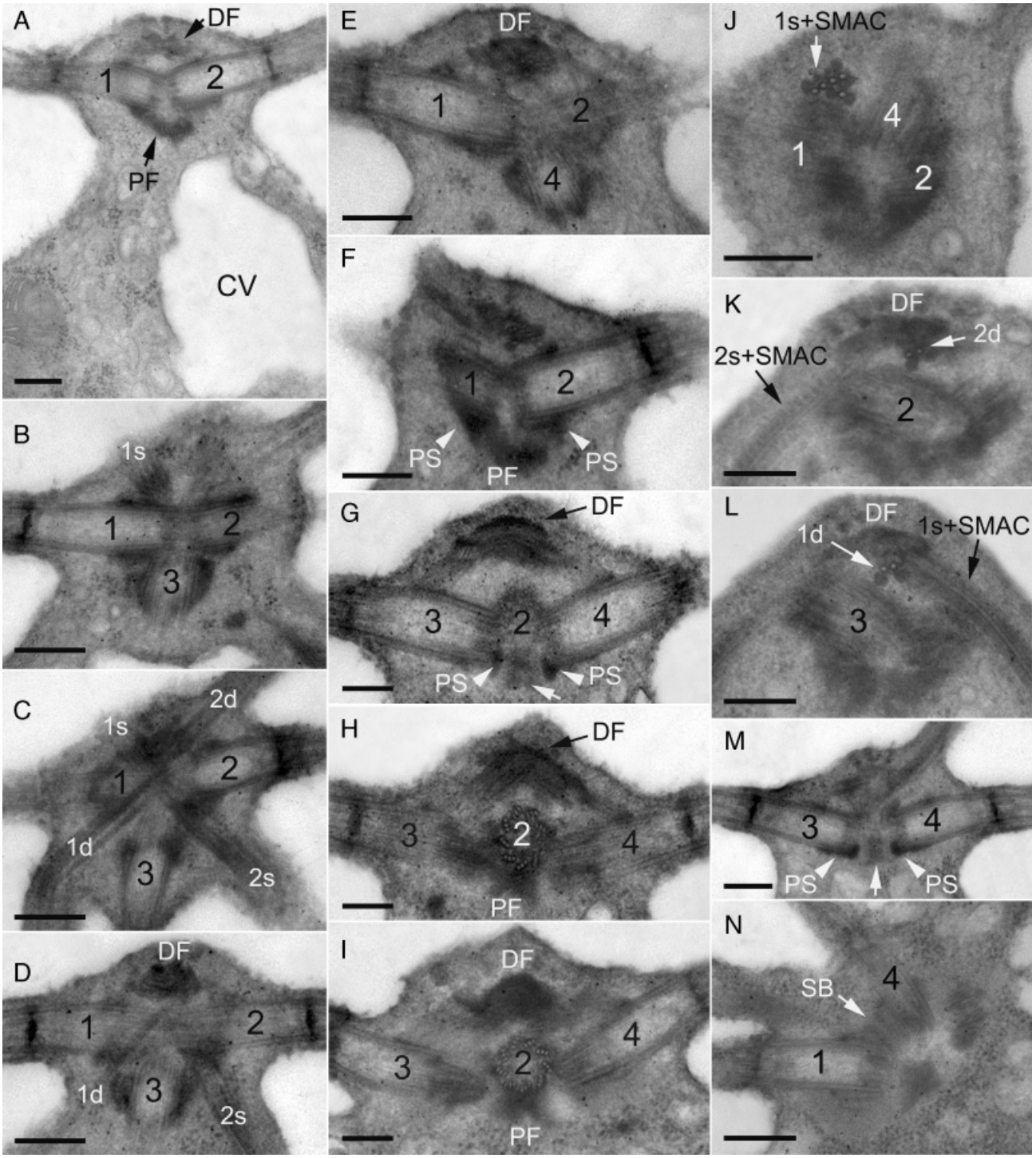
Phylogenetic analyses. Phylogenetic trees based on SSU rDNA sequences from 30 green algal species using different optimality criteria (MP, distance, and maximum likelihood) showed variable support for a number of algal groups (Class Ulvophyceae and class Chlorophyceae, including the orders Chaetophorales and Chaetopeltidales). The best tree under ML is shown in Fig. 4 including support values from analyses using four analytical methods (ML, Logdet distances, NJ, MP). Members of the order Chaetopeltidales formed a highly supported monophyletic group in all analyses, and included *P. americana*. In particular, *P. americana* was found sibling taxon to *Chaetopeltis orbicularis* with high bootstrap support (92%–96%). The two species of *Hormotilopsis* were

FIG. 3. Flagellar apparatus of *Pseudulvella americana* zoospores. (A) Longitudinal median section through a zoospore showing the flagellar apparatus and a contractile vacuole underneath it. (B, C) Serial cross-sections through the anterior end of a zoospore, viewed from anterior to posterior, showing the upper basal bodies directly opposite to one and other, and four cruciately arranged microtubular roots. (B) More posterior section showing proximal ends of upper-pair basal bodies. (C) More anterior section showing proximal ends of roots. (D–I) Selected longitudinal sections through the apical ends of zoospores. (D) Basal bodies 1 and 2 describe an angle of 180°. The distal fiber is obliquely sectioned; its central, electron-dense portion is prominent. (E, F) Noncontiguous serial sections, tip-to-base view of basal body 4. Basal bodies 1 and 2 describe an angle of 130°. (E) The distal fiber extends to each upper-pair basal body. (F) Wedge-shaped proximal sheaths subtend the upper-pair basal body. An arched proximal fiber connects the proximal surface of both proximal sheaths. (G–I) Serial obliquely longitudinal sections, tip-to-base view of basal body 2. The distal fiber sectioned in planes parallel to basal bodies 3 and 4 shows different profiles at different levels in the middle region. Basal bodies 3 and 4 form an angle of 140°, 150°, and 130° to each other in figures G, H, and I, respectively. (G) The central portion of the distal fiber adopts an ample, curved apical surface in this section. The arrow indicates the prominent proximal fiber in transverse section. (I) Upper surface of the distal fiber is angular in this section. (J) Transverse section through the 1 s root showing the five microtubules in a four-over-one configuration. Striated (Figs. 3, K and L) microtubule-associated components surround the root microtubules. (K, L) Nonconsecutive sections through the apical end of a zoospore, showing transverse sections of "d" roots; microtubular root 2d is viewed tip-to-base, root 1d is viewed base-to-tip. (K) Decoration of root 2d and adjacent root 2 s (including striated components). (L) Decoration of root 1d and adjacent root 1 s (including striated components). (M) Longitudinal median section through the lower basal bodies showing the proximal sheath subtending each one. The arrow indicates the proximal fiber. (N) Tangential and longitudinal section through the basal bodies 4 and 1, respectively, to show the striated band connecting both basal bodies at their lateral proximal end. CV, contractile vacuole, DF, distal fiber; PF, proximal fiber; PS, proximal sheath; SB, striated band; SMAC, striated microtubule-associated components; 1 and 2, upper basal bodies; 3 and 4, inner basal bodies, 1d and 2d, two stranded microtubular roots associated with the right side of basal bodies 1 and 2, respectively; 1s and 2s, five stranded microtubular roots associated with the left side of basal bodies 1 and 2, respectively. Scale bars, 0.3 μm.

not monophyletic and instead, *H. gelatinosa* was robustly resolved as sister to *Floydiella terrestris*.

DISCUSSION

Both the ultrastructural and molecular data indicate that *P. americana* belongs to the Chaetopeltidales. The pyrenoid architecture (matrix traversed by cytoplasmic channels) is found in all other members of Chaetopeltidales, except species of *Schizochlamys* (O'Kelly et al. 1994). The flagellar apparatus is characterized by cruciately arranged basal bodies and microtubular roots, an elaborate tetralobate distal fiber, prominently decorated "s" roots, "d" roots with four fibers at their proximal ends, and wedge-shaped proximal sheaths subtending the upper-pair basal bodies.



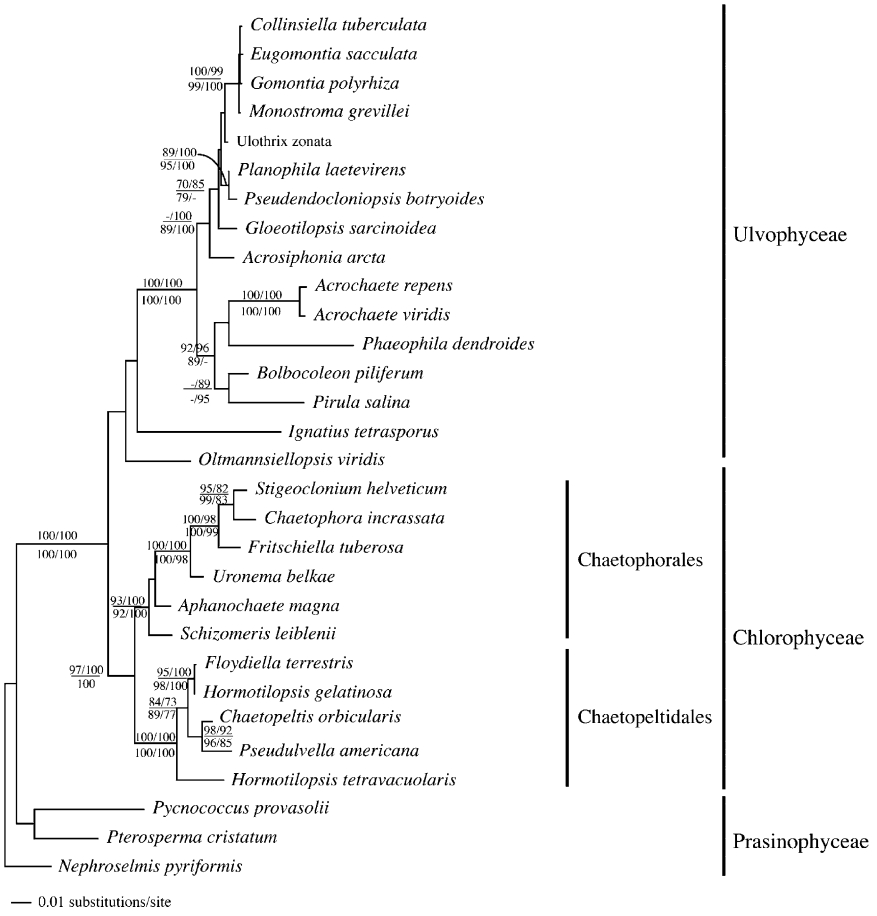


FIG. 4. Phylogenetic analysis of a number of green algal lineages based on nuclear SSU rDNA. Maximum likelihood (ML) tree based on GTR + I + Γ4 found using PAUP*. Numbers above the branches correspond to bootstrap support values from the ML analyses (on the left) and the Log-Det distance analysis (on the right), when values are higher than 65. Bootstrap values (>65) from the MP (on the left) and neighbor-joining (on the right) analyses are shown below the branches.

The characters of the "s" and "d" microtubular roots are similar to those present in some members of Chaetophorales *sensu stricto*; all of the other characters are found in members of Chaetopeltidales and not elsewhere (O'Kelly et al. 1994). Other chaetopeltidalean algae reproduce asexually via quadriflagellate zoospores that germinate by spherical expansion, without germination tubes, as does *P. americana* (O'Kelly et al. 1994).

The habit of *P. americana* closely resembles that of species of *Chaetopeltis*, and it is sister to *Chaetopeltis* in the gene sequence trees. Also, the zoospores of neither *Chaetopeltis* sp. nor *P. americana* contain rhizoplasts. However, *P. americana* differs from *C. orbicularis* Berthold, which co-occurred with *P. americana* in our samples, in its larger thalli with polystromatic central regions, smaller cells, cells much more regularly arranged in radial files, well-defined cell walls in TEM, and apparent absence of pseudocilia (Fig. 5). The regularly arranged radial files of cells, and the polystromatic thalli, are both diagnostic characters of *P. americana* (Fig. 5). Moreover, although there are several published species of *Chaetopeltis*, several of these are currently thought to be synonyms of *C. orbicularis*, the type species (Wujek and Thompson 1999). Consequently, we retain *P. americana* in *Pseudulvella* rather than transferring the species to *Chaetopeltis* as Collins (1909) did.

P. americana also apparently differs from *Chaetopeltis* in the absence of scales on the zoospores, in the expression of an obtuse (rather than a 90°) angle described by the 1-4 and 2-3 basal body pairs, and in the apparent absence of pseudocilia. However, none of these characters is well understood at present. The

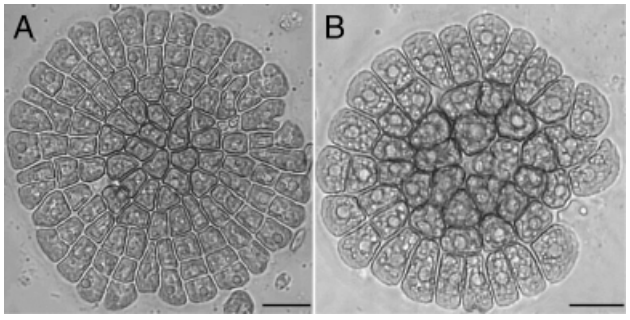


FIG. 5. Light micrographs of disk-shaped thalli from *Pseudulvella americana* (A) and *Chaetopeltis orbicularis* (B). Scale bars, 20 μm.

scales of chaetopeltidalean algae have, so far, only been observed in free-swimming zoospores of *Chaetopeltis* (O'Kelly and Floyd 1984b), *Floydiella* (O'Kelly et al. 1994, as *Planophila*), and *Hormotilopsis* (O'Kelly et al. 1994). Scales were absent from the zoospores of *Phyllogloea*, which were examined before release from the zoosporangia, as have been the zoospores of *P. americana*. Also, the basal body 1-4 and 2-3 pairs formed a 90° angle in the free-swimming zoospores of *Chaetopeltis*, *Floydiella,* and *Hormotilopsis*, but a ca. 120° angle in the unreleased zoospores of *Chaetopeltis*, *Floydiella*, and especially *Phyllogloea* (O'Kelly et al. 1994). At present, these characters appear to represent developmental stages common to many, if not all, Chaetopeltidales, rather than character states that discriminate among taxa within Chaetopeltidales (O'Kelly et al. 1994). Likewise, although neither pseudocilia nor basal body complexes have been observed in vegetative cells of *P. americana*, the factors controlling expression of pseudocilia in those species of Chaetopeltidales in which they occur are not well known. Therefore, we cannot yet say that Snow's record of "evanescent bristles" in *P. americana* is in error, although we can say that, if pseudocilia are present, they are much more infrequently expressed than in *Chaetopeltis*.

O'Kelly et al. (1994) created the Chaetopeltidales with six genera: *Chaetopeltis, Dicranochaete, Hormotilopsis, Planophila pro parte, Phyllogloea*, and *Schizochlamys*. Subsequent molecular studies, beginning with those of Nakayama et al. (1996) and Booton et al. (1998), confirmed the existence and robustness of the Chaetopeltidales clade, but have questioned the monophyly of the genus *Hormotilopsis*. We have replicated these findings. Wujek and Thompson (1999) placed the genera *Oligochaetophora* and *Polychaetophora* in synonymy with *Chaetopeltis* on the basis of morphological and culture studies. Friedl and O'Kelly (2002), using a combination of ultrastructural and molecular methods, placed the type species of *Planophila*, *P. laetevirens* Gerneck, in the Ulotrichales (Ulvophyceae) and created the genus *Floydiella* for the chaetopeltidalean species *Planophila terrestris* Groover & Hostetter. *F. terrestris* is closely related, in ultrastructure and gene sequence trees, to *Hormotilopsis gelatinosa* Triemer & Bold, type species of *Hormotilopsis*, although the vegetative morphologies of the two algae are distinctively different. With the addition of *Pseudulvella*, the genera of Chaetopeltidales are now as follows: *Chaetopeltis* (type genus), *Dicranochaete*, *Floydiella* (inquirendae, possibly = *Hormotilopsis*), *Hormotilopsis* (polyphyletic), *Oligochaetophora* (later synonym of *Chaetopeltis*), *Phyllogloea*, *Polychaetophora* (later synonym of *Chaetopeltis*), *Pseudulvella*, and *Schizochlamys*.

P. americana has now been reported from USA (Snow 1899, Wujek et al. 1998), India (Philipose 1947), and Argentina (Tell 1972, and this study). These disjunct records probably mean that the species is widely, even globally, distributed in suitable habitats, but it is either of infrequent occurrences or is commonly overlooked.

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