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New taxa of the Porphyridiophyceae (Rhodophyta): *Timspurckia oligopyrenoides* gen. et sp. nov. and *Erythrolobus madagascarensis* sp. nov.

Eun Chan Yang[1,5], Joe Scott[2]*, John A. West[3], Evguenia Orlova[2], Dave Gauthier[4], Frithjof C. Küpper[5], Hwan Su Yoon[1] and Ulf Karsten[6]

[1]*Bigelow Laboratory for Ocean Sciences, P.O. Box 475, 180 McKown Point Road, West Boothbay Harbor, ME 04575, USA*
[2]*Department of Biology, College of William and Mary, Williamsburg, VA 23187, USA*
[3]*School of Botany, University of Melbourne, Parkville, Victoria 3010, Australia*
[4]*Department of Biological Sciences, Old Dominion University, Norfolk VA 23529, USA*
[5]*Culture Collection of Algae and Protozoa, Scottish Association for Marine Science, Scottish Marine Institute, Dunstaffnage Marine Laboratory, Dunbeg, Oban, Argyll, PA37 1QA, Scotland, UK*
[6]*Institute of Biological Sciences – Applied Ecology, University of Rostock, Albert-Einstein-Strasse 3, D-18057 Rostock, Germany*

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Two new marine unicellular red algae are described: *Timspurckia oligopyrenoides* gen. et sp. nov. isolated from southeastern Australia and *Erythrolobus madagascarensis* sp. nov. isolated from Madagascar. *Timspurckia oligopyrenoides* cells are spherical, 7–11 μm in diameter, greyish red to reddish brown and surrounded by a conspicuous fibrillar matrix about 2 μm thick. Cells exhibit positive phototaxis. In the single chloroplast the lobes extend from several pyrenoids to occupy most of the cell. A peripheral thylakoid is absent. The pyrenoid matrices are filled with tubular thylakoids and are usually surrounded by starch sheaths in the adjacent cytoplasm. The nucleus is peripheral with a nucleolus appressed to the nuclear envelope usually closest to the cell center. Golgi bodies are associated with mitochondria usually at the cell periphery. *Erythrolobus madagascarensis* cells are spherical, 5–8 μm in diameter, greyish red to reddish brown and surrounded by a thin conspicuous fibrillar sheath. Cells exhibit positive phototaxis. Each cell has a single chloroplast with several lobes extending from an eccentric pyrenoid forming a complex parietal layer. A peripheral thylakoid is absent. The pyrenoid matrix is filled with tubular thylakoids, and a starch sheath is visible in the adjacent cytoplasm. The nucleus is eccentric and has a central nucleolus. Golgi bodies are associated with mitochondria usually at the cell periphery. Numerous conspicuous, electron-transparent, fibrous vesicles are always present in the cells. The sequence divergences of *psa*A and *psb*A genes and phylogeny support that *E. madagascarensis* sp. nov. and *T. oligopyrenoides* gen. et sp. nov. are newly uncovered natural entities within the class Porphyridiophyceae along with *Erythrolobus coxiae*, *Porphyridium* spp. and *Flintiella sanguinaria*. The low-molecular-weight carbohydrates (LMWCs) floridoside, digeneaside and trehalose are present in both taxa. Thus, three independent features – ultrastructural characters, plastid gene sequences and LMWC patterns – support the suggested phylogeny and taxonomy of these red algae.

Key Words: Carbohydrates, Chloroplast, Golgi, Phototaxis, Porphyridiophyceae, Pyrenoids, Ultrastructure, Unicellular red algae

INTRODUCTION

Recently, important changes have been made in the systematics of unicellular red algae with molecular, biochemical and ultrastructural methodologies. Until several years ago these algae were all placed in a single order, Porphyridiales, within the phylum Rhodophyta, class Bangiophyceae (Scott *et al.* 2006). Currently the 10 recognized genera of unicellular red algae in the subphylum Rhodophytina are placed in three classes, Porphyridiophyceae, Rhodellophyceae and Stylonematophyceae (Yoon *et al.* 2006; Scott *et al.* 2008; Yokoyama *et al.* 2009). A comprehensive overview of red algal unicells is provided in Ott (2009).

In addition to clear-cut molecular differences, several ultrastructural characters also help distinguish each class, especially the Golgi body association with other organelles. In cells of the three Porphyridiophyceae genera *Porphyridium*, *Flintiella* and *Erythrolobus* (Scott *et al.* 2008), the Golgi bodies are invariably associated with endoplasmic reticulum (ER) and a mitochondrion; whereas, the five genera in Rhodellophyceae have an association involving either ER (*Rhodella* and *Corynoplastis*; Yokoyama *et al.* 2009) or the functionally equivalent outer membrane of the nuclear envelope (*Neorhodella*, *Dixoniella* and *Glaucosphaera*; Scott *et al.* 2008). Only one of the two genera in Stylonematophyceae (*Rhodosorus* but not *Rhodospora*) has been examined by the transmission electron microscope [TEM; see discussion in Scott *et al.* (2006)]. In unpublished observations (Scott), the Golgi bodies in *Rhodosorus*, similar to *Rhodella*, *Corynoplastis* and multicellular Stylo-

* Corresponding author (jlscot@gmail.com).

nematophyceae, were associated with ER only. Each of the remaining three Rhodophytina classes also has a specific Golgi body–associated organelle: the ER and mitochondrion in Florideophyceae and Bangiophyceae and ER alone in Compsopogonophyceae (West *et al.* 2007a, b).

In red algal taxonomy the occurrence or lack of specific carbohydrate components such as low-molecular-weight photosynthates has been considered useful for distinguishing groups at several levels (Kremer 1980; Barrow *et al.* 1995). Although the physiological state of an algal species and the environmental conditions strongly influence the intracellular concentrations of low-molecular-weight carbohydrates (LMWC; Karsten *et al.* 1993, 2005), the principal biochemical capability to produce particular compounds such as heterosides (floridoside, digeneaside) or polyols is useful for chemotaxonomic considerations.

In this work we present molecular results, LMWC content and light and electron microscopic observations on two new unicellular red algae obtained from mangrove habitats and isolated into laboratory culture.

MATERIAL AND METHODS

Isolation and culture conditions

Two isolates (JAW 3827 and JAW 4318) from a mangrove habitat in southeastern Australia, *Timspurckia oligopyrenoides* gen. et sp. nov., and one isolate (JAW 4329) from mangroves in Madagascar, *Erythrolobus madagascarensis* sp. nov., were investigated. Methods for collection, isolation and maintenance of cultures are presented in West and Zuccarello (1999) and West (2005). To promote growth of target unicellular red algae, low nutrient levels were used initially [2 ml modified Provasoli media (MPM) enrichment per litre of sterile 30 practical salinity units (psu)-seawater]. Careful weekly observations with a dissecting stereomicroscope detected developing unicell colonies that were removed by a 100-µl micropipette or microforceps and subcultured in 50 × 70-mm crystallizing dishes containing 30 psu seawater with 10 ml MPM enrichment per litre. Most cultures were maintained in 18–22°C, 5–20 µmol photons m^{-2} s^{-1} cool-white fluorescent lighting at 12:12 LD daily cycle. Some cultures were also grown in 30-psu von Stosch enriched seawater (von Stosch 1964) in Pyrex No. 3250 dishes in front of north-facing windows (37°27′N, 76°71′W) during all seasons of the year.

Light microscopy

Live cells were placed on a slide and sealed under a coverslip with VALAP (vasoline:lanolin:paraffin 1:1:1) and observed and photographed using bright-field and Normarski optics on Zeiss GFL and Leica DMRB microscopes. Phototaxis studies involved removal of cell clumps from cultures grown in MPM under fluorescent lighting and placement in Pyrex No. 3240 dishes wrapped in aluminum foil such as to allow unilateral exposure to window light. Dishes were observed after 7–9 d of growth with a stereomicroscope and photographed with a Nikon D40X digital camera.

Time-lapse videomicroscopy

Cell motility was observed using procedures described in Ackland *et al.* (2007).

Confocal microscopy

Live cells were settled on coverslips coated with 0.01% PEI (polyethylenimine). Plastid autofluorescence was excited with a 488-nm argon laser and emittance captured between 665 and 695 nm using a Leica TCS 4D confocal microscope. Images consist of an average of 20 optical sections 3 µm in depth.

TEM

Cells from cultures grown in window light were filtered onto poly-L-lysine coated 0.45-µm Millipore™ filters and fixed for 2 h at ambient temperature in 2% glutaraldehyde in a 0.1 M phosphate buffer solution (pH 6.8) with 0.25 M sucrose. Following buffer rinses, samples were postfixed 1 h in the same buffer in 1% OsO_4 at 4°C, 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 2 h. Samples were then further dehydrated in a graded acetone series, infiltrated gradually and embedded in EmBed 812 resin (Electron Microscopy Sciences) and polymerized at 70°C for 1–3 d. Thin sections were cut with an RMC MT6000-XL ultramicrotome. Sections were stained 1 min with lead acetate. A Zeiss EM 109 electron microscope was used for observation and photography.

LMWC analyses

LMWCs were separated and quantified by high-performance liquid chromatography (HPLC). For these analyses, 10–15 mg dry weight (dw) of the red algal samples were extracted in 1 ml of 70% (v/v) ethanol and heated for 3 h in a water bath at 70°C. After centrifugation for 5 min at 6200 × g, 700 ml of the supernatant were evaporated to dryness under vacuum (Speed Vac Concentrator SVC 100H). Dried extracts were redissolved in 700 ml of distilled water, sonicated for 1 min and vortexed for 30 s. After centrifugation at 16,000 × g, the supernatants were analysed with an isocratic Agilent HPLC system equipped with a differential refractometer. LMWCs were separated using two types of columns and quantified with an isocratic Agilent HPLC system equipped with a differential refractometer (Karsten *et al.* 1991, 2005). Separation of LMWCs was performed on a resin-based column (Bio Rad Aminex Fast Carbohydrate Analysis, 100 × 7.8-mm outer diameter) after passing through a Phenomenex Carbo-Pb^{2+} (4 × 3-mm inner diameter) guard cartridge and eluted with 100% H_2O at a flow rate of 1 ml min^{-1} at 70°C (Karsten *et al.* 1991). In addition, a resin-based Phenomenex (Rezex ROA-Organic Acid, 300 × 7.8-mm outer diameter) column protected with a Phenomenex Carbo-H^+ guard cartridge (4 × 3-mm inner diameter), was used (Karsten *et al.* 2005). On this column LMWCs were eluted with 5 mM H_2SO_4 at a flow rate of 0.4 ml min^{-1} at 75°C. Peaks on both columns were identified by comparison of the respective retention times with those of commercial standard compounds and

quantified by peak areas. The concentrations were expressed in μmol g^{-1} dry mass.

Analyses of *psa*A and *psb*A sequences

Genomic DNA was extracted from each culture strain using a DNeasy Plant Mini Kit (Qiagen), according to the manufacturers' instructions. PCR and sequencing were performed with specific primer sets for two plastid genes; *psa*A was amplified and sequenced using psaA130F-psaA1110R and psaA971F-psaA1760R (Yoon *et al.* 2002; Yang & Boo 2004) and *psb*A using psbAF-psbAR2 (Yoon *et al.* 2002). Amplified DNA was purified with the QIAquick PCR Purification Kit (Qiagen) and sent to a commercial sequencing company.

The electropherogram output for each specimen was edited using the program Chromas v.1.45 (http://www.technelysium.com.au/chromas.html). For the phylogenetic analyses, 27 *psa*A and *psb*A sequences were aligned including five newly sequenced from this study (*psa*A, GU295437–GU295441; *psb*A, GU295442–GU295446) and 22 sequences (Yoon *et al.* 2006) from GenBank using program Se-Al v.2.0a11 (http://tree.bio.ed.ac.uk/software/seal). The amino acid sequence alignments were used to assist in the DNA alignment. The intron sequences from *psa*A gene were excluded in subsequent analyses.

The evolution model was selected by the program ModelGenerator v.0.85 (Keane *et al.* 2006). Maximum likelihood (ML) analyses were preformed using RAxML v.7.0.3 (Stamatakis 2006). Tree likelihoods were estimated using 100 independent replications using a general time reversible (GTR) substitution model with rate heterogeneity among sites (gamma distribution, Γ) and proportion of invariable site (I). Automatically optimized SPR rearrangement and GTRMIX algorithm (unfixed GTR + Γ model parameters) were used for best tree search. Maximum parsimony (MP) analyses were conducted using the PAUP* v.4.0b10 (Swofford 2002). The heuristic search was carried out with 1000 replicates, random sequences addition and TBR branch swapping.

Bootstrap support values of ML (MLBt) and MP (MPBt) were calculated with 1000 replicates to test the stability of monophyletic groups.

Bayesian analysis was conducted with MrBayes v.3.2 (Ronquist & Huelsenbeck 2003) using GTR + Γ + I model, the same as ML analysis. The GTR rates, Γ and I value were not fixed. Two independent runs of 2 million generations were performed with four chains, and trees were sampled every 100 generations. The burn-in point was identified by the average of standard deviation from split frequencies (<0.01) between two independent runs. Trees sampled after burn-in point were used to infer the Bayesian posterior probability (BPP) calculation.

RESULTS

T. oligopyrenoides gen. et sp. nov. E.C. Yang, J.L. Scott & J.A. West

Cellulae sphaericae 7–11 μm diametro, matrice extracellulari pellucida conspicua (2 μm crassa), griseo-rubrae vel rubro-brunneae, phototaxe positiva. Chloroplastus singulus lobis multis extensis a pyrenoidibus multis per totam cellulam dispositis; pyrenoidibus thylacoides tubulares continentibus, cytoplasmati expositis, plerumque vagina amyli circumcinctis; granis amyli quoque in locis alteris cellularum, plerumque juxta lobos chloroplasti; thylacoidibus peripheralibus nullis. Corpora Golgi uno mitochondrio semper consociata, plerumque in peripheriam cellulae posita. Nucleus peripheralis nucleolo singulo ad partem involucri nuclearis centro cellulae proximam adpresso. Carbonii hydrata parvi ponderis floridosidum, digeneasidum, et trehalosum.

Spherical cells 7–11 μm in diameter, conspicuous (2 μm thick) clear extracellular matrix, greyish red to reddish brown, exhibiting positive phototaxis. The single chloroplast has numerous lobes extending from multiple pyrenoids located throughout the cell. Pyrenoids, containing tubular thylakoids, are exposed to the cytoplasm and are usually surrounded by a starch sheath. Starch grains also present elsewhere in cells, usually adjacent to chloroplast lobes. Peripheral thylakoids absent. Golgi bodies always closely associated with a mitochondrion and usually located at the cell periphery. Nucleus peripheral with a single nucleolus appressed to the region of the nuclear envelope usually closest to the cell center. Low molecular weight carbohydrates are floridoside, digeneaside and trehalose.

HOLOTYPE: CCAP 1393/2 (JAW 4318). Live culture: Culture Collection of Algae and Protozoa, Scottish Association for Marine Science, Scottish Marine Institute, Dunstaffnage Marine Laboratory, Dunbeg, Oban, Argyll, PA37 1QA, UK.

ICONOTYPE: Figs 1–4, 6, 9–11 of this article.

ISOTYPE: Dried specimen on paper, NSW 796127, Royal Botanic Gardens, Mrs. Macquaries Road, Sydney, NSW 2000, Australia.

TYPE LOCALITY: Mud sample, Stony Creek Backwash, Williamstown, Victoria, Australia (37°49′S, 144°53′E), 13 March 2003.

PARATYPE: CCAP 1393/1 (JAW 3827). Mud sample, Stony Creek Backwash, Williamstown, Victoria, Australia (37°49′S, 144°53′E), 15 February 1998.

CRYOPRESERVED CULTURE: CCMP 3278, Bigelow Laboratory for Ocean Sciences, P.O. Box 475, 180 McKown Point Road, West Boothbay Harbor, ME 04575, USA.

COLOUR: The specimens in laboratory culture range from greyish red to a reddish brown [p. 8, 5D, to p. 10, 6B, of Kornerup and Wanscher (1978)].

ETYMOLOGY: Dr Tim Spurck (1955–2009) was a wonderful research colleague and friend who provided superb insights and technical assistance on numerous cell biological techniques to many staff in School of Botany, University of Melbourne. He died in his sleep of a coronary complication on the night of 12 January 2009 at the age of 53. He is sorely missed by many of us. We have chosen the genus name *Timspurckia* to honour him.

The species name 'oligopyrenoides' refers to the variable number (one to four) of pyrenoids seen in different cells.

***E. madagascarensis* sp. nov. E.C. Yang, J.L. Scott & J.A. West**

Cellulae sphaericae 5–8 μm diametro, matrice extracellulari tenuissima (<1 μm), griseo-rubrae vel rubrobrunneae, phototaxe positiva. Chloroplastus singulus lobis aliquot extensis ab una pyrenoide eccentrica thylacoides tubulares continente; pyrenoidibus cytoplasmati expositis, saepe vagina amyli circumcinctis; granis amyli quoque in locis alteris cellulae, plerumque juxta lobos chloroplasti; thylacoidibus peripheralibus nullis. Corpora Golgi uno mitochondrio semper arcte consociata, plerumque in peripheriam cellulae posita. Nucleus eccentricus nucleolo singulo centrale. Vesicula multa fibrosa circa 0.8 μm diametro, membrana limitata et electronibus translucida, in cellulis omnibus praesentia. Carbonii hydrata parvi ponderis floridosidum, digeneasidum, et trehalosum.

Cells spherical, 5–8 μm in diameter, very thin (<1 μm) extracellular matrix, greyish red to reddish brown, positively phototactic. The single chloroplast has several lobes extending from the single eccentric pyrenoid that contains tubular thylakoids. The pyrenoid is exposed to cytoplasm and often surrounded by a starch sheath. Starch grains also present elsewhere in cell, usually adjacent to chloroplast lobes. Peripheral thylakoids absent. Golgi bodies always closely associated with a mitochondrion and usually located at the cell periphery. Eccentric nucleus with a single central nucleolus. Numerous membrane-bound electron transparent fibrous vesicles about 0.8 μm diameter present in all cells. Low molecular weight carbohydrates are floridoside, digeneaside and trehalose.

HOLOTYPE: CCAP 1393/3 (JAW4329). Live culture: Culture Collection of Algae and Protozoa, Scottish Association for Marine Science, Scottish Marine Institute, Dunstaffnage Marine Laboratory, Dunbeg, Oban, Argyll, PA37 1QA, UK.

ICONOTYPE: Figs 5, 8, 12–15 of this article.

ISOTYPE: Dried specimen on paper, NSW 796126, Royal Botanic Gardens, Mrs. Macquaries Road, Sydney, NSW 2000, Australia.

CRYOPRESERVED CULTURE: CCMP 3276, Bigelow Laboratory for Ocean Sciences, P.O. Box 475, 180 McKown Point Road, West Boothbay Harbor, ME 04575, USA.

TYPE LOCALITY: Isolated from a culture of *Sphacelaria* sp. obtained on *Sargassum* sp., Angeva, Madagascar, 23°10.130′S, 43°36.575′E, 22 April 2003.

COLOUR: The specimens in laboratory culture range from greyish red to a reddish brown [p. 7, 4D, to p. 11, 4B, of Kornerup & Wanscher (1978)].

ETYMOLOGY: Species name is for the island of Madagascar, from which it was collected and isolated.

Light microscopy and cell motility

Some cells of *T. oligopyrenoides* JAW 4318 (Fig. 1) and JAW 3827 (Fig. 3) appeared to have a single, central pyrenoid, but most cells revealed two to four pyrenoids scattered throughout the cell visible at different focal planes. The cell diameter of both isolates was 7–11 μm. Cells grown in static culture formed one to several layers of cells attached to dish bottoms and were separated from each other by their thin extracellular matrices (Figs 1, 3). Several *E. madagascarensis* 4318 cells transferred from one culture dish to another were seen with elongate polysaccharide tails two to five times longer than the cell diameter (Fig. 2). *Erythrolobus madagascarensis* cells were 5–8 μm in diameter, and static cultures were different from those of *T. oligopyrenoides*. Coherent cell layering was not seen. Cells formed groups loosely attached to the bottom of the dish and formed a thick, red ring of cells at the culture medium–air interface. All cells had a single conspicuous central pyrenoid (Fig. 5).

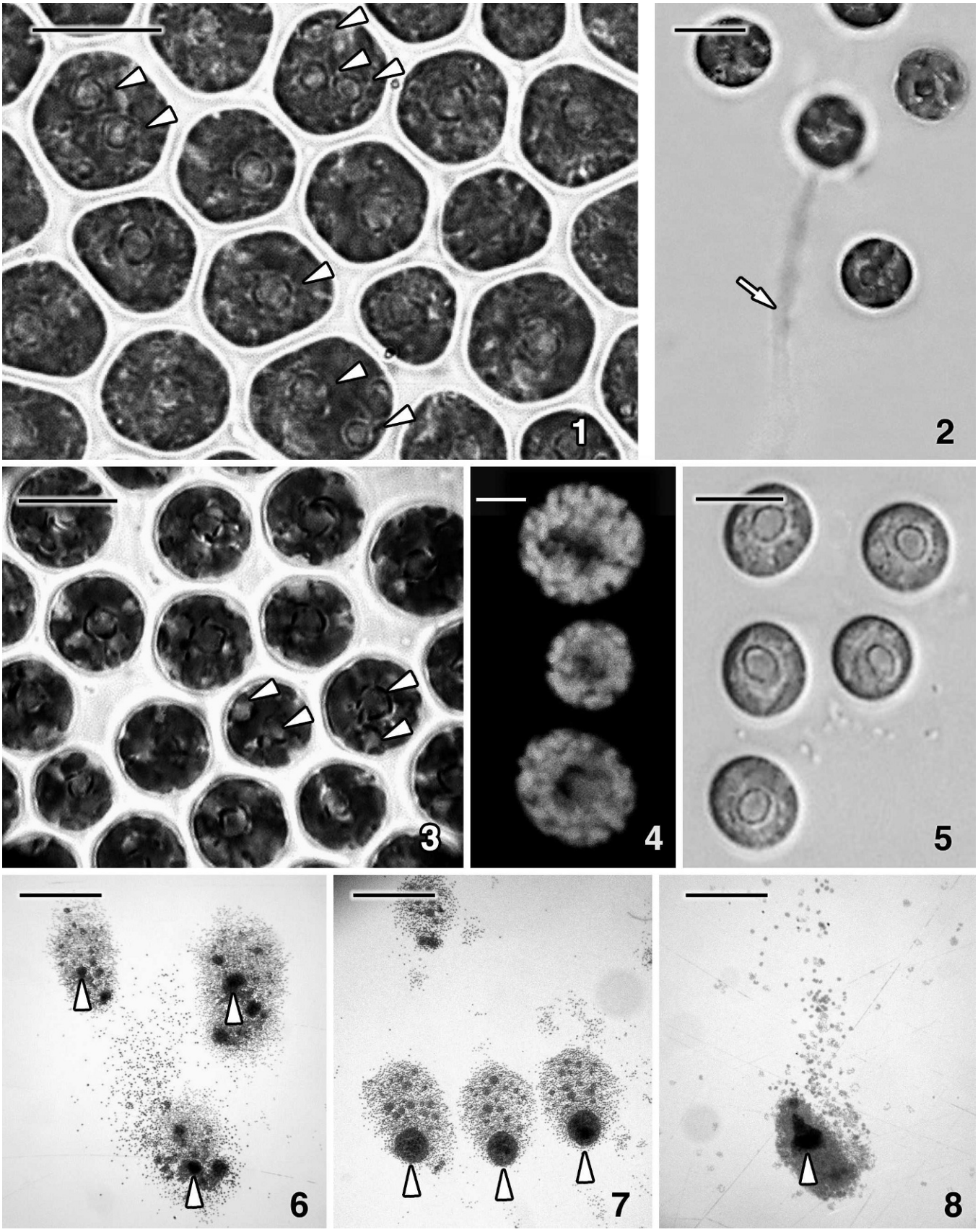
All isolates grown in culture dishes exposed to unilateral window light exhibited positive phototactic movement. After 7 d, *T. oligopyrenoides* JAW 3827 and JAW 4318 demonstrated good growth and migrated as clusters of cells towards the light (Figs 6, 7). *Erythrolobus madagascarensis* cells also showed good growth in 9 d, but fewer cells migrated toward the light (Fig. 8), and they appeared to travel farther than *T. oligopyrenoides* cells. As examined by time-lapse videomicroscopy, *T. oligopyrenoides* JAW 3827 cells with and without elongate polysaccharide tails showed a good motility independent of substrate contact (see Pickett-Heaps *et al.* 2001).

TEM

T. oligopyrenoides

Numerous fixation protocols were attempted to provide quality images of isolates JAW 3827 and JAW 4318. Only one was moderately successful, and the images used in this work are from JAW 4318, although the poorer images from JAW 3827 are virtually identical. The cell coat was thick (about 2 μm) and loosely fibrous (Figs 9, 11). A peripheral ER system with short tubules directed at right angles to the cell membrane, typical of nearly all other red algal unicells (Scott *et al.* 2006), was not observed. Based on determinations made from observing thin sections of many cells, it appears that in many cells the single chloroplast has two or more pyrenoids, each containing tubular thylakoids in the matrix. Most pyrenoids are enclosed by a starch sheath in the adjacent cytoplasm. Fig. 9 shows a cell that was fortuitously sectioned to reveal four pyrenoids along with the nucleus. A few pyrenoids (2.5-μm diameter) were larger than the nucleus, but most were smaller. Several chloroplast lobes are connected to each pyrenoid, resulting in a very intricate chloroplast (Fig. 9) occupying most of the cell. The numerous spherical, electron-dense bodies found interspersed among the thylakoids in Fig. 6 were determined to be artifacts. Phycobilisomes are clearly visible (Figs 9–11). A peripheral thylakoid is absent (Figs 9–11).

Cytoplasmic regions devoid of chloroplast lobes and pyrenoids were occupied by dispersed starch grains, mitochondrial profiles, a single peripheral 2.0-μm nucleus and small numbers of Golgi bodies. The nucleolus was closely associated with the inner membrane of the nuclear envelope (Figs 9, 10). Nuclei with a central nucleolus were never seen. The nucleolus was always along the nuclear envelope except the region facing the cell periphery.



Figs 1–4. *Timspurckia oligopyrenoides*. gen. et sp. nov. bright-field microscopy.
Fig. 1. Most cells appear to have just one pyrenoid (arrowheads), but several cells show two or three. These cells are separated from each other by a thick electron transparent extracellular matrix (JAW 4318). Scale bar = 10 μm.

Heterochromatin was present in small amounts. Golgi bodies were detected in only a few cells. Generally only one was seen in a single section, and it was always found at the cell periphery in close association with a mitochondrion (Fig. 11).

E. madagascarensis

Cells grown in static culture conditions had a thin, faintly visible extracellular matrix (Fig 12). As in *T. oligopyrenoides*, a peripheral ER system was not visible. The single multilobed chloroplast had a 1.5-μm eccentric pyrenoid with a moderately electron dense matrix containing numerous tubular thylakoids (Fig. 12). The pyrenoid was exposed to the cytoplasm, usually surrounded by a starch sheath. Starch was also found scattered throughout the cell closely appressed to the chloroplast lobes (Figs 12, 15). Examination of several hundred cells revealed that each cell has a single chloroplast possessing three to five lobes projecting from the pyrenoid. The lobes extended to and occupied most of the cell periphery. Phycobilisomes were clearly seen, and a peripheral encircling thylakoid was absent (Figs 12, 14, 15).

The irregularly spherical 1.5-μm nucleus was eccentric, lying in close proximity to the pyrenoid (Fig. 12). A single nucleolus 0.5–0.6 μm in diameter was found in the nucleus center (Figs 12, 15). Heterochromatin was never seen. Mitochondria, or profiles of a possible mitochondrial reticulum as seen in a single plane of sectioning [as seen in *Dixoniella* (as *Rhodella reticulata* Deason, Butler & Rhyne), Broadwater & Scott 1986], were found throughout the cell (Figs 12–15). Mitochondrial cristae were distinctly tubular. Golgi bodies were usually found at the cell periphery in areas not occupied by chloroplast lobes (Fig. 14) but occasionally were seen near the cell center (Fig. 12). Golgi cisternae were evenly spaced, and the cis-region of each Golgi body was invariably closely associated with a mitochondrion (Fig. 14).

Over a 2-yr period, seven to eight fixations using varying TEM protocols at different times of the day were performed in a mostly unsuccessful search for details of dividing cells. However, unusual, membrane-bound fibrillar vesicles up to 0.8 μm in diameter were consistently observed (Figs 12–14). Extremely fine, loosely packed electron dense fibers were intertwined within an electron transparent vesicle matrix (Figs 12–14). We never found evidence that these vesicles were Golgi derived, and we never observed vesicles in the process of fusing with the cell membrane and secreting their contents into the cell coat (exocytosis). In addition, a small population of smaller electron translucent vesicles enclosing a single electron dense sphere was visible in most cells.

Although dividing nuclei in metaphase through telophase were never observed in the hundreds of cells examined, a few cells showed signs of incipient mitosis. Fig. 15 shows a late prophase or early prometaphase nucleus. Adjacent sections revealed that the opposite ends of the somewhat elongated nucleus both had a conspicuous, single invagination 'capped' by a small cloud of material in which sat an electron dense body, determined to be ring shaped. Approximately two dozen of these polar structures were found, interpreted by us as the 'nucleus associated organelles' (NAOs) common in red algal mitosis (Scott & Broadwater 1990). Short microtubules were often observed emanating from the NAOs towards the nuclear pole (visible in Fig. 15). A few nuclei had polar invaginations containing several microtubules directed from the NAOs into the invaginations (not shown).

Sequence divergences and phylogeny

In the present study, 1365 base pairs of the *psa*A gene (excluding intron sequences) and 873 base pairs of *psb*A were aligned for 27 taxa representing all seven red algal classes. In the alignment of *psa*A, 641 positions (47%) were parsimony informative, and for *psb*A, 334 positions (38.3%) were parsimony informative.

The pairwise comparisons of sequence divergences from *psa*A and *psb*A genes showed clear grouping in different taxonomic levels (i.e. species, genus). For example, when the *psa*A sequence divergences were plotted against those of *psb*A, the intergeneric divergences (black circles in Fig. 16) were higher than interspecific divergences (black squares in Fig. 16). The newly described genus *Timspurckia* showed higher divergence with *Porphyridium* (P distance = 0.224) than other genera in the *psa*A gene (0.214 with *Erythrolobus*; 0.207 with *Flintiella*) along with the *psb*A gene (0.097 with *Porphyridium*; 0.071 with *Erythrolobus*; 0.074 with *Flintiella*). Interspecific differences among *Erythrolobus* species ranged from 0.112 to 0.150 in *psa*A and from 0.038 to 0.044 in *psb*A, *E. coxiae* vs *Erythrolobus* sp. JAW 4188 and *E. madagascarensis* vs *Erythrolobus* sp. JAW 4188, respectively.

←

Fig. 2. Some cells have a prominent polysaccharide tail (arrow) up to five times longer than the cell diameter (JAW 4318). Scale bar = 10 μm.

Fig. 3. Two or more pyrenoids (arrowheads) are seen in some cells (JAW 3827). Scale bar = 10 μm.

Fig. 4. Confocal microscopy showing autofluorescence of chloroplast indicating a multilobed structure but not showing pyrenoids (JAW 4318). Scale bar = 8 μm.

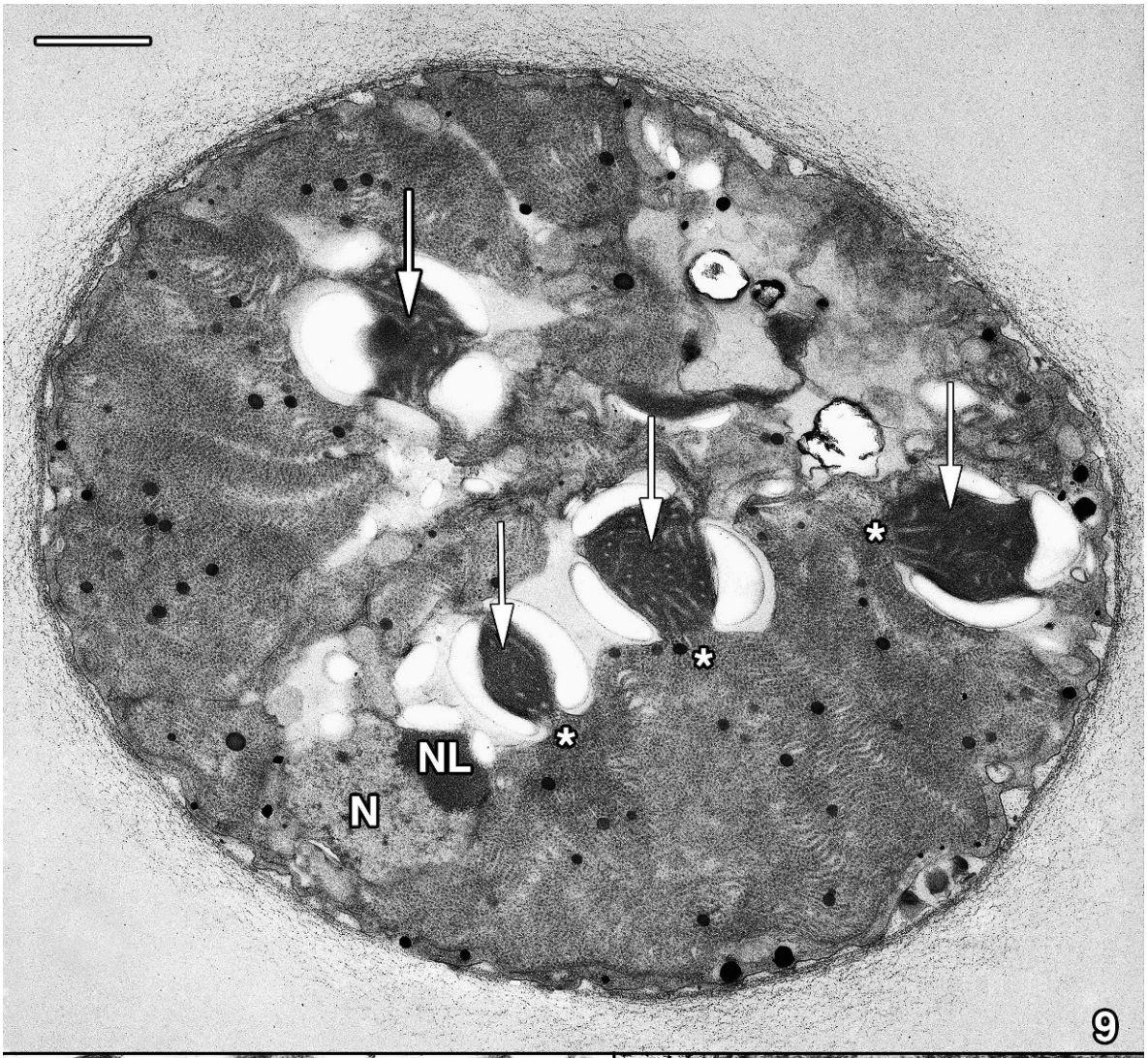
Fig. 5. *Erythrolobus madagascarensis* sp. nov. (JAW 4329). Normarski microscopy. Each cell has a prominent eccentric pyrenoid. Scale bar = 7 μm.

Figs 6–8. Positive phototaxis by cells of *Timspurckia oligopyrenoides*. gen. et sp. nov. and *Erythrolobus madagascarensis* sp. nov. grown in culture dishes exposed to unilateral light (top of each image).

Fig. 6. *Timspurckia oligopyrenoides*. gen. et sp. nov. (JAW 3827). Cells moved from original dark clumps (arrowheads) toward light (7 d). Scale = 1 mm.

Fig. 7. *Timspurckia oligopyrenoides*. gen. et sp. nov. (JAW 4318). Cells of this isolate also moved toward light from the original clumps (arrowheads) in the dish (7 d). Scale = 1 mm.

Fig. 8. *Erythrolobus madagascarensis* sp. nov. (JAW 4329). Fewer cells of this isolate showed movement away from the original clump (arrowhead) in the dish (9 d). Scale = 1 mm.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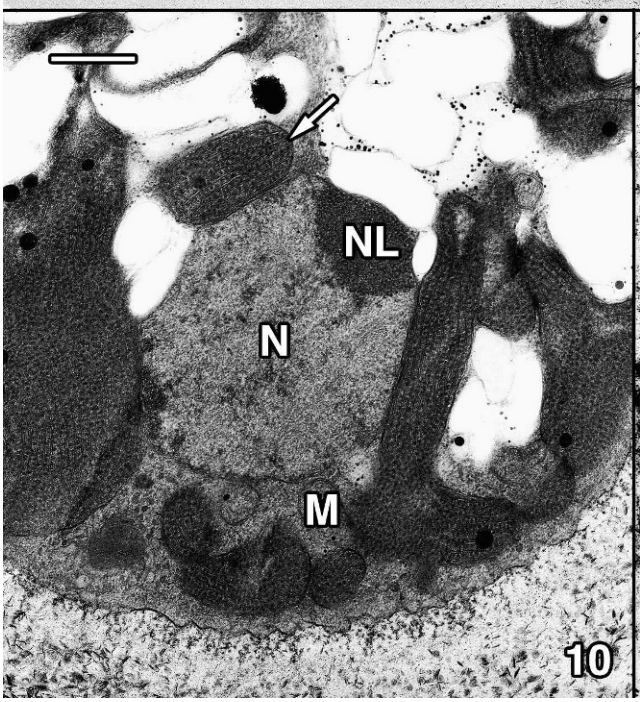
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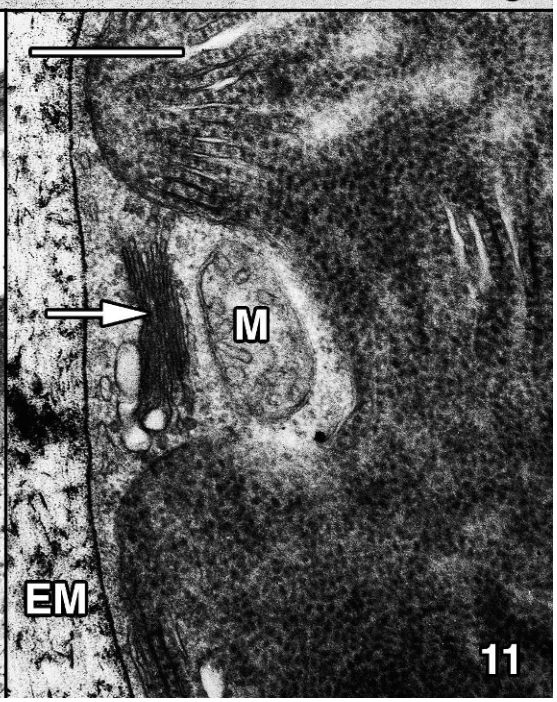
M
EM
11

All analyses of *psa*A + *psb*A data support the monophyly of the Porphyridiophyceae (98% MLBt, 79% MPBt and 1.0 BPP in Fig. 17). *Timspurckia* was clearly separated from other Porphyridiophyceae genera *Erythrolobus*, *Flintiella* and *Porphyridium*. A sister relationship between *Erythrolobus* and *Timspurckia* was supported by BPP but not MLBt or MPBt. The monophyly of *Erythrolobus* was strongly supported (100% MLBt, 99% MPBt and 1.0 for BPP), and *E. madacarensis* was basal within the genus. A sister relationship of *Erythrolobus* sp. JAW 4188 and *E. coxiae* was also strongly supported (Fig. 17).

LMWC patterns

Both *E. madagascarensis* and *T. oligopyrenoides* contain similar LMWC patterns with floridoside as the most abundant compound (178.8–346.7 μmol g^{-1} dw). However, in *Erythrolobus*, digeneaside concentrations between 32.3 and 38.5 μmol g^{-1} dw could be determined, but *Timspurckia* showed only trace concentrations of this heteroside (<1 μmol g^{-1} dw). In addition, in both species trace values of trehalose (0.2–2.2 μmol g^{-1} dw) were measured.

DISCUSSION

Cell motility and phototaxis

Cell motility of *T. oligopyrenoides* JAW 3827 [as *Porphyridium purpureum* (Bory de Saint-Vincent) Drew & Ross] was described briefly by Pickett-Heaps *et al.* (2001). The cells showed two types of movement. Cells with a tail showed a sinusoidal movement, and those without a tail move slower with frequent starts and stops. In both types the cells were spherical and showed a directional motion that did not require substrate contact. No external appendages were observed to be involved in cell movement. The tails often were up to five times longer than the cell diameter, did not contribute to movement and were dragged along behind the cells in a wave motion. Normally the cells with tails moved faster (1.0–1.5 μm s^{-1}) than those without tails (0.35–0.5 μm s^{-1}).

Scott *et al.* (2008) summarized the various types of observed red algal unicell motility. Three genera exhibit a 'slow rambling, shuffling motility' [*Rhodella maculata* Evans, *R. violacea* (Kornmann) Wehrmeyer and *Neorhodella cyanea* Scott, Yokoyama, Billard, Fresnel & West], and three have a 'vigorous directional motility' (*P. purpureum*, *E. coxiae* and *Dixoniella grisea*). The four other genera studied were regarded as 'stationary' (*Rhodosorus marinus* Geitler, *Rhodospora sordida* Geitler, *F. sanguinaria* Ott in Bourrelly and *Glaucosphaera vacuolata* Korshikov). The recently described genus *Corynoplastis* (Rhodellophyceae; Yokoyama *et al.* 2009) has a 'slow random gliding movement'. At this time, most described red algal unicellular genera including *T. oligopyrenoides* have been analysed for cell motility by time-lapse video microscopy. It is obvious that a particular motility pattern does not correspond to a particular class. For example, in the Rhodellophyceae *Neorhodella* exhibited 'slow rambling, shuffling motility', *Dixoniella* had 'vigorous directional motility' and *Glaucosphaera* had no motility (Pickett-Heaps *et al.* 2001). A more detailed perspective of phototaxis mechanisms in unicellular reds such as *Porphyridium* is provided by Nultsch & Schuchart (1980).

Cell motility of *T. oligopyrenoides* JAW 4318 and *E. madagascarensis* JAW 4329 was not analysed, although both isolates along with *T. oligopyrenoides* JAW 3827 show positive phototaxis.

General ultrastructure of the Porphyridiophyceae

Erythrolobus was established as a third genus of the class Porphyridiophyceae based on both ultrastructural and molecular characters (Scott *et al.* 2006; Yoon *et al.* 2006). Both *E. coxiae* and *Porphyridium* spp. possess a conspicuous pyrenoid traversed by thylakoids; whereas, *F. sanguinaria* lacks a pyrenoid. The major differences between *Erythrolobus* and *Porphyridium* relate to the nature of the chloroplasts. *Porphyridium* has a large central stellate chloroplast with an embedded pyrenoid. *Erythrolobus coxiae* possesses a chloroplast comprised of multiple peripheral lobes extending from a central 'naked' pyrenoid that is in contact with the cytoplasm and usually is enveloped by a starch sheath. All three genera are characterized by the absence of a peripheral encircling thylakoid (see Table 1). Peripheral thylakoids are present in the unicell *Rhodosorus* (Stylonematophyceae) and in two of the five genera of the Rhodellophyceae (*Dixoniella* and *Glaucosphaera*; Scott *et al.* 2008).

Erythrolobus coxiae also differs from *Porphyridium* spp. in one other ultrastructural feature, the location of the nucleolus in the peripheral nucleus. Similar to all other red algal unicells (except *T. oligopyrenoides*; see below), the nucleolus in *E. coxiae* is in the center or near-center of the nucleus; whereas, in *Porphyridium* spp. it is always appressed to the region of the nuclear envelope closest to the cell center (Gantt & Conti 1965; Gantt *et al.* 1968; Schornstein & Scott 1982; Scott *et al.* 2006). We are not aware of any functional attribute of this curious but consistent trait dissimilarity.

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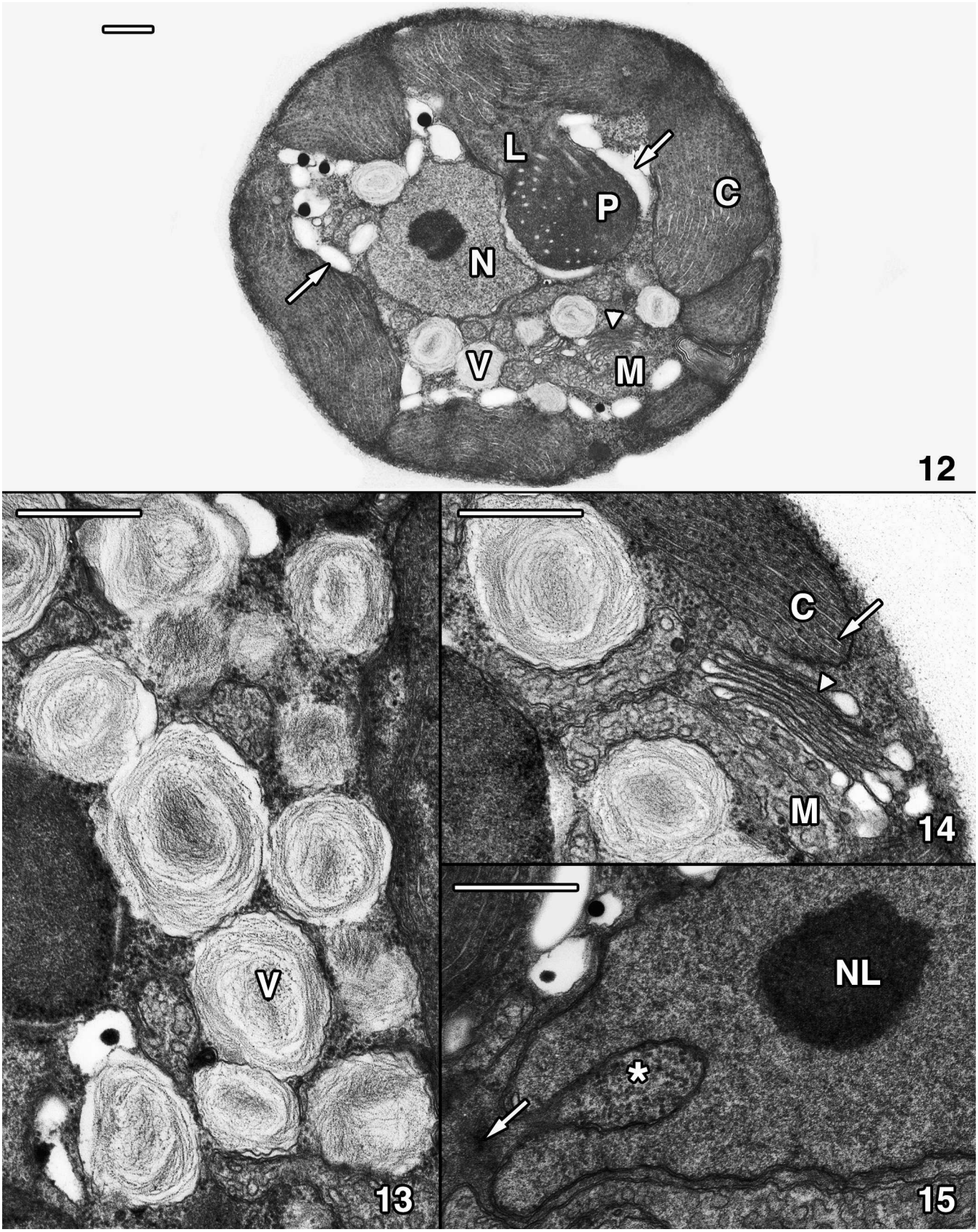
Figs 9–11. *Timspurckia oligopyrenoides*. gen. et sp. nov. (JAW 4318) cell ultrastructure.
Fig. 9. Low magnification electron micrograph of a medially sectioned cell. Four starch ensheathed pyrenoids (arrows) are seen. Several chloroplast lobes are connected to the pyrenoids (*). The nucleus (N) is near the cell periphery and contains a nucleolus (NL) appressed to the nuclear envelope. Small electron dense spheres on the chloroplasts are artifacts. Scale bar = 1 μm.
Fig. 10. Nucleus of a cell showing the typical nucleolus facing the cell center. Note a mitochondrion (M) and the lack of a peripheral thylakoid (arrow). Scale bar = 1 μm.
Fig. 11. Golgi body (arrow) associated with a mitochondrion (M) at the cell periphery with fibrous extracellular matrix (EM) visible. Scale bar = 1 μm.



Figs 12–15. *Erythrolobus madagascarensis* sp. nov. (JAW 4329) cell ultrastructure.

Fig. 12. Low-magnification electron micrographs of two medially sectioned cells. The nucleus (N) is eccentrically located in the cell, and its nucleolus (NL) is centrally positioned in the nucleus. The starch-ensheathed pyrenoid (P) of each cell is eccentrically positioned in the cell and is partially surrounded by the parietal chloroplast (C). One chloroplast lobe (L) extends from the pyrenoid (P). The pyrenoid

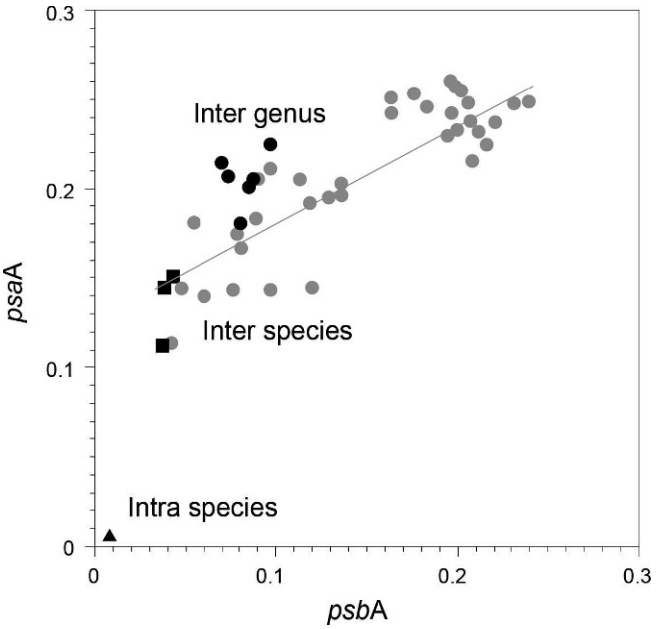


Fig. 16. Pairwise sequence divergences (P distance) comparison of *psa*A and *psb*A genes. The average sequence divergence of the Porphyridiophyceae was 0.186 ± 0.05 in *psa*A and 0.071 ± 0.02 in *psb*A, respectively. Intergeneric divergences (black circles from the Porphyridiophyceae and grey circles from the other five classes of the Rhodophyta) of each class and interspecies (three squares) and intraspecies divergences (one triangle) of the Porphyridiophyceae were plotted. Species of the Cyanidiophyceae are regarded as independent genera because of high divergence rates (taxonomic revision is underway; Yoon & Bhattacharya, unpublished data). Distinguishable generic, interspecific and intraspecific levels of divergence were showed within the Porphyridiophyceae. Overall correlation between *psa*A and *psb*A (except intraspecific divergence) was fitted as a line (*psa*A=0.13 + 0.54*psb*A, $r^2 = 0.69$).

The Golgi bodies in *E. coxiae*, *Porphyridium* spp. and *F. sanguinaria* are invariably closely associated with a mitochondrion, similar to all investigated algae in the classes Florideophceae and Bangiophyceae but not in the Rhodellophyceae, Stylonematophyceae and Compsopogonophyceae. Since only a few subcellular characters have proven to be reliable in unicellular red algal systematics (Scott *et al.* 2008), which of these characters are convincing that *E. madagascarensis* and *T. oligopyrenoides* represent new taxa in the Porphyridiophyceae? The major ultrastructural and carbohydrate characters that define the current members of the Porphyridiophyceae are presented in Table 1. Appendix 1 provides a key to unicellular red algal genera using ultrastructural characters.

T. OLIGOPYRENOIDES: While the ultrastructural differences between *E. coxiae* and *E. madagascarensis* are somewhat subtle, those seen in *T. oligopyrenoides* compared with the other genera in class Porphyridiphyceae are more obvious, largely due to the multiple, conspicuous pyrenoids. Neither *Porphyridium* nor *Erythrolobus* possesses more than one pyrenoid (*Flintiella* lacks one), although two pyrenoids have been found in a few, old *E. coxiae* cells (Scott *et al.* 2006), and four or more are reported in undescribed isolates of presumptive *Erythrolobus* cells (A. Yokoyama, unpublished ultrastructural studies). The only other unicells having more than a single pyrenoid are *Neorhodella cyanea* Scott, Yokoyama, Billard, Fresnel & West; Scott *et al.* 2008) and *Corynoplastis japonica* Yokoyama, Scott, Zuccarello, Kajikawa, Hara & West; Yokoyama *et al.* 2009). The pyrenoids in these two genera are quite unlike 'typical' red algal cell pyrenoids, being found at each slender tip of the numerous chloroplast lobes terminating in the cell center.

Also, since peripheral chloroplast lobes extend from a pyrenoid in both *E. coxiae* and *E. madagascarensis*, the overall shape of the chloroplast in *T. oligopyrenoides* is obviously more complicated than in the *Erythrolobus* species. We also believe that the unusual nature of the nucleus in *T. oligopyrenoides* is noteworthy. The only unicellular red algal genus we are aware of that has a nucleolus positioned at the innermost periphery of a nucleus, as discussed earlier, is *Porphyridium*. Therefore, *T. oligopyrenoides* shares one subcellular character with *Porphyridium* and another one (naked pyrenoids) with *Erythrolobus*.

E. MADAGASCARENSIS: In *E. coxiae*, the pyrenoid is usually surrounded by a starch sheath, the pyrenoid matrix contains tubular thylakoids and the parietal chloroplast is formed by several lobes extending from the pyrenoid. All observed *E. coxiae* cells had a central pyrenoid and a peripheral nucleus (Scott *et al.* 2006), but these two organelles are clearly eccentric in *E. madagascarensis*. Another difference is that cells of *E. madagascarensis* contain numerous fibrous vesicles; those of *E. coxiae* do not. We have never observed these vesicles in close association with Golgi bodies and have never seen them being secreted (exocytosis), even though we looked at hundreds of cells processed at various times in the light/dark cycle from cultures grown both in window light and in culture incubators. Perhaps the discharge of vesicles occurs during the cell division phases that we did not observe. No other unicellular red algae are known to have vesicles even closely similar to those seen in *E. madagascarensis* (Broadwater & Scott 1994; Scott *et al.* 2006, 2008). However, they do closely resemble the fibrous vesicles reported in the spermatangia of *Smithora naiadum* (Anderson) Hollenberg (Hawkes 1988), which appear to be Golgi derived. We have no explanation for the origin and function of these vesicles, but they do appear to differentiate *E. coxiae* from *E. madagascarensis*.

←

matrix is filled with tubular thylakoids. Fibrous vesicles (V), starch (arrows) and one Golgi body (arrowhead) as well as one mitochondrion (M) are visible. Scale bar = 0.8 μm.

Fig. 13. A population of fibrous vesicles (V) is always present in cells throughout the light/dark cycle. Scale bar = 1 μm.

Fig. 14. A Golgi body (arrowhead) in close association with a mitochondrion (M) is located near the cell periphery. A peripheral thylakoid is absent in a chloroplast lobe (arrow). Scale bar = 1 μm.

Fig. 15. One pole of a prophase/prometaphase nucleus is seen with a pronounced invagination (*) near a nucleus-associated organelle (NAO; arrow). NL, nucleolus. Scale bar = 1 μm.

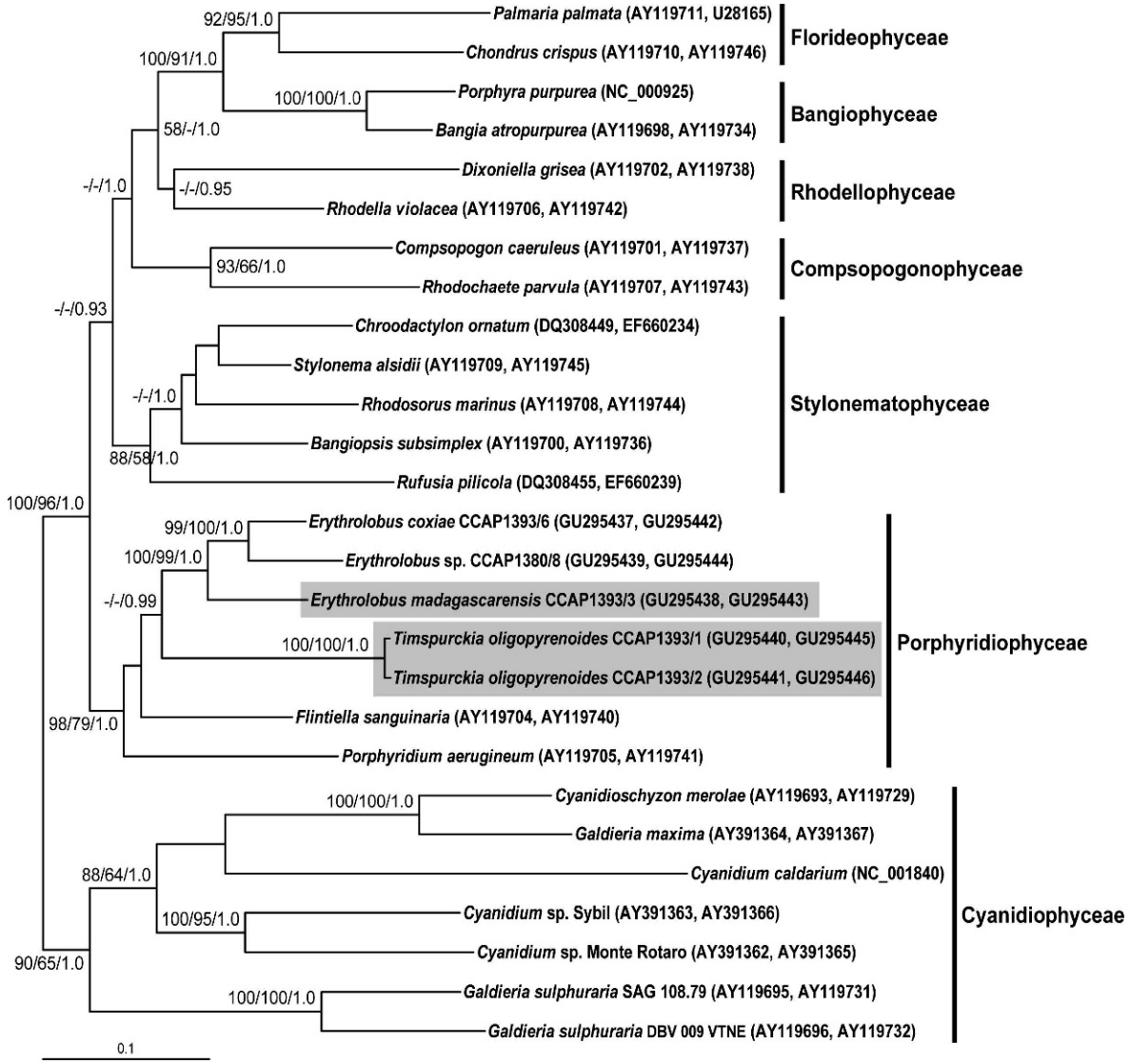


Fig. 17. Maximum likelihood tree of *Erythrolobus madagascarensis* and *Timspurckia oligopyrenoides* based on *psa*A + *psb*A data (−lnL = 25,256.94). Numbers near each clade refer the maximum likelihood and maximum parsimony bootstrap values and Bayesian posterior probability (MLBt/MPBt/BPP). The taxon names are followed by two GenBank accession numbers for *psa*A and *psb*A; however, plastid genome accession numbers (NC_000925 and NC_001840) are shown once.

Molecular characters of the Porphyridiophyceae

Overall tree topology based on *psa*A + *psb*A gene phylogeny presented in this study is congruent with the previous study that used a nine-gene data set (Yoon *et al.* 2006). Seven classes were identified, and the Porphyridiophyceae was a strong monophyletic group that includes four genera with one newly established genus *Timspurckia* even though the internal relationships were not fully resolved. *Timspurckia* showed high divergence rates that were similar to other red algal genera in divergence comparison (Fig. 16). Therefore, we proposed *Timspurckia* as a new genus based on morphological and molecular data. *Erythrolobus madagascarensis*, however, shows grouping with other *Erythrolobus* species in the phylogeny, with species level of divergence. Conclusively, molecular data support that *T. oligopyrenoides* and *E. madagascarensis* are newly uncovered natural entities within the class Porphyridiophyceae.

LMWCs of the Porphyridiophyceae

LMWC content has also been useful in defining each of the three classes containing unicellular red algae. Mannitol is present in all examined members of the Rhodellophyceae; whereas, digeneaside and sorbitol (*Rhodosorus*) or sorbitol and dulcitol (*Rhodospora*) are in the two unicells of the Stylonematophyceae (Karsten *et al.* 1999, 2003). Table 2 in Karsten *et al.* (2003) erroneously listed *Rhodospora sordida* (3628) as *Rhodosorus sordida*, but the correct designation for 3628 is found in that paper's discussion. Scott *et al.* (2006) reported the presence of both floridoside and digeneaside in *E. coxiae*. Unfortunately, this was overlooked by Scott *et al.* (2008), who stated that all members of

Table 1. Principal ultrastructural and biochemical features of species in the Porphyridiophyceae: *Erythrolobus*, *Flintiella*, *Porphyridium* and *Timspurckia*.

	Porphyridium spp.	*Flintiella sanguinarium*	*Erythrolobus coxiae*	*Erythrolobus madagascarensus*	*Timspurckia multipyrenoidosa*
Golgi association	mitochondrion-ER	mitochondrion-ER	mitochondrion-ER	mitochondrion-ER	mitochondrion-ER
Chloroplast peripheral thylakoid	(−)	(−)	(−)	(−)	(−)
Pyrenoid	(+)	(−)	(+)	(+)	(+)
Pyrenoid numbers	1	0	1; 2 or more in old cells	1	2 or more
Pyrenoid location	central, embedded[1]	NA[2]	central, naked[3]	eccentric, naked	random locations, naked
Nucleus location	peripheral	peripheral	peripheral	eccentric	peripheral
Nucleolus position	nucleus periphery[4]	nucleus center	nucleus center	nucleus center	nucleus periphery[5]
Persistent fibrous vesicles	(−)	(−)	(−)	(+)	(−)
LMWC[6]	floridoside	floridoside	floridoside, digeneaside	floridoside, digeneaside, trehalose	floridoside, digeneaside, trehalose

[1] Embedded in center of chloroplast, not exposed to cytoplasm/starch sheath.
[2] NA, not applicable.
[3] Naked, exposed to cytoplasm/starch sheath.
[4] Facing cell center.
[5] Facing everywhere except cell periphery.
[6] LMWC, low-molecular-weight carbohydrates.

the Porphyridiophyceae (*Erythrolobus*, *Porphyridium and Flintiella*) contain only floridoside. It has been determined that the isolates used in this study, *T. oligopyrenoides* JAW 4318, JAW 3827 and *E. madagascarensis* JAW 4329, all contain high concentrations of floridoside (178.8–346.7 μmol g^{-1} dw) together with low to intermediate contents of digeneaside (<38.5 μmol g^{-1} dw) plus traces of trehalose. In addition, other *Erythrolobus* isolates from Guam and several localities in Australia all consistently contain floridoside together with digeneaside (data not shown). Some but not all show trehalose as well. However, since the trehalose signals are extremely small and near the detection limit of the refractive index HPLC detector, the presence or absence of this LMWC cannot always be verified. Nevertheless, a revised statement of LMWC distribution within the Porphyridiophyceae is called for; it is the only class containing unicellular red algae whose members possess only floridoside (*Porphyridium* and *Flintiella*) or floridoside and digeneaside (*E. coxiae*, *E. madagascarensis* and *T. oligopyrenoides*). The significance of trehalose as a chemotaxonomic marker has to be evaluated in future studies using more sensitive methods. Since methodological refinements in LMWC chemistry have been made in the past few years, reexamination of all red algal unicells would be most welcome.

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Appendix 1. Key to unicellular red algae using ultrastructural features.

Rhodospora has not yet been investigated by TEM.

1. Golgi associated with mitochondrion 2
1. Golgi not associated with mitochondrion 5
2. Pyrenoids absent . . . . . *Flintiella*
2. Pyrenoids present 3
3. Embedded pyrenoids . . . . . *Porphyridium*
3. Naked pyrenoids 4
4. Nucleolus located in nucleus center . . . . *Erythrolobus*
4. Nucleolus located at nucleus periphery . . *Timspurckia*
5. Golgi associated with nucleus 6
5. Golgi associated with ER 8
6. Pyrenoids absent . . . . . *Glaucosphaera*
6. Pyrenoids present 7
7. Single large pyrenoid . . . . . *Dixoniella*
7. Pyrenoids small, numerous, on central tips of choloroplast lobes . . . . . *Neorhodella*
8. Single pyrenoid 9
8. Pyrenoids small, numerous, on central tips of choloroplast lobes . . . . . *Corynoplastis*
9. Pyrenoid matrix lacking thylakoids . . . . . *Rhodella*
9. Pyrenoid matrix with thylakoids . . . . . *Rhodosorus*

Corrigendum

Corrections to the paper by Yang E.C., Scott J., West J.A., Orlova E., Gauthier D., Küpper F.C., Yoon H.S. and Karsten U. (2010). New taxa of the Porphyridiophyceae (Rhodophyta): *Timspurckia oligopyrenoides* gen. et sp.nov. and *Erythrolobus madagascarensis* sp. nov. *Phycologia* 49(6): 604–616.

The designation of living cultures as the holotypes for the names of the new taxa in the above paper did not conform with Article 8.4 of the International Code of Botanical Nomenclature (McNeill *et al.* 2006) and resulted in those names not being validated. The purpose of this corrigendum is to validate those names.

An unrelated issue is that in the paper the copy editor for *Phycologia* abbreviated the generic names at the critical point where the new names were being diagnosed, which may lead to possible confusion in the citation of these taxa in the literature.

The correct full spellings of generic names are shown below:

Timspurckia oligopyrenoides **gen. et sp. nov. E.C. Yang, J.L. Scott et J.A. West**

The Latin diagnosis is provided in Phycologia 49: 606 (2010), and figures 1–4, 6, 9–11 are given in the same article by Yang et al. cited above.

Erythrolobus madagascarensis **sp. nov. E.C. Yang, J.L. Scott et J.A. West**

The Latin diagnosis is provided in Phycologia 49: 607 (2010), and figures 5, 8, 12–15 are given in the same article by Yang et al. cited above.

The designation of living cultures as the holotypes of *Timspurckia oligopyrenoides* and *Erythrolobus madagascarensis* was not in conformity with Art. 8.4 of the ICBN, which reads as follows: *Type specimens of names of taxa must be preserved permanently and may not be living plants or cultures. However, cultures of fungi and algae, if preserved in a metabolically inactive state (e.g. by lyophilization or deep-freezing), are acceptable as types.*

Cryopreserved material is now being designated as the holotype of the two newly proposed taxa. We call attention to the fact that what was intended to as the paratype for *Timspurckia oligopyrenoides* in Yang et al. (2010) is now the holotype because that material is cryopreserved.

Timspurckia oligopyrenoides **gen. et sp. nov. E.C. Yang, J.L. Scott et J.A. West**

HOLOTYPE: Cryopreserved culture CCMP 3278, Bigelow Laboratory for Ocean Sciences, P.O. Box 475, 180 McKown Point Road, West Boothbay Harbor, ME 04575, USA. This culture is JAW 3827 isolated by J. West from a mud sample obtained at the type locality: Stony Creek Backwash, Williamstown, Victoria, Australia (37° 49′S, 144° 53′E), 15 February 1998.

ISOTYPE: Dried specimen (NSW 796127) on paper from live culture JAW 3827, Royal Botanic Gardens, Mrs. Macquaries Road, Sydney, NSW 2000, Australia.

LIVE CULTURE : CCAP 1393/1 (JAW 3827), Culture Collection of Algae and Protozoa, Scottish Association for Marine Science, Scottish Marine Institute, Dunstaffnage Marine Laboratory, Dunbeg, Oban, Argyll, PA37 1QA, UK.

LIVE CULTURE: CCAP 1393/2 (JAW 4318), Culture Collection of Algae and Protozoa, Scottish Association for Marine Science, Scottish Marine Institute, Dunstaffnage Marine Laboratory, Dunbeg, Oban, Argyll, PA37 1QA, UK Victoria isolated by J. West from a mud sample obtained at the type locality: Stony Creek Backwash, Williamstown, Victoria, Australia (37° 49′S, 144° 53′E), 13 March 2003.

Erythrolobus madagascarensis **sp. nov. E.C. Yang, J.L. Scott et J.A. West**

HOLOTYPE: Cryopreserved culture: CCMP 3276, Bigelow Laboratory for Ocean Sciences, P.O. Box 475, 180 McKown Point Road, West Boothbay Harbor, ME 04575, USA, from live culture JAW4329 isolated by J. West from a culture of *Sphacelaria* sp. obtained on *Sargassum* sp. collected S. Loiseaux de Goër, Angeva, Madagascar, 23° 10.130′S, 43° 36.575′E, 22 April 2003.

ISOTYPE: Dried specimen (NSW 796126) on paper from live culture JAW 4329, Royal Botanic Gardens, Mrs. Macquaries Road, Sydney, NSW 2000, Australia.

LIVE CULTURE: CCAP 1393/3 (JAW4329). Culture Collection of Algae and Protozoa, Scottish Association for Marine Science, Scottish Marine Institute, Dunstaffnage Marine Laboratory, Dunbeg, Oban, Argyll, PA37 1QA, UK.

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John A. West, School of Botany, University of Melbourne, Parkville VIC 3010, Australia, e-mail: jwest@unimelb.edu.au