Phycologia

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/uphy20

Rhodachlya westii sp. nov. (Rhodachlyales, Rhodophyta), a new species from Brazil, revealed by an integrative taxonomic approach

Luanda Pereira Soares , Silvia M. P. de Beauclair Guimarães , Mutue Toyota Fujii , Yocie Yoneshigue-Valentin , Maria Gardênia Sousa Batista & Nair S. Yokoya

To cite this article: Luanda Pereira Soares , Silvia M. P. de Beauclair Guimarães , Mutue Toyota Fujii , Yocie Yoneshigue-Valentin , Maria Gardênia Sousa Batista & Nair S. Yokoya (2020): *Rhodachlya westii sp. nov*. (Rhodachlyales, Rhodophyta), a new species from Brazil, revealed by an integrative taxonomic approach, Phycologia

To link to this article: https://doi.org/10.1080/00318884.2020.1763771

PHYCOLOGIA
https://doi.org/10.1080/00318884.2020.1763771

Rhodachlya westii sp. nov. (Rhodachlyales, Rhodophyta), a new species from Brazil, revealed by an integrative taxonomic approach

Luanda Pereira Soares [1], Silvia M. P. de Beauclair Guimarães [1], Mutue Toyota Fujii [1], Yocie Yoneshigue-Valentin [2], Maria Gardênia Sousa Batista [3], and Nair S. Yokoya [1]

[1]*Nucleus for Research in Phycology, Institute of Botany, Av. Miguel Estéfano 3687, São Paulo 04301-902, Brazil*
[2]*Institute of Biology, Federal University of Rio de Janeiro, Av. Carlos Chagas Filho 373, Rio de Janeiro 21941-902, Brazil*
[3]*Center of Nature Sciences, State University of Piauí, Rua João Cabral 1295/1296, Teresina 64002-150, Brazil*

ABSTRACT

A small red alga was isolated into culture from rhodoliths collected on the Piauí coast, northeastern Brazil. Molecular data from the plastid-encoded *rbc*L gene, combined with morphological and ultrastructural evidence, demonstrated that it belongs to the genus *Rhodachlya* and supports the proposal of a new species, *R. westii*. The new species was characterised by basal and erect uniseriate filaments, cells with parietal H-shaped or lobed plastids with one or two prominent pyrenoids, absence of unicellular hairs, and with monosporangia sessile or on branched stalks. Ultrastructure revealed pit plugs with two cap layers without a cap membrane, typical of Rhodachlyales. In our *rbc*L phylogeny, *R. westii sp. nov.* was recovered in a well-supported clade containing *R. hawaiiana* and *R. madagascarensis* (generitype) with interspecific divergence ranging from 5% to 10%, respectively. Characters previously used for species-level taxonomy (e.g. cell dimensions, number of pyrenoids and presence or absence of colourless hairs) are often plastic and overlap. We conclude that *Rhodachlya* species can be accurately identified only based on DNA sequence data and their geographic distribution. *Rhodachlya westii* becomes the third species of the genus, representing the first record in the Atlantic Ocean. Our data suggest that the genus is probably widely distributed. In view of the scarcity of reliable morphological features available for comparison, molecular-assisted alpha taxonomy is needed for uncovering the long-overlooked diversity in this red algal group.

ARTICLE HISTORY
Received 15 April 2019
Accepted 29 April 2020
Published online 05 June 2020

KEYWORDS
Cryptic species; Nemaliophycidae; Phylogeny; Pit plug ultrastructure; *rbc*L; Species delimitation

INTRODUCTION

The subclass Nemaliophycidae of the red algal class Florideophyceae currently includes 11 orders and is composed mostly of marine organisms (Guiry & Guiry 2020; Yoon *et al.* 2010). Over the last few decades, seven new orders have been proposed using molecular data: Balbianiales (Evans *et al.* 2019; Sheath & Müller 1999), Balliales (Choi *et al.* 2000), Colaconematales (Harper & Saunders 2002), Corynodactylales (Saunders *et al.* 2017a), Entwisleiales (Scott *et al.* 2013), Rhodachlyales (West *et al.* 2008) and Thoreales (Müller *et al.* 2002). Multigene phylogenies of the Nemaliophycidae have revealed that it is a strongly supported assemblage, with all orders monophyletic and well supported; however, the inter-ordinal relationships are not fully resolved (Lam *et al.* 2016; Yang *et al.* 2016).

Some members of Nemaliophycidae can be characterised as small, red filamentous algae, usually found as epiphytes or endophytes, commonly exhibiting asexual reproduction by monosporangia. These simple rhodophyceans are often termed as 'acrochaetioid' algae and marine members are represented mainly by the genera: *Acrochaetium* Nägeli, *Colaconema* Batters, *Grania* (Rosenvinge) Kylin, *Rhodochorton* Nägeli, and *Rhodothamniella* Feldmann (Woelkerling 1983). The term acrochaetioid used herein is only a morphological designation and does not reflect phylogenetic affinities, since other unrelated taxa are also termed acrochaetioid algae (e.g. 'Chantransia' stages of Batrachospermales and Thoreales, Necchi & Oliveira 2011; Zucchi & Necchi 2003). They constitute a group with a confusing taxonomic history, represented mainly from their small size and few reliable morphological characters. Plastid morphology, number of pyrenoids, mode of attachment and life history are some characters traditionally used to distinguish species, although the literature is controversial (Garbary 1987; Schneider 1983; Woelkerling 1983). For instance, Woelkerling (1971) found the number of pyrenoids as the most stable character in acrochaetioid species from Australia, whereas Stegenga & Vroman (1976) pointed out that pyrenoids can be induced by high light.

Overlapping morphological attributes make an accurate species delimitation in acrochaetioid algae notoriously difficult. To overcome such limitations, it is essential for species assignments to employ molecular-assisted alpha taxonomy (MAAT) which uses DNA sequences, followed by morphological analyses (Cianciola *et al.* 2010). With the advent of molecular tools, the systematics of acrochaetioid algae has undergone changes. Saunders *et al.* (1995) investigated the phylogenetic affinities of the Acrochaetiales–Palmariales complex, resolving

CONTACT Luanda Pereira Soares luanda87@gmail.com
Supplemental data for this article can be accessed on the publisher's website.

the genera *Meiodiscus* G.W.Saunders & McLachlan and *Rhodothamniella*, both previously assigned to *Audouinella* Bory, *Kylinia* Rosenvinge and *Rhodochorton*, as members of Palmariales. Harper & Saunders (1998, 2002) brought significant findings to the troublesome delineation of this group, revealing a better circumscription of Acrochaetiales, with the transfer of species to a new order, Colaconematales. Clayden & Saunders (2008) used the nuclear large subunit ribosomal DNA (LSU) to resurrect the genus *Grania* for specimens previously assigned to *Acrochaetium efflorescens* (J.Agardh) Nägeli. Later, the genus *Rhododrewia* S.L.Clayden & G.W.Saunders was proposed to accommodate *A. porphyrae* (K.M.Drew) G.M.Smith and other related names (Clayden & Saunders 2014).

Another example of hidden diversity uncovered by molecular data in acrochaetioid algae is the order Rhodachlyales. West *et al.* (2008) isolated two red algae growing on seagrass from Madagascar and initially assigned them to the common acrochaetioid genera *Acrochaetium* and *Colaconema*. Molecular evidence revealed that the isolates were a distinct entity, and based on several approaches they proposed the order Rhodachlyales to accommodate the new genus *Rhodachlya*. Rhodachlyales is distinguished by the absence of a cap membrane between the cap layers, platelike outer and inner caps, and absence of peripheral encircling thylakoid (West *et al.* 2008). Currently, two species are recognised in *Rhodachlya*: the generitype *R. madagascarensis* J.A.West, Scott, K.A.West, Karsten, Clayden & Saunders; and, *R. hawaiiana* Kurihara, J.A.West, Conklin & Sherwood (Guiry & Guiry 2020; Kurihara *et al.* 2012). In addition to molecular data, features such as cell dimensions, frequency of colourless hairs and spore germination pattern, were used to distinguish the species (Kurihara *et al.* 2012). To date, *Rhodachlya* is known only from Madagascar and Hawaii. Although a molecular approach is particularly important in groups that have few morphological characters, species-level studies using MAAT are still scarce in marine acrochaetioid algae.

As part of an ongoing survey of epiphytic algae from the Brazilian coast, we isolated a small acrochaetioid red alga and analysed it using the plastid-encoded *rbc*L gene. Using molecular, morphological, and ultrastructural evidence, we describe this red alga as a new species of *Rhodachlya* and the first record of the genus in the Atlantic Ocean.

MATERIAL AND METHODS

Samples of rhodoliths were collected from 15 m depth manually by SCUBA diving in Pedra do Sal (2.5 km off the coast), Delta do Parnaíba, Piauí State, northeastern Brazil (02°50'S, 41°39'W), on 10 June 2016. Algal samples were transported to the Laboratory of Algal Culture 'Marilza Cordeiro Marino' of the Institute of Botany, São Paulo, Brazil. Algal samples were washed with sterilised seawater and cultured in medium composed of sterilised seawater (34 psu, pH 8.0) enriched with quarter strength Von Stosch's nutrient media (VSES/4; Oliveira *et al.* 1995), modified with a reduction of 50% in vitamin concentrations (Yokoya 2000). Germanium dioxide (GeO_2; 1 mg l^{-1}) was added to the culture medium to prevent diatom growth. Culture conditions were 23 ± 3 °C, 15–30 µmol photons m^{-2} s^{-1}, and a 14:10 h light:dark cycle, without aeration. Medium was renewed every two weeks. Before medium renewal, cultures were observed with a stereo dissecting microscope to detect the growth of crusts or filaments, which were isolated carefully and cultured in 'baby food' vessels with 50 ml of VSES/4 medium with GeO_2. Over three months, several algae were isolated, including filaments of an acrochaetioid alga. Three months after isolation, these filaments were cultured in medium composed of sterilised seawater enriched with half-strength Von Stosch's solution (VSES/2) without GeO_2; other culture conditions were as described above. To promote spore liberation, filaments were cultured in 50 × 75 mm crystallising dishes containing 80 ml of VSES/2 renewed every two weeks at 23 ± 3 °C, 17–19 µmol photons m^{-2} s^{-1}, and a 14:10 h light:dark cycle, with gentle agitation using an orbital shaker.

Morphological observations were performed from fresh cultured material. Drawings were made using a Leica DMLS camera lucida (Leica Microsystems, Wetzlar, Germany). Type and voucher specimens were deposited in the Herbarium of the Institute of Botany (SP; Thiers 2020).

Samples for transmission electron microscopy (TEM) were fixed in a solution containing 2% fresh formaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 24 h, rinsed three times for 30 min each in 0.1 M cacodylate buffer (pH 7.2), post-fixed for 1 h with 1% OsO_4 in 0.1 M cacodylate buffer plus 0.8% potassium ferrocyanide, rinsed twice in 0.1 M cacodylate buffer, and twice in deionised water, and, finally, dehydrated in an ethanol series, for 30 min in each solution, and three times in 100% ethanol. Samples were slowly infiltrated in epoxy resin (Poly/Bed®, Polysciences® Inc., Warrington, Pennsylvania, USA), embedded in silicon moulds and polymerised at 60 °C for 48 h. Ultrathin sections (70–100 nm) were obtained in a C7 Ultramicrotome (Leica Microsystems, Wetzlar, Germany), collected over 300# copper grids, and stained with lead citrate (Reynolds 1963) and uranyl acetate. Images were obtained with a transmission electron microscope (Morgagni, FEI Company, Hillsboro, Oregon, USA), operating at 80 KV.

Silica gel-dried samples were ground in a Precellys®24 tissue homogeniser (Bertin Instruments, Montigny-le-Bretonneux, France), and DNA was extracted using the NucleoSpin® Plant II Kit (Macherey-Nagel, Düren, Germany), following manufacturer's instructions. The plastid-encoded large subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase gene (*rbc*L) was amplified by PCR in three overlapping fragments using the primers F57-R753, F577-R1150, F993-RrbcS-start (Freshwater & Rueness 1994). PCR conditions were as described in Soares *et al.* (2019). Successful amplifications were purified using the Illustra™ GFX™ PCR DNA Purification Kit (GE Healthcare, Buckinghamshire, UK), according to manufacturer's protocols and commercially sequenced (Macrogen Inc., Seoul, Korea) with the amplification primers.

Bidirectional reads were manually assembled to generate consensus sequences using BioEdit v7.2.6.1 (Hall 1999). Two newly generated *rbc*L sequences from two isolates of the same rhodolith were deposited in GenBank. Additionally, 29 sequences of representatives of all orders of Nemaliophycidae were obtained from GenBank (Table S1).

Members of Sporolithales, *Heydrichia woelkerlingii* Townsend, Chamberlain & Keats, *Sporolithon durum* (Foslie) Townsend & Woelkerling, and *Sporolithon ptychoides* Heydrich were selected as outgroups. Genetic divergences were estimated using uncorrected *p*-distances in MEGA 7 (Kumar *et al.* 2016). Phylogenetic reconstructions were performed using maximum-likelihood (ML) and Bayesian inference (BI) analyses. PartitionFinder v2.1.1 (Lanfear *et al.* 2017) was used to determinate the best partitioning scheme for the *rbc*L dataset and the best-fit model for each codon position, under the corrected Akaike information criterion (AICc). Maximum-likelihood analysis was conducted using RAxML v8.2.10 (Stamatakis 2014) on the online server CIPRES Science Gateway v3.3 (Miller *et al.* 2010), using the GTR-gamma model with 1000 bootstrap (BS) replicates (Felsenstein 1985). Bayesian inference was carried out in MrBayes v3.2.6 (Ronquist *et al.* 2012), using the same partition scheme as the ML analysis, with two parallel runs of four chains of Markov Chain Monte Carlo (MCMC). Each run started with a random tree, using six million generations, sampling one tree every 1000 generations. The analytic parameters were determined to be sufficient when the average deviation of split frequencies was less than 0.01. The burn-in period was determined graphically by plotting the likelihood scores against generations to establish the stationary phase in a scatter (XY) plot. Trees before stationarity were discarded, and the remaining trees were used to construct the 50% majority-rule consensus tree to determine the Bayesian posterior probabilities (PP).

RESULTS

Molecular analyses

The final *rbc*L dataset had 1203 nucleotide positions, of which 542 bp were variable. None of the newly generated sequences matched any other taxon available in GenBank, and our two samples were identical. Phylogenetic analyses resulted in congruent topologies for ML and BI, and only the ML tree is presented (Fig. 1). *Rhodachlya* formed a fully supported clade, sister to the monotypic Corynodactylales, but without support. Our phylogeny resolved the isolate from Brazil as sister to *R. hawaiiana* in a well-supported clade (BS = 100, PP = 0.9), with an interspecific genetic distance of 5%. Sequence divergence between *R. madagascarensis* (generitype) and the Brazilian isolates was 10%.

Our molecular and morphological data show that the undescribed acrochaetioid species from Brazil is a member of the Rhodachlyales. In light of these results, we describe the entity as a new species of *Rhodachlya*. All morphological observations were made using the cultured holotype.

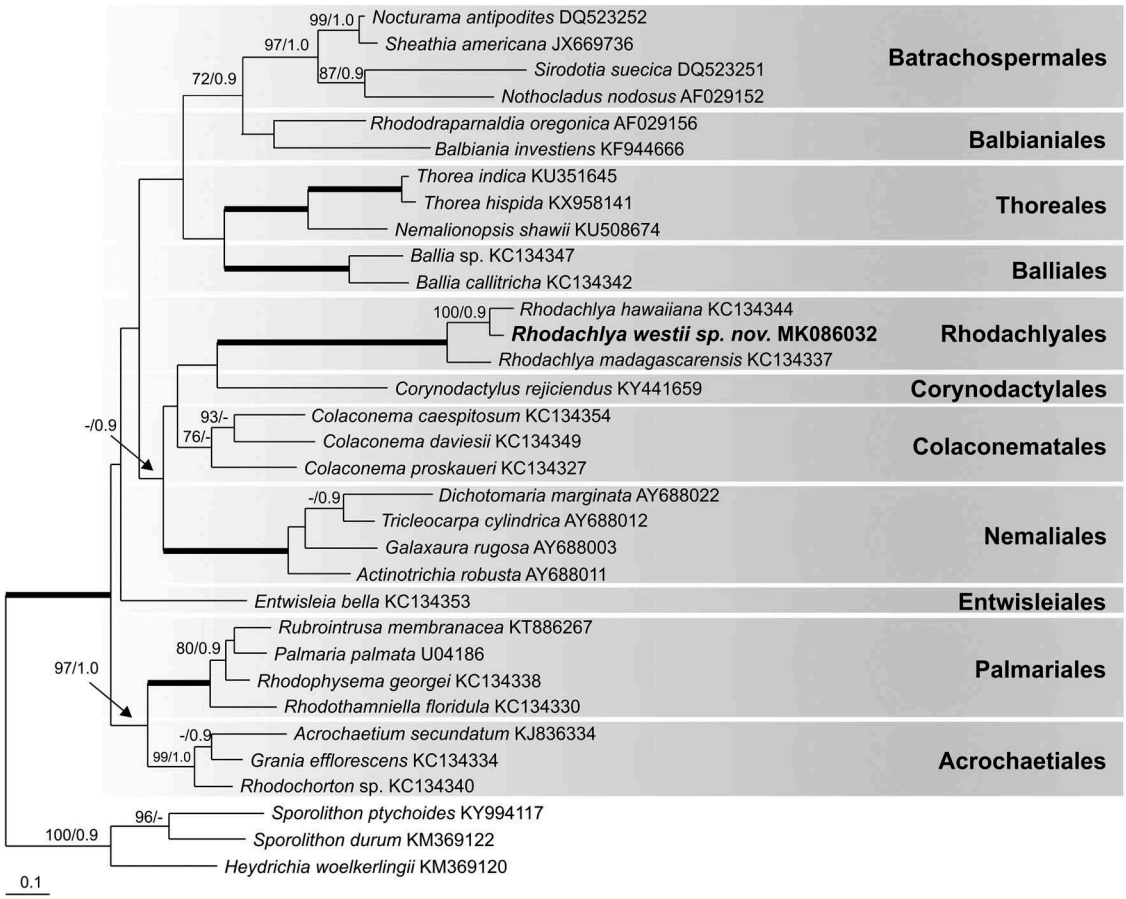


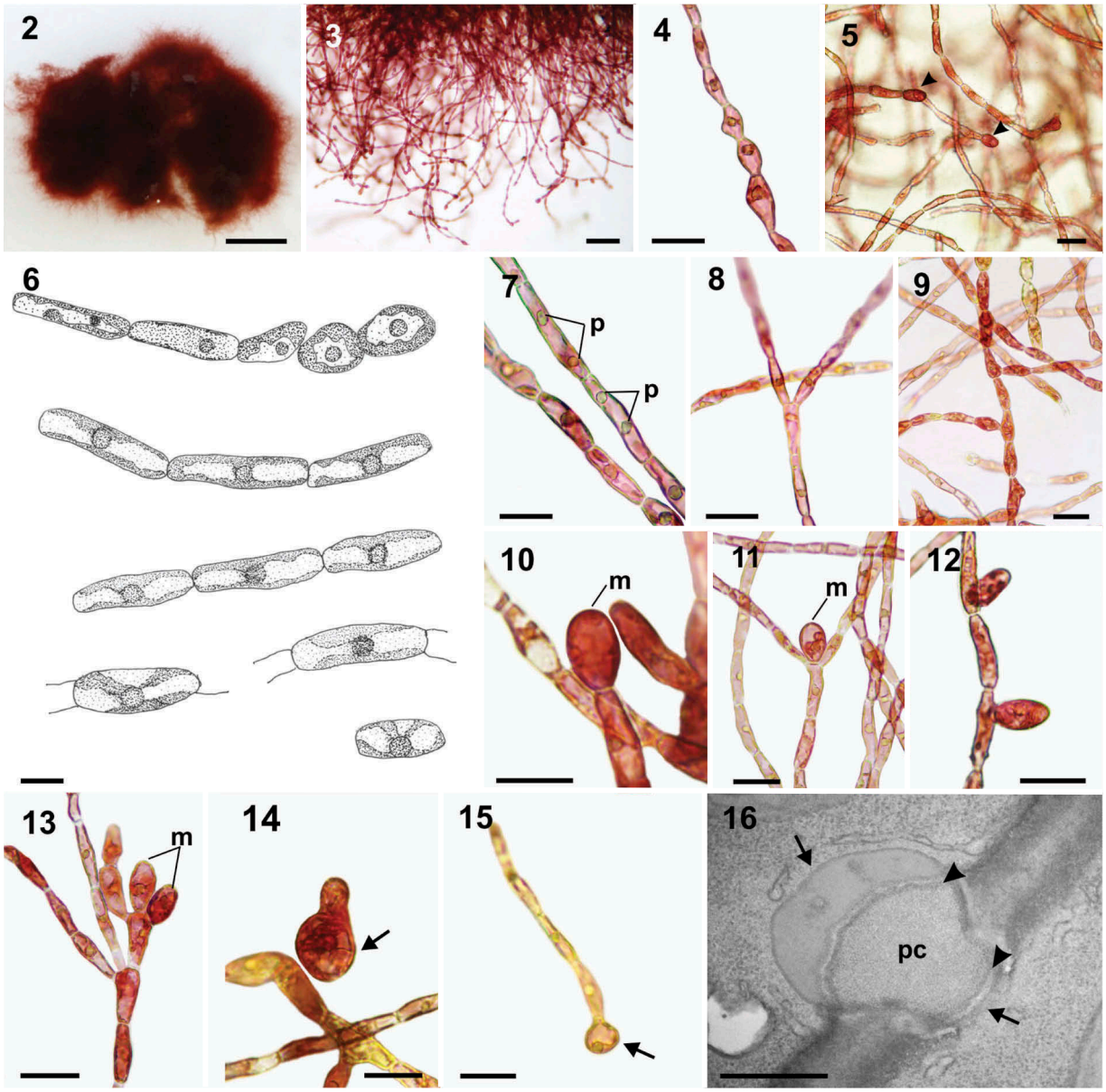
Fig. 1. ML phylogeny of Nemaliophycidae inferred from *rbc*L sequences. All orders currently included in Nemaliophycidae are represented. Values shown at the nodes indicate bootstrap support (BS) for ML and posterior probabilities (PP) for BI. Only values of BS ≥ 70% and PP ≥ 0.9 are shown; dashes (-) denote no support. The new species from this study is shown in bold. Thick bold branches correspond to full support in both analyses.

Rhodachlya westii L.P.Soares, N.S.Yokoya, S.M.Guimarães, Yonesh. & M.T.Fujii *sp. nov.*

Figs 2–16

DIAGNOSIS: Small marine red alga, forming dense red tufts in culture, with uniseriate, elongated, sparsely branched filaments. Intercalary vegetative cells elongated or irregular in outline. Each cell with a single parietal H-shaped or lobed plastid with one or two prominent pyrenoids. Hyaline hairs absent. Monosporangia spherical to ovoid, sessile, or on branched stalks.

HOLOTYPE: SP469734, deposited at the Maria Eneyda P. Kaufman Fidalgo Herbarium, Institute of Botany, São Paulo, Brazil, collected on 10 June 2016 by N.S. Yokoya. Culture isolate CCIBt4451, deposited in the Culture



Figs 2-16. Development in culture, and ultrastructure of *Rhodachlya westii sp. nov*. from Brazil (holotype SP469734).
Fig. 2. Habit of a tuft in culture. Scale bar = 0.5 cm.
Fig. 3. Higher magnification of basal and erect filaments. Scale bar = 100 µm.
Fig. 4. Cells of a basal filament; note some enlarged cells and single pyrenoids. Scale bar = 25 µm.
Fig. 5. Cells of erect filaments with more uniform dimensions. Note two young monosporangia (arrowheads). Scale bar = 25 µm.
Fig. 6. Schematic drawing of cells showing prominent pyrenoids and H-shaped, or lobed, parietal plastids; note a basal filament on top. Scale bar = 10 µm.
Fig. 7. Cells of erect filaments with two pyrenoids (p). Scale bar = 20 µm.
Fig. 8. Detail of an erect filament, showing dichotomous branching. Scale bar = 25 µm.
Fig. 9. Detail of an erect filament with branching at right angles. Scale bar = 25 µm.
Fig. 10. Terminal monosporangium (m). Scale bar = 25 µm.
Fig. 11. Monosporangium (m) in axil of branches. Scale bar = 25 µm.
Fig. 12. Lateral monosporangia in series. Scale bar = 25 µm.
Fig. 13. Monosporangia (m) in clusters. Scale bar = 25 µm.
Fig. 14. Spore germination with retention of protoplast (arrow). Scale bar = 25 µm.
Fig. 15. Young sporeling with original basal spore (arrow). Scale bar = 25 µm.
Fig. 16. TEM image of pit plug ultrastructure, showing plug core (pc), outer caps (arrows) and inner caps (arrowheads). Scale bar = 500 nm.

Collections of Algae, Fungi and Cyanobacteria, Institute of Botany, São Paulo, Brazil. *rbc*L sequence = GenBank accession MK086032.

ISOTYPE: SP469735, deposited at the Maria Eneyda P. Kaufman Fidalgo Herbarium, Institute of Botany, São Paulo, Brazil (*rbc*L sequence = GenBank accession MK086033).

TYPE LOCALITY: Delta do Parnaíba, Parnaíba, Piauí State, Brazil (02°50'S, 41°39'W), subtidal (15 m deep).

ETYMOLOGY: The species name '*westii*' honours Dr. John Arthur West from the University of Melbourne for his contributions to the knowledge of Rhodophyta, especially acrochaetioid algae isolated in culture.

Vegetative and reproductive morphology

The isolates formed floating, dense, red tufts up to 17 mm in diameter (Fig. 2), consisting of basal and erect filaments, that were uniseriate, randomly oriented, and sparsely branched (Fig. 3). Basal filaments were composed of irregularly enlarged and contorted cells that were barrel-shaped, reaching up to 25 µm long and 15 µm wide (Fig. 4). Cells of erect filaments were 15–38 µm long and 8–10 µm wide (Figs 5, 6). Each intercalary vegetative cell had an H-shaped, occasionally lobed, parietal chloroplast, with one or two prominent pyrenoids (Figs 6, 7). Branching was sparse, irregular to dichotomous (Fig. 8), or sometimes disposed at right angle (Fig. 9). Hyaline hairs were absent. Monosporangia were spherical to ovoid and sessile (Figs 10–12), or on branched stalks (Fig. 13). Monosporangia were solitary or occasionally in clusters of 2–3 (Fig. 13), terminal (Figs 10, 11) or lateral (Fig. 12), 18–25 µm long and 10–15 µm wide. After four weeks in culture, monospores, 13–15 µm in diameter, were released and germinated with retention of protoplast after germ tube formation (Fig. 14). Sporelings were also visible after this period (Fig. 15). Other reproductive structures were not observed. Pit plugs were composed of the plug core, inner and outer cap layers, without an intervening cap membrane; the outer cap layer was thickened on one side of the pit plug (Fig. 16).

DISCUSSION

Taxonomic challenges in acrochaetioid algae, owing to their simple vegetative and reproductive structures, call for the use of molecular-assisted alpha taxonomy to accurately assess species diversity (Cianciola *et al.* 2010; Pante *et al.* 2015). Our molecular findings, based on the *rbc*L gene, strongly support the uniqueness of the acrochaetioid isolates from Brazil at the species level. Molecular, morphological and ultrastructural data confirm the generic placement in *Rhodachlya*. *Rhodachlya westii* is the third species of the genus and the first record in the Atlantic Ocean. A comparison of vegetative and reproductive features of *R. westii* and the other two species of *Rhodachlya* is shown in Table 1. Morphologically, *R. westii* is similar to *R. hawaiiana*, but *rbc*L sequence divergence indicate that they are genetically distinct entities. Smaller dimensions of intercalary vegetative cells, occurrence of two pyrenoids, and the absence of unicellular hairs could separate *R. westii* from *R. hawaiiana*. However, these characters are highly variable and overlap (see Table 1), and we do not believe that they would be sufficiently stable to clearly distinguish them.

The reliability of morphological attributes traditionally used to delineate acrochaetioid algae was a long-standing debate (Garbary 1987; Woelkerling 1971, 1983). This is reflected in the several classification schemes for the acrochaetioid genera *Acrochaetium*, *Colaconema*, *Grania*, and *Rhodochorton* (for more detailed accounts see Stegenga 1979; Woelkerling 1983). Even in the most recent studies, morphological features were tentatively used for species delineation (Clayden & Saunders 2014; Kurihara *et al.* 2012; West *et al.* 2008). The genus *Rhodachlya* was proposed by West *et al.* (2008) and remained monotypic until Kurihara *et al.* (2012) described *R. hawaiiana*. Spore germination pattern, together with cell dimensions and rarity of colourless hairs, were used to distinguish *R. hawaiiana* from the generitype *R. madagascarensis* (Kurihara *et al.* 2012).

According to the scheme proposed by West (1972), the spore germination pattern of *R. westii* corresponds to Type II, which was also observed in *R. hawaiiana* (Kurihara *et al.* 2012). In Type II germination, the spore retains a protoplast after germ tube formation. The generitype *R. madagascarensis* presents a Type I germination pattern in which the entire content of the spore moves into the germ tube, and a transverse wall is formed. Woelkerling (1971, 1983) showed some evidence that spore germination is a character of little taxonomic importance in acrochaetioid algae. Furthermore, Clayden & Saunders (2008) observed that tetraspores and carpospores exhibit both germination types in the acrochaetioid species *Grania efflorescens*. Although the type of germination differs among *Rhodachlya* species, studies comparing the germination pattern are scarce and there is no evidence to consider it a reliable character.

It has been demonstrated that cell dimensions are influenced by several factors, such as salinity (White & Boney

Table 1. Comparison of diagnostic taxonomic characters for the three species of *Rhodachlya*.

	R. *westii sp. nov.*	*R. hawaiiana*	*R. madagascarensis*
Habit	on rhodolith	on *Gelidium* sp.	on *Posidonia*
Cell dimensions (µm)	8–10 × 15–38	8–18 × 25–50	7–9 × 24–29
Plastid	parietal, H-shaped or lobed	parietal, H-shaped or axial, lobed, stellate	parietal, H-shaped
Pyrenoid	1–2	1	1
Hairs	absent	infrequent, 2.5 µm wide	frequent, 4–5 × 300–500 µm
Monosporangia	solitary or in clusters, 10–15 × 18–25 µm	–	solitary or in clusters, 7–10 × 15–19 µm
Spore (µm)	13–15	10–20	9–11
Spore germination	Type II	Type II	Type I
Type locality	Piauí, Brazil	Hawaii Island, USA	Ifaty, Madagascar
Reference	this study	Kurihara *et al.* (2012)	West *et al.* (2008)

1969), temperature (Garbary 1979a), and host identity for endophytes (Garbary 1979b). However, Clayden & Saunders (2014) used cell size to help distinguish *Rhododrewia* from *Acrochaetium* species. Currently available data for *Rhodachlya* species show that vegetative and reproductive cell dimensions overlap (Table 1), but a statistical comparison could reveal significant differences among them. The reliability of colourless hairs is doubtful since there is evidence that hairs can develop with high light intensity, e.g. in species of *Acrochaetium* (Stegenga & Vroman 1976) and *Colaconema* (Stegenga & Borsje 1976; West 1972). Accordingly, the taxonomic value of colourless hairs, considered by Kurihara *et al.* (2012) in *R. hawaiiana*, is questionable.

With respect to plastid structure, Stegenga (1979) suggested that young cells, the second to sixth cell bellow the apical cell, provided the most representative morphology. Although plastid morphology can vary with culture conditions and with cell age (West 1970), the three species of *Rhodachlya* present a typical H-shaped parietal plastid. Therefore, plastid morphology and position seem consistent enough to establish generic boundaries in acrochaetioid algae, as previously suggested by Harper & Saunders (2002) and Saunders *et al.* (2018). Woelkerling (1983) pointed out that the occurrence and number of pyrenoids cannot be used to help delineate acrochaetioid genera, but seems more suitable for identifying species. This feature was considered diagnostic by Woelkerling (1971, 1973) in the *Audouinella* complex where acrochaetioid species were organised into three sections: pyrenoids absent, only one pyrenoid, more than one pyrenoid. However, Stegenga & Vroman (1976) showed that pyrenoids can be induced by high light intensity. According to their original descriptions, both *R. madagascarensis* and *R. hawaiiana* have only a single pyrenoid per cell (Kurihara *et al.* 2012; West *et al.* 2008); but cells with one or two prominent pyrenoids were observed in our isolates of *R. westii*.

The Nemaliophycidae include orders with cells possessing two cap-layered pit plugs (Le Gall & Saunders 2007; Pueschel & Cole 1982; Saunders & Bailey 1997; Saunders & Hommersand 2004). A distinctive character of the pit plug in Rhodachlyales is the absence of a cap membrane between the outer and inner caps (West *et al.* 2008), differing from other acrochaetioid groups (e.g. Acrochaetiales and Colaconematales). Pit plug morphology of *R. westii* is similar to *R. hawaiiana* and *R. madagascarensis* (Kurihara *et al.* 2012; West *et al.* 2008). The ultrastructural characterisation, along with molecular and morphological data, placed our isolates in *Rhodachlya*. In our preparations, the outer cap was thicker than the inner cap, as also observed by West *et al.* (2008) in *R. madagascarensis*. Pit plugs observed in *R. westii* were moderately electron-dense on one side, although this was possibly a fixation artefact (Pueschel & Cole 1982; West *et al.* 2008). Similarly, Pueschel & Cole (1982) found that some exceptional pit plugs of *Rhodophysema georgei* Batters (Palmariales, Nemaliophycidae) had one enlarged cap layer.

When the order Rhodachlyales and the genus *Rhodachlya* were proposed, West *et al.* (2008) used molecular data to recognise their isolate as a novel lineage, since they had morphology similar to *Acrochaetium* and *Colaconema* species. Similarly, other genera with simple morphology were revealed based on molecular evidence. In Acrochaetiales, *Grania* was resurrected to accommodate *A. efflorescens* (Clayden & Saunders 2008), *Rhododrewia* was segregated from an *Acrochaetium* species complex (Clayden & Saunders 2014), and *Ottia* J.R.Evans, M.L.Vis & G.W.Saunders was proposed for the freshwater *Audouinella meiospora* (Skuja) Garbary (Entwisle *et al.* 2018). As discussed above, it is evident that morphological features can be variable (e.g. cell dimensions, number of pyrenoids, and occurrence of colourless hairs) and thus have limited value in species-level taxonomy. We consider that *Rhodachlya* species can be accurately distinguished only based on DNA sequence data.

Since the morphological species concept has been historically used in macroalgae (Guiry 2012), this lack of morphological distinguishability raises the issue of species concepts for algal taxonomists. The influx of molecular data has revealed divergent evolutionary lineages with morphological stasis (Verbruggen 2014) and, currently, the idea of species as separately evolving lineages of metapopulations is largely accepted (De Queiroz 2007; Fišer *et al.* 2018; Mayden 1997). Species boundaries in macroalgae are difficult to assess mainly as a result of plastic morphologies and the absence of diagnostic characters (Cianciola *et al.* 2010; Saunders & Hommersand 2004). Verbruggen (2014) argued that one should not assume that because two individuals look alike, they are going to belong to the same species. As pointed out by Leliaert *et al.* (2014) and Verbruggen (2014), it could be that many algal species can be discovered only by DNA sequence data. Thus, relying on morphology alone often does not permit the detection of new species, leading to an increasing number of cryptic species described in diverse red algal groups (Díaz-Tapia *et al.* 2020; Jesus *et al.* 2019; Schneider *et al.* 2017; Soares *et al.* 2019; Zuccarello *et al.* 2018). Saunders *et al.* (2017b) is a good example of the extensive hidden diversity revealed by routine DNA-based surveys. They described 10 new genera and 10 new species in the family Kallymeniaceae, some of which were erected primarily by molecular analyses (e.g. the genus *Austrokallymenia* Huisman & G.W.Saunders).

Species delimitation based on DNA sequences usually relies on the comparison of nucleotide differences (divergence percentage) in different evolutionary lineages (Leliaert *et al.* 2014; Schneider *et al.* 2017). The plastid-encoded *rbc*L gene has been used in diverse red algal genera to separate morphologically similar entities (e.g. *Bangiopsis* Schmitz: West *et al.* 2014; *Gracilaria* Greville: Soares *et al.* 2018; *Galene* D'Archino & Zuccarello: Schneider *et al.* 2019) or even cryptic species (*Dasya* C.Agardh: Schneider *et al.* 2017; *Madagascaria* J.A.West & KiKuchi: Soares *et al.* 2019; *Lophurella* Schmitz: Díaz-Tapia *et al.* 2020). Ignoring cryptic or pseudo-cryptic species can lead to an underestimation of species diversity, critical for conservation policies, or even an overestimation of the geographical range of a species (Bickford *et al.* 2007; Chenuil *et al.* 2019). Molecular taxonomy has revealed that macroalgal genera considered to be of restricted distribution can have single species in different ocean basins. For example, in *Madagascaria*, a microscopic crustose genus that was restricted to Madagascar and Japan (Zuccarello *et al.* 2010),

a new species was recently proposed from Brazil (Soares *et al.* 2019). Moreover, species that were thought to be widespread based on morphology now have been shown to often have narrower distributions (Díaz-Tapia *et al.* 2018). Molecular evidence demonstrates how morphology-based identification is difficult and often fails to accurately resolve species in the Rhodophyta.

Within *Rhodachlya* and Rhodachlyales, currently data are based on a limited number of specimens, and therefore, can represent a limited perspective on generic diversity. But, even if a wider study were undertaken, the striking morphological simplicity would make it difficult to distinguish species. Whether the characters historically employed in species-level taxonomy are fully reliable will remain debatable until more taxa are studied in a molecular phylogenetic context. Combining an integrative approach and wide sampling, it is likely that *Rhodachlya* is more widespread. *Rhodachlya* remains overlooked due to its small size and resemblance to common acrochaetioid algae, such as *Acrochaetium* and *Colaconema*. The new species *R. westii* constitutes one more example of an overlooked and cryptic species being uncovered by routine DNA-based tools.

The sparse knowledge of acrochaetioid algae in Brazil prompted us to study this challenging algal group. Our data revealed a new species and the first record of the genus *Rhodachlya* in the Atlantic Ocean. Acrochaetioid algae are often ignored in floristic surveys due to their limited morphological differentiation. Thus, an integrated taxonomic approach is a more suitable tool for revealing previously overlooked diversity in this red algal group.

ACKNOWLEDGEMENTS

We would like to thank the National Center for Structural Biology and Bioimaging (CENABIO, UFRJ) and Dra. Marcia Attias, from the Institute of Biophysics (UFRJ), for assistance with electron microscopy. NSY, MTF and YYV thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the Productivity Fellowship (310672/2016-3, 304899/2017-8 and 304953/2014-8, respectively). We also thank Giuseppe Zuccarello and David Garbary for their valuable comments that greatly improved the manuscript.

FUNDING

This study was partially funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/AUXPE-CIMAR 1991/2014/23038.001431/2014-75) and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2016/50370-7).

ORCID

Luanda Pereira Soares http://orcid.org/0000-0002-4207-5022
Silvia M. P. de Beauclair Guimarães http://orcid.org/0000-0003-1066-9640
Mutue Toyota Fujii http://orcid.org/0000-0001-6752-1570
Yocie Yoneshigue-Valentin http://orcid.org/0000-0001-9539-0395
Maria Gardênia Sousa Batista http://orcid.org/0000-0001-8281-1277
Nair S. Yokoya http://orcid.org/0000-0001-8322-3178

REFERENCES

Bickford D., Lohman D.J., Sodhi N.S., Ng P.K.L., Meier R., Winker R., Ingram K.K. & Das I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution* 22: 148–155. DOI: 10.1016/j.tree.2006.11.004.

Chenuil A., Cahill A.E., Délémontey N., Du Luc E.D.S. & Fanton H. 2019. Problems and questions posed by cryptic species. A framework to guide future studies. In: *From assessing to conserving biodiversity. History, philosophy and theory of the life sciences*, Vol. 24, (Ed. by E. Casetta, J.M. Silva & D. Vecchi), pp. 77–106. Springer, Cham, Switzerland. 455 pp.

Choi H.G., Kraft G.T. & Saunders G.W. 2000. Nuclear small-subunit rDNA sequences from *Ballia* spp. (Rhodophyta): proposal of Balliales ord. nov., Balliaceae fam. nov., *Ballia nana* sp. nov. and *Inkyuleea* gen. nov. (Ceramiales). *Phycologia* 39: 272–287. DOI: 10.2216/i0031-8884-39-4-272.1.

Cianciola E.N., Popolizio T.R., Schneider C.W. & Lane C.E. 2010. Using molecular-assisted alpha taxonomy to better understand red algal biodiversity in Bermuda. *Diversity* 2: 946–958. DOI: 10.3390/d2060946.

Clayden S.L. & Saunders G.W. 2008. Resurrecting the red algal genus *Grania* within the order Acrochaetiales (Florideophyceae, Rhodophyta). *European Journal of Phycology* 43: 151–162. DOI: 10.1080/09670260801896630.

Clayden S.L. & Saunders G.W. 2014. A study of two *Acrochaetium* complexes in Canada with distinction of *Rhododrewia gen. nov.* (Acrochaetiales, Rhodophyta). *Phycologia* 53: 221–232. DOI: 10.2216/13-224.1.

De Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* 56: 879–886. DOI: 10.1080/10635150701701083.

Díaz-Tapia P., Maggs C.A., Macaya E. & Verbruggen H. 2018. Widely distributed red algae often represent hidden introductions, complexes of cryptic species or species with strong phylogeographic structure. *Journal of Phycology* 54: 829–839. DOI: 10.1111/jpy.12778.

Díaz-Tapia P., Maggs C.A., Nelson W., Macaya E.C. & Verbruggen H. 2020. Reassessment of the genus *Lophurella* (Rhodomelaceae, Rhodophyta) from Australia and New Zealand reveals four cryptic species. *European Journal of Phycology* 55: 113–128. DOI: 10.1080/09670262.2019.1659419.

Entwisle T.J., Evans J.R., Vis M.L. & Saunders G.W. 2018. *Ottia meiospora* (Ottiaceae, Rhodophyta), a new genus and family endophytic within the thallus of *Nothocladus* (Batrachospermales, Rhodophyta). *Journal of Phycology* 54: 79–84. DOI: 10.1111/jpy.12603.

Evans J.R., Amour N.S., Verbruggen H., Salomaki E.D. & Vis M.L. 2019. Chloroplast and mitochondrial genomes of *Balbiania investiens* (Balbianiales, Nemaliophycidae). *Phycologia* 58: 310–318. DOI: 10.1080/00318884.2019.1573349.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791. DOI: 10.1111/j.1558-5646.1985.tb00420.x.

Fišer C., Robinson C.T. & Malard F. 2018. Cryptic species as a window into the paradigm shift of the species concept. *Molecular Ecology* 27: 613–635. DOI: 10.1111/mec.14486.

Freshwater D.W. & Rueness J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) based on analysis of plastid *rbc*L nucleotide sequences. *Journal of Phycology* 31: 616–632. DOI: 10.1111/j.1529-8817.1995.tb02558.x.

Garbary D. 1979a. The effects of temperature on the growth and morphology of some *Audouinella* spp. (Acrochaetiaceae, Rhodophyta). *Botanica Marina* 22: 493–498. DOI: 10.1515/botm.1979.22.8.493.

Garbary D. 1979b. A revised concept for endophytic and endozoic members of the Acrochaetiaceae (Rhodophyta). *Botaniska Notiser* 132: 451–455.

Garbary D. 1987. The Acrochaetiaceae (Rhodophyta): an annotated bibliography. *Bibliotheca Phycologica* 77: 1–267.

Guiry M.D. 2012. How many species of algae are there? *Journal of Phycology* 48: 1057–1063. DOI: 10.1111/j.1529-8817.2012.01222.x.

Guiry M.D. & Guiry G.M. 2020. *AlgaeBase*. Worldwide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 20 March 2020.

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

Harper J.T. & Saunders G.W. 1998. A molecular systematic investigation of the Acrochaetiales (Florideophycidae, Rhodophyta) and related taxa based on nuclear small-subunit ribosomal DNA sequence data. *European Journal of Phycology* 33: 221–229. DOI: 10.1080/09670269810001736723.

Harper J.T. & Saunders G.W. 2002. A re-classification of the Acrochaetiales based on molecular and morphological data, and establishment of the Colaconematales, ord. nov. *European Journal of Phycology* 37: 463–475. DOI: 10.1017/S0967026202003840.

Jesus P.B., Costa A.L., Nunes J.M.C., Manghisi A., Genovese G., Morabito M. & Schnadelbach A.S. 2019. Species delimitation methods reveal cryptic diversity in the *Hypnea cornuta* complex (Cystocloniaceae, Rhodophyta). *European Journal of Phycology* 54: 135–153. DOI: 10.1080/09670262.2018.1522454.

Kumar S., Stecher G. & Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874. DOI: 10.1093/molbev/msw054.

Kurihara A., West J.A., Conklin K.Y. & Sherwood A.R. 2012. A second species of *Rhodachlya* (Rhodachlyales, Rhodophyta) from Hawaii, with a description of *R. hawaiiana* sp. nov. *Cryptogamie, Algologie* 33: 21–33. DOI: 10.7872/crya.v33.iss1.2011.021.

Lam D.W., Verbruggen H., Saunders G.W. & Vis M.L. 2016. Multigene phylogeny of the red algal subclass Nemaliophycidae. *Molecular Phylogenetics and Evolution* 94: 730–736. DOI: 10.1016/j.ympev.2015.10.015.

Lanfear R., Frandsen P.B., Wright A.M., Senfeld T. & Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772–773. DOI: 10.1093/molbev/msw260.

Le Gall L. & Saunders G.W. 2007. A nuclear phylogeny of the Florideophyceae (Rhodophyta) inferred from combined EF2, small subunit and large subunit ribosomal DNA: establishing the new red algal subclass Corallinophycidae. *Molecular Phylogenetics and Evolution* 43: 1118–1130. DOI: 10.1016/j.ympev.2006.11.012.

Leliaert F., Verbruggen H., Vanormelingen P., Steen F., López-Bautista J. M., Zuccarello G.C. & De Clerck O. 2014. DNA-based species delimitation in algae. *European Journal of Phycology* 49: 179–196. DOI: 10.1080/09670262.2014.904524.

Mayden R.L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In: *Species: the units of biodiversity* (Ed. by M.F. Claridge, H.A. Dawah & M.R. Wilson), pp. 381–424. Chapman & Hall, London, UK 442 pp.

Miller M.A., Pfeiffer W. & Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the 2010 Gateway Computing Environments Workshop (GCE)*, New Orleans. 8 pp.

Müller K.M., Sherwood A.R., Pueschel C.M., Gutell R.R. & Sheath R. G. 2002. A proposal for a new red algal order, the thoreales. *Journal of Phycology* 38(4): 807–820. DOI: 10.1046/j.1529-8817.2002.01055.x.

Necchi O., Jr. & Oliveira M.C. 2011. Phylogenetic affinities of "Chantransia" stages in members of the Batrachospermales and Thoreales (Rhodophyta). *Journal of Phycology* 47(3): 680–686. DOI: 10.1111/j.1529-8817.2011.00997.x.

Oliveira E.C., Paula E.J., Plastino E.M. & Petti R. 1995. Metodologias para o cultivo axênico de macroalgas marinas *in vitro*. In: *Manual de Métodos Ficológicos* (Ed. by K. Alveal, M.E. Ferrario, E.C. Oliveira & E. Sar), pp. 429–455. Universidad de Concepción, Concepción, Chile 863 pp.

Pante E., Schoelinck C. & Puillandre N. 2015. From integrative taxonomy to species description: one step beyond. *Systematic Biology* 64(1): 152–160. DOI: 10.1093/sysbio/syu083.

Pueschel C.M. & Cole K.M. 1982. Rhodophycean pit plugs: an ultrastructural survey with taxonomic implications. *American Journal of Botany* 69(5): 703–720. DOI: 10.1002/j.1537-2197.1982.tb13310.x.

Reynolds E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* 17(1): 208–212. DOI: 10.1083/jcb.17.1.208.

Ronquist F., Teslenko M., van der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M.A. & Huelsenbeck J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. DOI: 10.1093/sysbio/sys029.

Saunders G.W. & Bailey J.C. 1997. Phylogenesis of pit-plug-associated features in the Rhodophyta: inferences from molecular systematic data. *Canadian Journal of Botany* 75(9): 1436–1447. DOI: 10.1139/b97-858.

Saunders G.W., Bird C.J., Ragan M.A. & Rice E.L. 1995. Phylogenetic relationships of species of uncertain taxonomic position within the Acrochaetiales/Palmariales complex (Rhodophyta): inferences from phenotypic and 18S rDNA sequence data. *Journal of Phycology* 31: 601–611. DOI: 10.1111/j.1529-8817.1995.tb02556.x.

Saunders G.W. & Hommersand M.H. 2004. Assessing red algal supraordinal diversity and taxonomy in the context of contemporary systematic data. *American Journal of Botany* 91: 1494–1507. DOI: 10.3732/ajb.91.10.1494.

Saunders G.W., Huisman J.M., Vergés A., Kraft G.T. & Le Gall L. 2017b. Phylogenetic analyses support recognition of ten new genera, ten new species and 16 new combinations in the family Kallymeniaceae (Gigartinales, Rhodophyta). *Cryptogamie, Algologie* 38: 79–132. DOI: 10.7872/crya/v38.iss2.2017.79.

Saunders G.W., Jackson C. & Salomaki E.D. 2018. Phylogenetic analyses of transcriptome data resolve familial assignments for genera of the red-algal Acrochaetiales-Palmariales complex. *Molecular Phylogenetics and Evolution* 119: 151–159. DOI: 10.1016/j.ympev.2017.11.002.

Saunders G.W., Wadland K.L., Salomaki E.D. & Lane C.E. 2017a. A contaminant DNA barcode sequence reveals a new red algal order, Corynodactylales (Nemaliophycidae, Florideophyceae). *Botany* 95: 561–566. DOI: 10.1139/cjb-2017-0010.

Schneider C.W. 1983. The red algal genus *Audouinella* Bory (Nemaliales: Acrochaetiaceae) from North Carolina. *Smithsonian Contributions to the Marine Sciences* 22: 1–25. DOI: 10.5479/si.01960768.22.

Schneider C.W., Popolizio T.R., Kraft L.G.K. & Saunders G.W. 2019. New species of *Galene* and *Howella* gen. nov. (Halymeniaceae, Rhodophyta) from the mesophotic zone off Bermuda. *Phycologia* 58: 690–697. DOI: 10.1080/00318884.2019.1661158.

Schneider C.W., Quach P.K. & Lane C.E. 2017. A case for true morphological crypsis: Pacific *Dasya anastomosans* and Atlantic *D. cryptica sp. nov.* (Dasyaceae, Rhodophyta). *Phycologia* 56: 359–368. DOI: 10.2216/16-79.1.

Scott F.J., Saunders G. & Kraft G.T. 2013. *Entwisleia bella*, gen. et sp. nov., a novel marine 'batrachospermaceous' red alga from southeastern Tasmania representing a new family and order in the Nemaliophycidae. *European Journal of Phycology* 48: 398–410. DOI: 10.1080/09670262.2013.849359.

Sheath R.G. & Müller K.M. 1999. Systematic status and phylogenetic relationships of the freshwater genus *Balbiania* (Rhodophyta). *Journal of Phycology* 35: 855–864. DOI: 10.1046/j.1529-8817.1999.3540855.x.

Soares L.P., Guimarães S.M.P.B., Fujii M.T., Yoneshigue-Valentin Y., Batista M.G.S. & Yokoya N.S. 2019. Thallus ontogeny, morphology and molecular phylogeny of *Madagascaria atlantica sp. nov.* (Compsopogonophyceae, Rhodophyta), a diminutive crustose species uncovered in the Atlantic Ocean. *Aquatic Botany* 159: 1–6. DOI: 10.1016/j.aquabot.2019.103152.

Soares L.P., Gurgel C.F.D. & Fujii M.T. 2018. *Gracilaria suzanneae sp. nov.* (Gracilariales, Rhodophyta), a new flattened species from northeast Brazil based on morphological and molecular evidence. *Phycologia* 57: 345–353. DOI: 10.2216/17-102.1.

Stamatakis A. 2014. RaxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. DOI: 10.1093/bioinformatics/btu033.

Stegenga H. 1979. *Life histories and systematics of the Acrochaetiaceae*. Thesis. Vrije Universiteit Amsterdam, Amsterdam, Netherlands. 34 pp.

Stegenga H. & Borsje W.J. 1976. The morphology and life history of *Acrochaetium dasyae* Collins (Rhodophyta, Nemaliales). *Acta Botanica Neerlandica* 24: 15–29. DOI: 10.1111/j.1438-8677.1976.tb01049.x.

Stegenga H. & Vroman M. 1976. The morphology and life history of *Acrochaetium densum* (Drew) Papenfuss (Rhodophyta, Nemaliales). *Acta Botanica Neerlandica* 25: 257–280. DOI: 10.1111/j.1438-8677.1976.tb00239.x.

Thiers B. 2020. (continuously updated) *Index Herbariorum*: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/; searched on 20 March 2020.

Verbruggen H. 2014. Morphological complexity, plasticity, and species diagnosability in the application of old species names in DNA-based taxonomies. *Journal of Phycology* 50: 26–31. DOI: 10.1111/jpy.12155.

West J.A. 1970. The life history of *Rhodochorton concrescens* in culture. *British Phycological Journal* 5: 179–186. DOI: 10.1080/00071617000650241.

West J.A. 1972. Environmental control of hair and sporangial formation in the marine red alga *Acrochaetium proskaueri* sp. nov. *Proceedings of the International Seaweed Symposium* 7: 377–384.

West J.A., de Goër S.L. & Zuccarello G.C. 2014. A new species of *Bangiopsis: B. franklynottii* sp. nov. (Stylonematophyceae, Rhodophyta) from Australia and India and comments on the genus. *Algae* 29: 101–109.

West J.A., Scott J.L., West K.A., Karsten U., Clayden S.L. & Saunders G. W. 2008. *Rhodachlya madagascarensis gen. et sp. nov.*: a distinct acrochaetioid represents a new order and family (Rhodachlyales *ord. nov.*, Rhodachlyaceae *fam. nov.*) of the Florideophyceae (Rhodophyta). *Phycologia* 47: 203–212. DOI: 10.2216/07-72.1.

White E.B. & Boney A.D. 1969. Experiments with some endophytic and endozoic *Acrochaetium* species. *Journal of Experimental Marine Biology and Ecology* 3: 246–274. DOI: 10.1016/0022-0981(69)90050-1.

Woelkerling W.J. 1971. Morphology and taxonomy of the *Audouinella* complex (Rhodophyta) in southern Australia. *Australian Journal of Botany* 1: 1–91.

Woelkerling W.J. 1973. The morphology and systematics of the *Audouinella* complex (Acrochaetiaceae, Rhodophyta) in northeastern United States. *Rhodora* 75: 529–621.

Woelkerling W.J. 1983. The *Audouinella* (*Acrochaetium-Rhodochorton*) complex (Rhodophyta): present perspectives. *Phycologia* 22: 59–92. DOI: 10.2216/i0031-8884-22-1-59.1.

Yang E.C., Boo S.M., Bhattacharya D., Saunders G.W., Knoll A.H., Fredericq S., Graf L. & Yoon H.S. 2016. Divergence time estimates and the evolution of major lineages in the florideophyte red algae. *Scientific Reports* 6: 21361. DOI: 10.1038/srep21361.

Yokoya N.S. 2000. Apical callus formation and plant regeneration controlled by plant growth regulators on axenic cultures of the red alga *Gracilariopsis tenuifrons* (Gracilariales, Rhodophyta). *Phycological Research* 48: 133–142. DOI: 10.1111/j.1440-1835.2000.tb00208.x.

Yoon H.S., Zuccarello G.C. & Bhattacharya D. 2010. Evolutionary history and taxonomy of red algae. In: *Red algae in the genomic age. Cellular origin, life in extreme habitats and astrobiology*, Vol. 13, (Ed. by J. Seckbach & D. J. Chapman), pp. 27–42. Springer, Dordrecht, Netherlands. 500 pp.

Zuccarello G.C., Kikuchi N. & West J.A. 2010. Molecular phylogeny of the crustose Erythropeltidales (Compsopogonophyceae, Rhodophyta): new genera *Pseudoerythrocladia* and *Madagascaria* and the evolution of the upright erect. *Journal of Phycology* 46: 363–373. DOI: 10.1111/j.1529-8817.2010.00810.x.

Zuccarello G.C., West J.A. & Kamiya M. 2018. Non-monophyly of *Bostrychia simpliciuscula* (Ceramiales, Rhodophyta): multiple species with very similar morphologies, a revised taxonomy of cryptic species. *Phycological Research* 66: 100–107. DOI: 10.1111/pre.12207.

Zucchi M.R. & Necchi O., Jr. 2003. Blue-greenish acrochaetioid algae in freshwater habitats are "Chantransia" stages of Batrachospermales *sensu lato* (Rhodophyta). *Cryptogamie, Algologie* 24: 117–131.