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Light and Electron Microscopic Observations on *Erythrolobus coxiae* gen.et sp. nov. (Porphyrideophyceae, Rhodophyta) from Texas U.S.A.

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Low molecular weight carbohydrates, phycobilin pigments and cell structure using light and transmission electron microscopy were used to describe a new genus of unicellular red algae, *Erythrolobus coxiae* (Porphyridiales, Porphyrideophyceae, Rhodophyta). The nucleus of *Erythrolobus* is located at the cell periphery and the pyrenoid, enclosed by a cytoplasmic starch sheath, is in the cell center. The pyrenoid matrix contains branched tubular thylakoids and four or more chloroplast lobes extend from the pyrenoid along the cell periphery. A peripheral encircling thylakoid is absent. The Golgi apparatus faces outward at the cell periphery and is always associated with a mitochondrion. *Porphyridium* and *Flintiella*, the other members of the Porphyrideophyceae, also lack a peripheral encircling thylakoid and have an ER-mitochondria-Golgi association. The low molecular weight carbohydrates digeneaside and floridoside are present, unlike both *Porphyridium* and *Flintiella*, which have only floridoside. The phycobilin pigments B-phycoerythrin, R-phycocyanin and allophycocyanin are present, similar to *Porphyridium purpureum*. The cells have a slow gliding motility without changing shape and do not require substrate contact. The ultrastructural features are unique to members of the Porphyrideophyceae and recent molecular analyses clearly establish the validity of this new red algal class and the genus *Erythrolobus*.

Key Words: cell motility, *Erythrolobus*, *Flintiella*, floridoside/digeneaside, Porphyridiophyceae, *Porphyridium*, *Rhodosorus*, TEM

INTRODUCTION

Species of the unicellular red algae *Dixoniella*, *Flintiella*, *Glaucosphaera*, *Porphyridium*, *Rhodosorus* and *Rhodella* have been formally described and their fine structure investigated by transmission electron microscopy (TEM; Broadwater and Scott 1994) while the unicellular genus *Rhodospora* (Geitler 1927, Johansen *et al.* 2005) is described only at the light microscopic level. Several other new species and genera of unicellular red algae have also been isolated and briefly examined by molecular analysis and light and electron microscopy (Hara *et al.* 2000, Yokoyama *et al.* 2004) and formal description of several of these is currently underway (Yokoyama and Hara, personal communication).

Recently our understanding of phylogenetic interrelationships of red algal unicells has progressed significantly using molecular techniques in combination with several TEM features (Hara *et al.* 2000, Saunders and Hommersand 2004, Yoon *et al.* 2006). Ultrastructural features considered most valuable in unicellular red algal systematics include Golgi apparatus associations with other organelles, presence or absence of pyrenoids and pyrenoid attributes, the morphology and precise details of chloroplasts and certain details of nuclei and mitosis (Broadwater and Scott 1994).

In the mid-1970s a unicellular red alga believed to be a new genus was isolated from coastal Texas USA. Initial work describing growth characteristics and behavior in culture, phycobilin pigments and light and electron microscopy was completed but the results were not published (see Baca 1978). Recently, we conducted microscopic studies on this strain in order to formally describe this new coccoid red alga as *Erythrolobus coxiae* nov.gen et nov. sp.

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MATERIAL AND METHODS

Culture methods

Initial isolation and culturing is described in Baca (1978). The Texas (type) isolate was collected in the fall of 1975 on submerged rocks on South Padre Island at the U.S. Coast Guard Station, Texas USA. The Washington state isolate (paratype) was collected from the coast of Washington USA in 1974 by J.R. Waaland but was not utilized in this study. One of us (FDO) has maintained both collections since the 1970s and has deposited the type culture as *Erythrolobus coxiae* LB 2545 with The Culture Collection of Algae at the University of Texas, Austin, Texas USA and CCMP 1944 with the Bigelow Laboratory for Ocean Sciences, P.O. Box 475, 180 McKown Point Road, West Boothbay Harbor, ME 04575 USA.

Cells were grown in Pyrex No. 3250 or No. 3140 dishes in natural seawater at 30 psu with Modified Provasoli's Medium (MPM/2) (West 2005), 18-23°C, 12:12 LD daily photoregime, 10-25 μmol photons m^{-1} s^{-2} cool white fluorescent lighting. For faster growth cultures were placed on 70 rpm rotary shakers or a 17 rpm rocker.

Phycobilin pigment analysis

The description of phycobilin pigment analysis is provided in Gantt (1990).

Carbohydrate analysis

The low molecular weight carbohydrate analysis is outlined in Karsten *et al.* (1999, 2003).

Light microscopy - live cells

Live cells were placed on a slide and sealed under a coverslip with VALAP (vasoline:lanolin:parrafin 1:1:1). Cells were observed inversely with a Leica TCS SP2 confocal microscope using either a HCX PL APO 63/1.4 or PL Fluotar 100/1.3 objective; chloroplast autofluorescence was imaged using a 488 nm argon laser. Cell motility was monitored for 60 seconds with a ten-second time delay between image acquisitions. Cell velocity was calculated from these images.

Light microscopy - fixed cells

Cells were fixed with 4% EM grade formaldehyde (ProSciTech) and 1% glutaraldehyde (ProSciTech) in culture medium for 10 minutes. Fixed cells were washed 3X in culture medium then incubated with 20 μg mL^{-1} Hoechst 33258 (Sigma-Aldrich) in culture medium for approximately 5 minutes. Cells were washed 3X in medium then pipetted onto a No. 1 coverslip which was previously flamed and coated with 1% polyethylenimine (Sigma). Cells were allowed to settle and adhere to the coverslip for a few minutes before the addition of mounting media containing 50% glycerol/PBS with 0.1mg/ml of 1,4-Diazabicyclo[2.2.2]Octane (Sigma). Coverslips were inverted onto slides and sealed with VALAP. Images were captured on a Leica TCS SP2 confocal microscope using either a HCX PL APO 63/1.4 or PL Fluotar 100/1.3 objective; chloroplast autofluorescence was imaged using a 488 nm argon laser and Hoescht DNA fluorescence using a 405 nm diode laser. Images were processed in Adobe Photoshop.

Electron microscopy

Cells were gently scraped from dish bottoms and collected on 0.45 μm Millipore filters prior to fixation at ambient temperature in 2.5% glutaraldehyde in a 0.1 M phosphate buffer solution (pH 6.8) with 0.25 M sucrose. Following buffer rinses samples were post-fixed 1 hr in the same buffer in 1% OsO_4 at ambient temperature, rinsed thoroughly in H_2O, left in 50% acetone for 30 min and stored in a 70% acetone-2% uranyl acetate solution at ambient temperature for 30 min. Samples were then dehydrated in a graded acetone series, infiltrated gradually and embedded in EmBed 812 resin (Electron Microscopy Sciences, Fort Washington, PA 19034 USA) and polymerized at 70°C for 1-3 days. Thin sections were cut with an RMC MT6000-XL ultramicrotome, stained with lead acetate and viewed with a Zeiss EM 109 electron microscope.

RESULTS

Erythrolobus coxiae J.L. Scott, J.B. Baca, F.D. Ott & J.A. West gen. et sp. nov

Description

Cellulae motum labentem exhibentes, sphaericae vel ovoideae, 5-12 μm in diametro, binae vel coacervatae nunquam visae. Multiplicatio asexualis per fissionem. Matrix extracellularis tenuis. Chloroplastus vivide ruber parietalis, e lobis quattuor vel pluribus e pyrenoide centrali radiantibus constans. Lobi chloroplasti nucleum peripheralem in parte cingentes, sine thylacoide peripherali cingenti. Pyrenoides in cellulis veteribus elongata vel multiplicans, vagina amyli florideani circumcincta.

Matrix pyrenoidis thylacoides ramosas continens. Grana amyli per cellulas vetustiores dispersa. Vacuolae parvae per cytoplasma peripherale dispositae. Nucleus nucleolo centrali. Regio cis apparatus Golgi cum reticulo endoplasmico mitochondrioque consociata. Carbonii hydrata parvi ponderis floridosidum et digeneasidum. Phycobilina B-phycoerythrinum, R-phycocyaninum et allophycocyaninum.

Cells with gliding motility, spherical to ovoid, 5-12 μm in diameter and never seen as doublets or packets. Multiplication is asexual by fission. Thin extracellular matrix. Chloroplast bright red, parietal and composed of four or more lobes radiating outwardly from and around the central pyrenoid. Chloroplast lobes partially encircle the peripheral nucleus, lacking peripheral encircling thylakoid. Pyrenoid becoming elongated or multiple in old cells, surrounded by floridean starch sheath. Pyrenoid matrix containing branched thylakoids. Starch grains scattered throughout older cells. Vacuoles small, located throughout peripheral cytoplasm. Nucleus with central nucleolus. Cis-region of Golgi apparatus associated with endoplasmic reticulum and mitochondrion. The low molecular weight carbohydrates are floridoside and digeneaside. The phycobilins are B-phycoerythrin, R-phycocyanin and allophycocyanin (Fig. 12).

Etymology: The generic name refers to the red colored (erythro) and lobed (lobus) chloroplast; the specific epithet is to honor Elenor R. Cox, a distinguished phycologist on the faculty at Texas A & M University (College Station, Texas) from 1967 to 1993. She was the PhD advisor of Bart Baca.

Holotype: NSW 738978, Royal Botanic Gardens Sydney, Mrs Macquaries Rd, Sydney NSW 2000, Australia. On submerged stones at the U.S. Coast Guard Station (26°4′N 97°1′W), South Padre Island, Texas U.S.A. Date: June, 1975.

Type culture: UTEX LB 2545. UTEX Culture Collection of Algae, 1 University Station A6700, University of Texas, Austin, TX 78712-0183, U.S.A.

CCMP 1944, Bigelow Laboratory for Ocean Sciences, P.O. Box 475, 180 McKown Point Road, West Boothbay Harbor, ME 04575 USA.

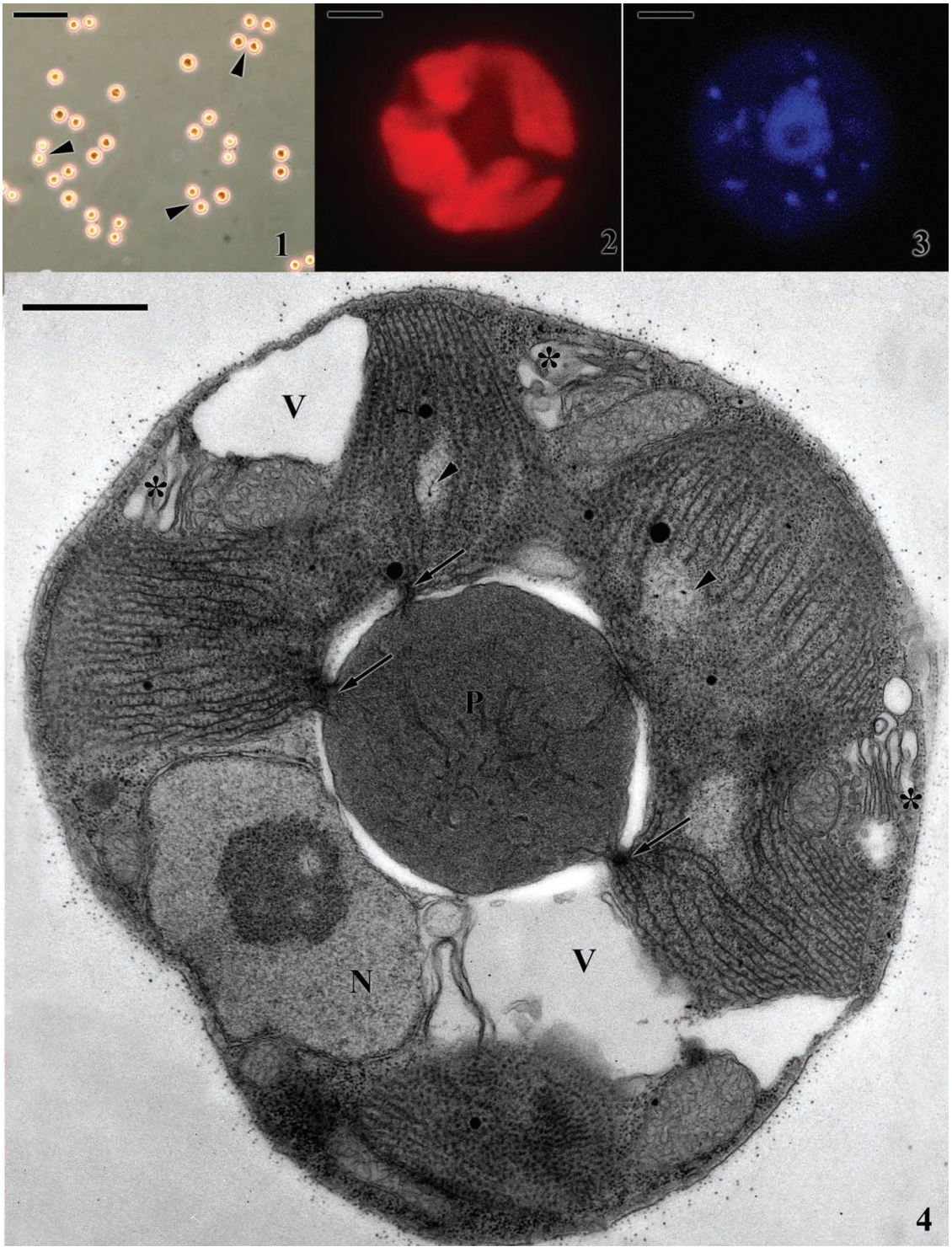
Light microscopy and cell motility

Actively growing cells in liquid culture were bright red and grow as a loose film. Baca (1978) reports that "the morphology of cells on agar is the same as in liquid culture, but cell motility is more evident on agar, and wandering cells can be seen moving away from clumps of vegetative cells." In our present study all the cells appeared to spread out from a tight colony and were observed to glide unidirectionally with a velocity range from 0.04 to 0.24 μm s^{-1}. Cells had either a constant speed or showed an increase/decrease over time. The cells moved in an out of focus during observations so substrate contact was not necessary. No external appendages were visible with light or electron microscopy.

Soon after division, daughter cells moved apart and were commonly seen as pairs separated from each other by variable distances (Fig. 1), often interconnected by a mucilage strand. Bicells and cell aggregates were never seen. Confocal microscopy reveals some details of the complex chloroplast morphology in relation to the nucleus (Figs 2, 3). Using a maximum projection of selected confocal fields, four or more of the autofluorescent chloroplast lobes appear to arise from the central pyrenod (not visible due to the absence of fluorescent pigments) to form a cup-shaped structure partially enclosing the nucleus (Fig. 2). Figure 3 shows the various DNA loci in the cell. Small, blue punctae are chloroplast and mitochondrial DNA regions. The nucleus occupies the top-most region of the cup, its nucleolus visible as a circular shape due to its lack of DNA.

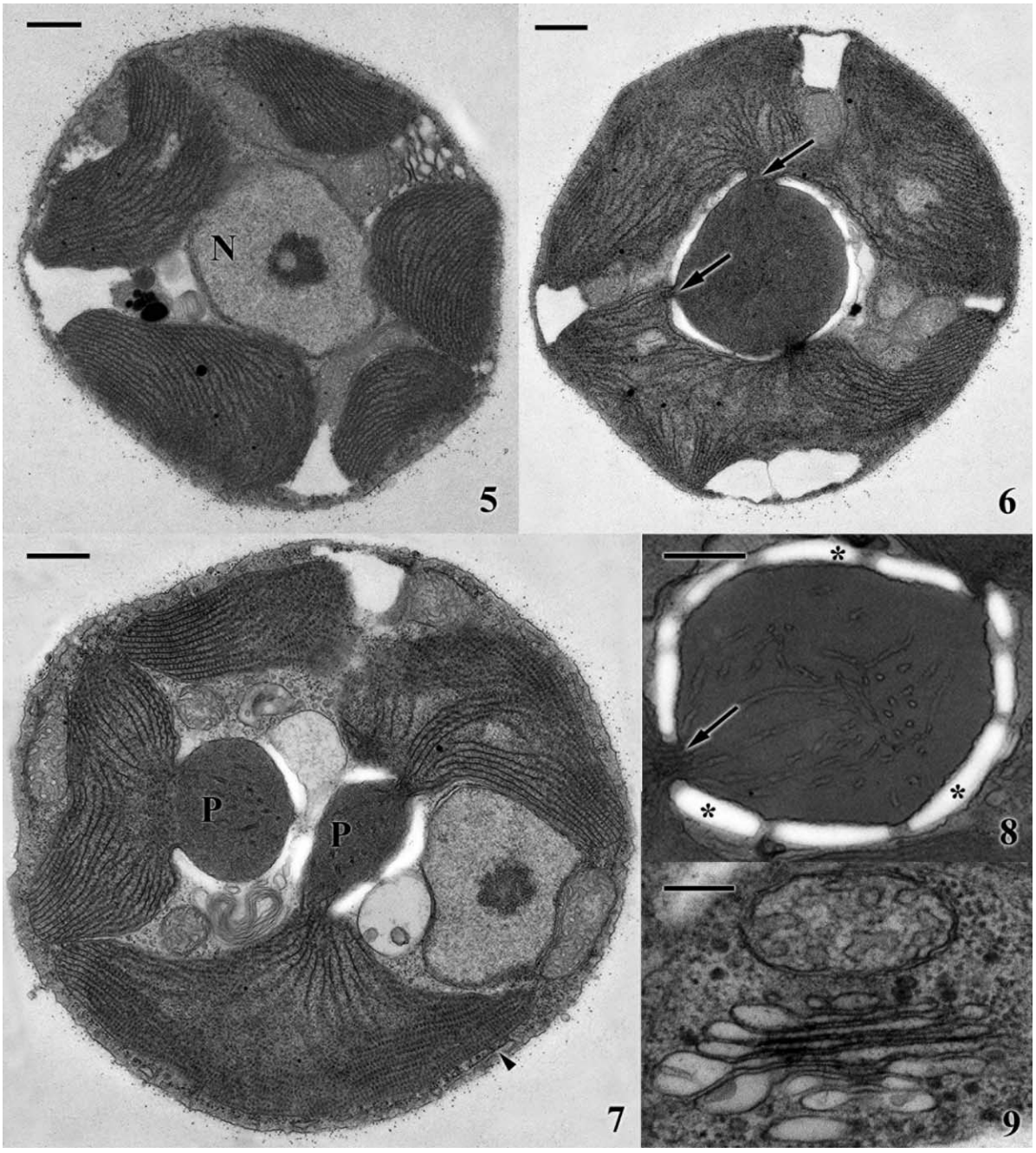
Transmission electron microscopy

The cell coat is not well preserved although electron dense granular material is usually noted close to the cell membrane (Figs 4-7). Sections passing through both the nucleus and the pyrenoid show that the former is located at the cell periphery while the latter is in the cell center (Figs 4, 7). The 2-3 μm nucleus is sub-spherical and contains a single, centrally located nucleolus (Figs 4, 5, 7). The nucleus is often seen close to the pyrenoid but is separated either by a pyrenoidal starch sheath (Fig. 4), chloroplast lobes, small vacuoles, or combinations of the three (Fig. 7). Cells undergoing mitosis or cytokinesis were never observed. The spherical pyrenoid, 3-3.5 μm in diameter, is usually slightly larger than the nucleus in young cells (Figs 4, 6), and in older, larger cells the pyrenoid was smaller, often misshapened (Fig. 7). Occasionally several pyrenoids were seen in older cells (possibly shown in Fig. 7 but otherwise observed but not presented here). Floridean starch always forms a pyrenoidal sheath and occasionally is seen along the nucleus. Tubular, branched phycobilisome-free membranes traverse the pyrenoid matrix (Figs 4, 7, 8), most likely derived from thylakoids entering through either



Figs 1-3. *Erythrolobus coxiae* phase and confocal microscopy. **Fig. 1.** Recently divided cells are found in separated pairs and are slightly smaller than single, older cells. Phase microscopy. Scale bar = 30 μm. **Fig. 2.** Chloroplast autofluorescence corresponding to cell in Fig. 3. Maximum projection of selected confocal sections highlighting four chloroplast lobes arising from the central pyrenoid (central non-autofluorescent region). Scale bar = 2 μm. **Fig. 3.** Confocal microscope image, maximum projection showing total Hoechst DNA staining. The central fluorescence staining of the nucleus is evident in addition to punctuate chloroplast fluorescence corresponding to chloroplast autofluorescence in Fig. 2. Scale bar = 2 μm.

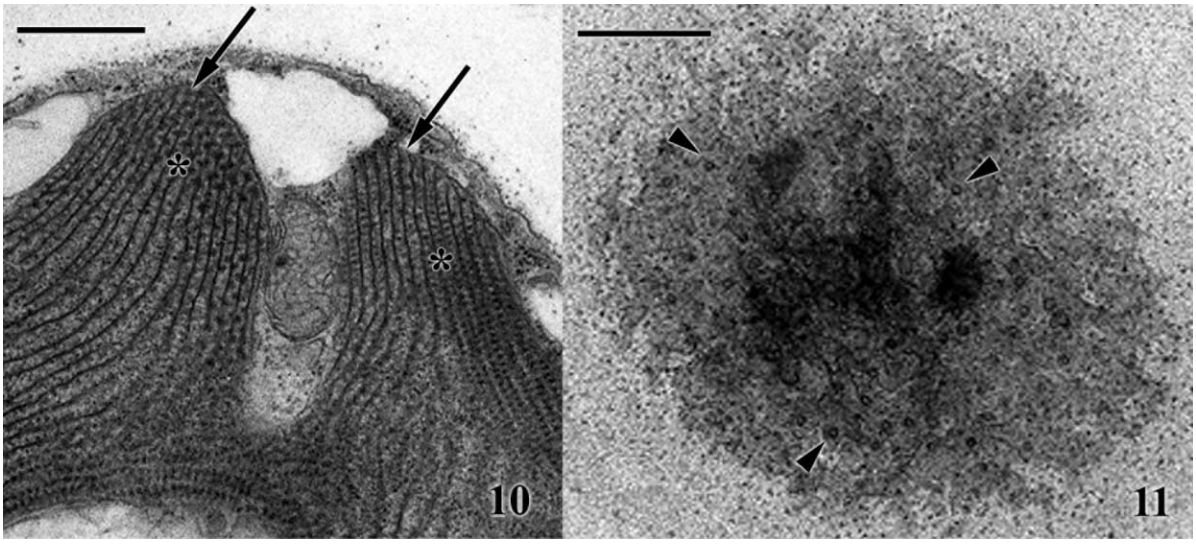
Fig. 4. *Erythrolobus coxiae* general ultrastructure. Medial section showing peripheral nucleus (N) with prominent nucleolus. The central pyrenoid (P) has a dense matrix penetrated by thylakoids and is enclosed by a starch sheath. Three of the five or more chloroplast lobes are connected to the pyrenoid by thin connections (arrows); a peripheral thylakoid is absent. Each Golgi body (*) is closely associated with a mitochondrion at its cis-region. Vacuoles, V. Scale = 0.5 μm.



Figs 5-9. *Erythrolobus coxiae* general ultrastructure. **Fig. 5.** Section through peripheral nucleus (N) revealing five encircling chloroplast lobes. Scale bar = 0.5 μm. **Fig. 6.** Medial section through central pyrenoid with chloroplast lobes connected in at least two regions (arrows). Scale bar = 0.5 μm. **Fig. 7.** Medial section through nucleus and either a single non-spherical pyrenoid or possibly two pyrenoids (P). The peripheral ER - tubule system is nearly inconspicuous (arrowhead). Scale bar = 0.5 μm. **Fig. 8.** Higher magnification of pyrenoid and enclosing starch sheath (*). Thylakoids that enter the pyrenoid matrix from chloroplast lobe (arrow) are tubular and apparently phycobilisome-free. Scale bar = 0.5 μm. **Fig. 9.** Higher magnification of Golgi - mitochondrion association. Scale bar = 150 nm.

narrow (Figs 4, 6) to broad (Figs 6, 7) connections to the chloroplast lobes, as determined by examining serial sections. As many as five chloroplast lobes were seen in favorable planes of sectioning (Fig. 5).

Thylakoids are evenly spaced and terminate close to the chloroplast envelope. A peripheral encircling thylakoid is absent (Figs 4-7, 10). Phycobilisomes are well preserved and appear to be hemidiscoidal (Fig. 10), similar to most red algae (Gantt 1990). DNA is seen as small electron transparent regions with electron dense fibrils and is located throughout central regions of the chloroplast lobes (Figs 4-6). Plastoglobuli localized to tight clus-



Figs 10-11. *Erythrolobus coxiae* general ultrastructure. **Fig. 10.** Periphery of chloroplast lobes (arrows) showing absence of peripheral thylakoid and two planes of sectioning through phycobilisomes (*). Scale bar = 0.5 μm. **Fig. 11.** Glancing section through cell showing transversely-cut tubules (arrowheads) extending from peripheral ER system. Scale bar = 40 nm.

ters at the periphery of chloroplast lobes were not observed.

Cis-regions of each Golgi apparatus are invariably closely associated with a single mitochondrion (Figs 4, 5, 9). In all our observations the Golgi apparatus is seen only at the cell periphery. Unlike several other unicellular red algae and sporangia of filamentous red algae (discussed in Broadwater *et al.* 1995), close apposition or fusion of Golgi cisternae was never observed. Mitochondrial profiles with obviously tubular cristae (Figs 5-7, 9) were found throughout the cell. Small vacuoles were seen at the cell periphery in all cells examined (Figs 4-7, 10). Medial cell sections showed a somewhat inconspicuous peripheral ER system located beneath the cell membrane (Fig. 7) but sections grazing the cell surface revealed the tiny interconnections between the ER system and the cell membrane, typical of most all unicellular red algae studied by transmission electron microscopy (Patrone *et al.* 1991, Scott *et al.* 1992, Broadwater and Scott 1994).

Phycobilin pigments

The absorption maxima of these pigments confirm that B-phycoerythrin (545, 560 nm), R-phycocyanin (555, 619 nm) and allophycocyanin (650 nm) are present (Fig. 12).

Low molecular weight carbohydrates

Erythrolobus coxiae isolate (Ott 528) UTEX LB 2545 has floridoside (373.1 μmol g^{-1} DW) and digeneaside (154.3 μmol g^{-1} DW) and isolate (Ott 530) CCMP 1943 also has

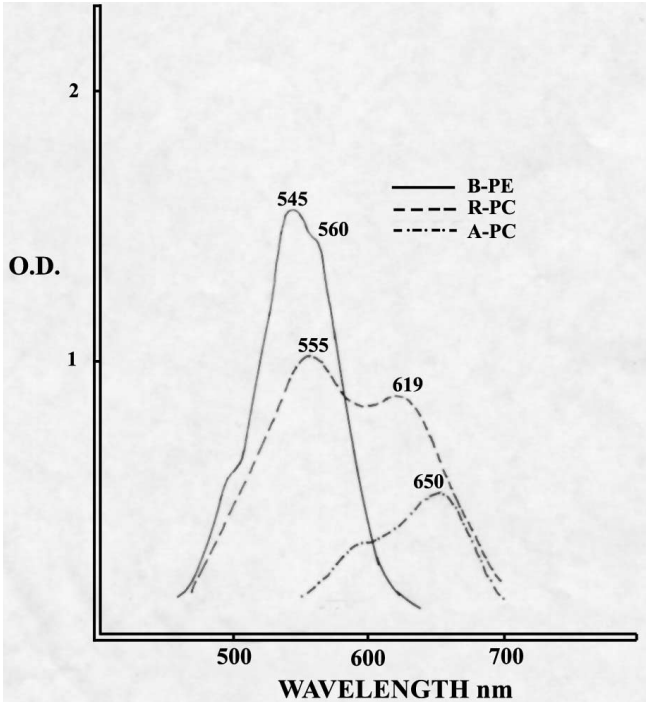


Fig. 12. The absorption maxima of the phycobilin pigments confirm that B- phycoerythrin (545, 560 nm), R-phycocyanin (555, 619 nm) and allophycocyanin (650 nm) are present.

floridoside (329.3 μmol g^{-1} DW) and digeneaside (160.8 μmol g^{-1} DW).

DISCUSSION

Cell motility

Baca (1978) first observed motility in *Erythrolobus coxi-*

ae and we also have noted gliding motility at 0.04-0.24 μm m^{-2} s^{-1}. Moreover, *Porphyridium purpureum* cell motility has been well studied (Hill *et al.* 1980; Lin, *et al.* 1975; Nultsch *et al.* 1979; Nultsch and Schuchart 1980; Pickett-Heaps *et al.* 2001) and shows blue light phototaxis and can move at rates up to 1.0 μm m^{-2} s^{-1}. Pickett-Heaps *et al.* (2001) also reported gliding motility in *Erythrolobus* sp. (JW3827-Fiji, as *Porphyridium purpureum*) and *Erythrolobus* sp. (JW3797-Australia, as *Rhodella*-like) at speeds of 0.35-1.5 μm m^{-2} s^{-1} either with or without mucilage tails. Strains 3797 and 3827 were shown to be *Erythrolobus* sp. through SSU molecular analysis (A. Yokoyama, personal communication). *Flintiella sanguinaria* Ott showed no motility (Pickett-Heaps *et al.* 2001). Other unicellular reds such as *Rhodella maculata* Evans, *R. violacea* (Kornmann) Wehrmeyer and *Dixoniella grisea* (Geitler) Scott, Broadwater, Saunders, Thomas and Gabrielson, also showed a gliding motility (Pickett-Heaps *et al.* 2001). By contrast, *Rhodosorus* shows protoplast rotation within the cell wall but does not have cell motility (Pickett-Heaps *et al.* 2001; Wilson *et al.* 2002). Amoeboid spores with pseudopodia are observed in some other red algae (Ackland *et al.* 2006; Pickett Heaps *et al.* 2001) but are not observed in the Porphyridiales. Atlthough cell motility is very common in many red algae the mechanism is not understood.

Transmission electon microscopy

Hara *et al.* (2000) first recognized four major lineages within subclass Bangiophycidae and designated them as Porphyridiales L-1 (Lineage 1, Cyanidiales), L-2, L-3 and L-4. Müller *et al.* (2001) accepted these views but modified the terminology to designate the lineages as Porphyridiales (1), (2) and (3). The most recent major contribution to red algal phylogeny was published by Yoon *et al.* (2006). They relied mainly on DNA and protein molecular analyses but also included ultrastructural and low molecular weight carbohydrate data. Two new subphyla in phylum Rhodophyta were proposed, the Cyanidiophytina and Rhodophytina, the latter with six classes, the Bangiophyceae, Florideophyceae, Compsopogonophyceae, Rhodellophyceae, Stylonematophyceae and Porphyridiophyceae. The last three classes are comprised of unicellular, colonial and small branched or unbranched uniseriate to multiseriate filamentous algae and correspond to the Porphyridiales (1), (2) and (3), respectively.

The only published references to the genus *Erythrolobus* are in Spero and Moree (1981), where it is mentioned as a potential food source for *Gymnodinium*, and in (Yoon *et al.* 2006) where various genes and proteins of numerous red algal genera were used to define the major lineages of red algae. A formal Latin description has never been published. Providing such a description was our principal goal in this paper but we also wished to determine the extent to which ultrastructural features could be used in corroborating phylogenetic trees based largely on molecular data (Hara *et al.* 2000; Muller *et al.* 2001; Saunders and Hommersand 2004; Yokoyama *et al.* 2004; Yoon *et al.* 2006).

The lineage consisting of *Erythrolobus*, *Flintiella* and *Porphyridium* constitutes the new class Porphyrideophyceae, with one order, Porphyridiales (Yoon *et al.* 2006). This conclusion is fully supported by comparing certain ultrastructural features shared by the three genera (Table 1) and is one reason that we chose to use the phylogenetic treatment by Yoon *et al.* (2006) in this discussion, especially since this is the only study that has included *Erythrolobus* in a molecular analysis.

Pyrenoid and nucleolus characteristics in the Porphyridiales apparently are of little taxonomic value at the ordinal level. For example, *Flintiella* lacks a pyrenoid, *Erythrolobus* has a pyrenoid with a cytoplasmic starch sheath while *Porphyridium* has a pyrenoid deeply embedded in the chloroplast matrix. The position of the nucleolus in *Flintiella* and *Erythrolobus* is consistently in the center of the nucleus, as it is in most other red algal unicells (e.g. Patrone *et al.* 1991; Scott *et al.* 1992; Broadwater *et al.* 1995), but was always seen appressed to the nuclear envelope region facing the cell center and chloroplast in two species of *Porphyridium* (Gantt and Conti 1965; Gantt *et al.* 1968; Schornstein and Scott 1982).

However, the Golgi apparatus and certain chloroplast features appear to be quite reliable in characterizing this order. Each genus has endoplasmic reticulum (ER) and a mitochondrion closely associated with the cis-region of the Golgi apparatus (ER-mitochondrion-Golgi association). This unique organization of organelles is universally found in all florideophycean and bangiophycean algae examined by transmission electron microscopy (TEM; Scott 1984; Pueschel 1990; Broadwater and Scott 1994). More importantly, it is not found in any genera in the Rhodellophyceae and Stylonematophyceae, the only other classes containing unicells besides the Porphyrideophyceae (West *et al.* 2005; Yokoyama, unpublished results) although we must qualify this last statement.

Broadwater and Scott (1994) listed the unicell *Rhodosorus* (class Stylonematophyceae) as also having an

Table 1. Selected ultrastructural features in Porphyridiales

Genus	Golgi associations cisternae	Fused Golgi thylakoid	Peripheral presence	Pyrenoid site	Pyrenoid matrix	Pyrenoid position	Nucleolus
Erythrolobus	ER: Mitochondrion	(-)	(-)	(+)	Central[1]	Thylakoids	Center of nucleus
Flintiella	ER: Mitochondrion	(-)	(-)	(-)	NA[2]	NA	Center of nucleus
Porphyridium	ER: Mitochondrion	(-)	(-)	(+)	Embedded[3]	Thylakoids	Facing chloroplast

[1] Central, located in center of cell exposed to cytoplasm and starch sheath
[2] NA, not applicable
[3] Embedded, located in center of chloroplast and not exposed to cytoplasm or starch sheath

ER-mitochondrion-Golgi association. What is perplexing is that the only publications on *Rhodosorus* ultrastructure (Giraud 1962; Ford 1984; Wilson *et al.* 2002) did not describe the Golgi apparatus, so the only possible source of information concerning this organelle must be traced back to the Broadwater and Scott (1994) publication which shows one micrograph of what was believed to be a cell of *Rhodosorus* (Fig. 4, Broadwater and Scott 1994) with an ER-mitochondrion-Golgi association (see also Table 1 in the 1994 paper). A reexamination of that micrograph and others from the same fixation of a putative *Rhodosorus* culture, obtained from the UTEX Culture Collection of Algae before 1990 (Scott, unpublished), strongly indicates that what was maintained as a *Rhodosorus* culture actually either was *Erythrolobus*, or a *Rhodosorus* culture contaminated by *Erythrolobus*. In his unpublished dissertation, Baca (1978) compared *Erythrolobus* with *Rhodosorus* because these two unicells bear a resemblance more to each other than to any other red algal unicellular genera, at least with regard to the larger organelles. Each has a centric to eccentric pyrenoid bordering the cytoplasm, the pyrenoid matrix is traversed by thylakoids, several chloroplast lobes extend from the pyrenoid and the nucleus is located at the cell periphery. Relying on just these features could lead to misidentification. Therefore, at this time we conclude that nothing is known about the Golgi apparatus in *Rhodosorus*. Personal experience has shown that this genus is not easily prepared for TEM by conventional preparation techniques. Modified techniques are currently being employed to hopefully obtain better quality images of *Rhodosorus*.

Another clue to the correct identity of the *Rhodosorus* reported by Broadwater and Scott (1994) relates to chloroplast details. As seen in this study, the chloroplast of *Erythrolobus* does not have a peripheral encircling thylakoid, and thus is similar to the chloroplasts of *Flintiella* and *Porphyridium* (Table 1). To the contrary, the chloroplast of *Rhodosorus* possesses a peripheral thylakoid, as is evident in the studies by Ford (1984) and Wilson *et al.* (2002) and in unpublished recent work by Scott. Figure 4 in Broadwater and Scott (1994) review of unicellular red algae clearly shows the absence of a peripheral thylakoid. Chloroplasts of all members of the Stylonematophyceae currently examined by TEM possess a peripheral thylakoid (West *et al.* 2005). Only two other unicellular genera have peripheral thylakoids, *Dixoniella* (Scott *et al.* 1992) and *Glaucosphaera* (Broadwater and Scott 1994). A peripheral thylakoid is found in all florideophycean algae and in the sporophyte (conchocelis) stage of bangiophycean algal genera (Pueschel 1990). Curiously, clusters of plastoglobuli (PG) located at the outermost regions of chloroplasts, a feature seen in most red algal unicells (West *et al.* 2005), were not seen in *Erythrolobus*. PG clusters are also absent in *Flintiella* (Scott 1986) and *Porphyridium* (Gantt and Conti 1965; Gantt *et al.* 1968; Schornstein and Scott 1982), showing further agreement of cell features among these three algae.

To conclude the comparison of *Erythrolobus* with *Rhodosorus*, several other distinguishing attributes need to be mentioned. Light and electron microscopic observations of the cell coat of these two genera clearly distinguishes one from the other; the cell coat of *Rhodosorus* is easily seen as a mucilaginous, fibrillar sheath (Baca 1978; Wilson *et al.* 2002; Scott, unpublished) while the coat of *Erythrolobus* is much less conspicuous (Baca 1978, this paper). Also, following cell division daughter cells of *Rhodosorus* often remain closely opposed to each other (Baca 1978; Wilson *et al.* 2002), whereas those of *Erythrolobus* immediately separate (Baca 1978; Ott and Scott, personal observations). In addition, as seen in this study, vacuoles in *Erythrolobus* are small and located at

the cell periphery, similar to both *Flintiella* and *Porphyridium*. Vacuoles in *Rhodosorus* are much larger and occupy much of the cell interior (Ford 1984; Wilson *et al.* 2002; Scott, unpublished).

Phycobilin pigments

The phycobilin pigments of *Erythrolobus* are quite similar to those of *Porphyridium purpureum* (Bory de Saint-Vincent) K. Drew & Ross (Gantt 1990). These results were confirmed by Elizabeth Gantt (personal communication) using cultures provided by Baca (1978). No pigment analyses have been published for *Flintiella*, the other genus of the Porphyridiophyceae.

Low molecular weight carbohydrates

Erythrolobus coxiae contains digeneaside and floridoside but *Porphyridium* and *Flintiella* contain only floridoside (Karsten *et al.* 1999, 2003). This needs to be reconfirmed with other new *Erythrolobus* sp. strains (Yokoyama, unpublished).

In summary, a new genus of unicellular red algae is described, *Erythrolobus coxiae*. Based on both molecular (Yoon *et al.* 2006) and ultrastructural data, the placement of *Erythrolobus* in order Porphyridiales, class Porphyrideophyceae, is well founded.

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