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A new species of *Pyropia* (Rhodophyta, Bangiaceae), from the Pacific coast of Mexico, based on morphological and molecular evidence

LUZ ELENA MATEO-CID[1]*, ANGELA CATALINA MENDOZA-GONZÁLEZ[1], JHOANA DÍAZ-LARREA[2], ABEL SENTÍES[2], FRANCISCO F. PEDROCHE[3] & JUAN DIEGO SÁNCHEZ HEREDIA[4]

[1] *Departamento de Botánica, Escuela Nacional de Ciencias Biológicas, IPN. Carpio y Plan de Ayala s/n. Mexico, D.F. 11340.*
[2] *Departamento de Hidrobiología. Universidad Autónoma Metropolitana-Iztapalapa. A.P. 55-535, Mexico, D.F. 09340, Mexico.*
[3] *Departamento de Ciencias Ambientales. Universidad Autónoma Metropolitana-Lerma, Mexico.*
[4] *Facultad de Biología. Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, Mexico.*

* *Corresponding author: E-mail: lmateoc@ipn.mx*

Abstract

Pyropia raulaguilarii sp. nov. is described from Michoacán, tropical Mexican Pacific, on basis of comparative morphology and nrSSU, *rbc*L sequence analysis. It is distinguished from other *Pyropia* species by the foliose and lanceolate gametophyte, a monoecious thallus and the zygotosporangia in packets of 2x2x4. The phylogenetic analysis showed that the two Pacific Mexican samples, from Caletilla and Carrizalillo (Michoacán), were almost identical and formed a distinctive and well supported clade segregated from other species of *Pyropia* from Brazil, USA and Mexico. The Mexican entity is morphologically and genetically distinct from other *Pyropia* species, suggesting that this species should be assigned to a new taxon.

Key words: Bangiales, molecular phylogeny, nrSSU, *rbc*L, marine red algae.

Introduction

Species of *Porphyra* C.Agardh have few characters for distinguishing species, however, these characters alone have proved to be misleading based on the discovery, using molecular sequences, of many cryptic taxa among species with very similar morphologies (e.g. Brodie & Irvine 1997, Broom *et al.* 2002, 2004, Neefus *et al.* 2002, Teasdale *et al.* 2002, Lindstrom & Fredericq 2003, Niwa *et al.* 2005, Brodie *et al.* 2007, Lindstrom 2008). Almost all of these studies have used either nrSSU or plastid *rbc*L gene. The use of the more conservative nrSSU locus has generally resulted in phylogenies with a well-supported ‘‘backbone’’ but little differentiation of closely related taxa. The protein-coding *rbc*L gene clearly distinguishes species, but support for ancient divergences is often less than the one obtained in analyses based on the nrSSU gene (Lindstrom & Fredericq 2003, Nelson *et al.* 2006).

“*Porphyra* sensu lato” as the cited papers include species no longer considered part of *Porphyra* (sensu stricto) (Sutherland *et al.* 2011). Molecular studies have revealed extensive cryptic speciation within the genus *Porphyra* (Broom *et al.* 1999, 2004, 2010, Jones *et al.* 2004) and has been used in the genetics (Kuang *et al.* 1998) and breeding of *Porphyra* species since the end of the twentieth century (Jia *et al.* 2000, Xu *et al.* 2011). Most previous reports were focused on the identification, protection, construction, and classification of different strains (Iitsuka *et al.* 2002, Kunimoto *et al.* 2003, Liu *et al.* 2004, Sun *et al.* 2005, Weng *et al.* 2005, Qiao *et al.* 2007).

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Recently, Sutherland *et al.* (2011) carried out an important revision that constitutes a major change in understanding relationships and evolution in Bangiales, and combined analyses of the nuclear SSU rRNA and chloroplast *rbc*L regions recognizing five new genera and two others that were resurrected. The genus *Porphyra* is now restricted to five described species and a number of undescribed species. Other foliose taxa previously placed in *Porphyra* are now recognized to belong to the genera *Boreophyllum* S.C.Lindstr. *et al.*, *Clymene* W.A.Nelson, *Fuscifolium* S.C.Lindstr., *Lysithea* W.A.Nelson, *Miuraea* Kukuchi *et al.*, *Pyropia* J.Agardh and *Wildemania* De Toni.

The genus *Pyropia* is characterized by a) gametophytic thalli monostromatic, linear, ovate, orbicular or funnel shaped, b) margins entire or dentate, planar, undulate, or ruffled, c) color variable, d) blades sessile or with brief stipe, e) vegetative cells embedded in colorless, firm gelatinous matrix, f) cells with one or two stellate chloroplasts, each one with central pyrenoid, g) plants monoecius or dioecius, h) sexual thalli monoecious with groups of cells forming either spermatangia and zygotosporangia, these often in streaks or rectangular patches, or divided into separate male and female sectors by a vertical or horizontal line, i) in dioecious thalli, the spermatangia and zygotosporangia forming as continuous areas along the margins of the blade, and j) the conchocelis phase is a microscopic and filamentous stage (Sutherland *et al.* 2011).

During recent research on the diversity of benthic marine algae of the state of Michoacán, tropical Pacific Mexico coast, an undescribed red alga was collected. This entity is characterized by thalli with monostromatic blades, arising from a single discoid attachment, and sexual thalli monoecious with spermatangia and zygotosporangia in patches. This internal organization corresponds to the genus *Pyropia*.

Materials & Methods

Morphological analysis. Thalli of *Pyropia* were observed at two localities in southern Michoacán: Caletilla and El Carrizalillo. Slides of fertile specimens were prepared for microscopic observation from material preserved in 5% formalin-seawater. Whole mount slides with cross and transverse sections were made by hand with razor blades, these sections were stained in 1% aqueous aniline blue, acidified by the addition of 1N HCl, according to Mateo-Cid *et al.* (2005) and permanently mounted on microscope slides with 70% Karo Syrup® with 1% phenol added as a preservative. Photomicrographs were taken with a Sony MPEGMOVIEVX (Tokyo, Japan) coupled to an Olympus CX31 Microscope (Manila, Filipinas). Images of specimens were taken with a digital SONY camera (MPEGMOVIEVX). Digital images were edited and assembled on plates using Adobe Photoshop 7.0 (San Jose, CA, USA).

Molecular analysis. Samples used for molecular analysis were dried in silica gel. Total DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. Sequences of the nrSSU and the *rbc*L genes from Bangiales taxa were obtained. The specimens sequenced in this study, plus other available sequences used for the phylogenetic analysis, are shown in Table 1, including their accession numbers in the National Center for Biotechnology Information (NCBI) GenBank data base.

The *rbc*L and nrSSU regions were amplified with primers suggested by Freshwater & Rueness (1994) and Saunders *et al.* (1996), respectively, by using the Taq PCR Core Kit (Quiagen). All polymerase chain reaction (PCR) products were electrophoresed on 1% agarose gel to verify product size. PCR products were purified with Qiagen QIAquick Purification Kit following the manufacturer's instructions. Sequencing was carried out using PCR primers with the BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, NJ, USA) on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The authors performed the nucleotide BLAST (Basic Local Alignment Search Tool) of the sequences with the program implemented in the NCBI (National Center for Biotechnology Information) home page. Sequences editing was performed with the Sequence Navigator (Applied Biosystems) and aligned according to the CLUSTAL algorithm (Thompson *et al.* 1994).

Phylogenetic analysis. Three data sets were constructed -nrSSU data set, the *rbc*L data set, and a combined data set- with the genes concatenated. Outgroup species (*Dione arcuata* W.A.Nelson, *Minerva aenigmata* W.A.Nelson and *Miuraea migitae* (N. Kikuchi *et al.*) N. Kikuchi *et al.* were selected because of a close phylogenetic relationship with the ingroup (Sutherland *et al.* 2011).

Phylogenetic relationships were inferred with PAUP* 4.0b10 (Swofford 2002) and MrBayes v.3.0 beta 4 (Huelsenbeck & Ronquist 2001). Maximum-parsimony trees (MP) were constructed using the heuristic search option, tree-bisection-reconnection branch swapping algorithm, unordered and unweight characters, and gaps treated as missing data. Furthermore, distance analysis was conducted under Neighbour-Joining (NJ) algorithm.

Support values for the relationships discovered in analyses were calculated by performing bootstrap analyses (Felsenstein 1985) as implemented in PAUP*. Twenty thousand heuristic search replicates were executed using the TBR branch-swapping algorithm. Consistency (CI) and Homoplasy (HI) indexes were computed to evaluate the level of homoplasy in the most parsimonious tree.

Bayesian trees were constructed for both the single gene data sets and the concatenated data set. The model used was the general-time-reversible model of nucleotide substitution with invariant sites and gamma distributed rates for the variable sites (GTR + I + G) for all three data sets. This model was selected based on maximum likelihood ratio tests implemented by the software Model Test version 3.06 (Posada & Crandall 1998) with a significance level of 0.01. Analyses were started from random trees, and consisted of two runs, each of four chains (one hot and three cold), of 4 million generations for each data set. The stationary level was reached at generation 17000. Therefore, trees saved until generation 16500 were the ‘burn in’ of the chain, and inferences about the phylogeny were based on those trees sampled after generation 16500. To visualize the burn-in of -LnL or model parameters, we use the software Tracer v1.4 (Rambaut & Drummond 2007). A 50% consensus tree as implemented by PAUP* was computed after the ‘burnin’. The range of *rbc*L divergence values within and among species was calculated using uncorrected ‘*p*’ distances using PAUP*.

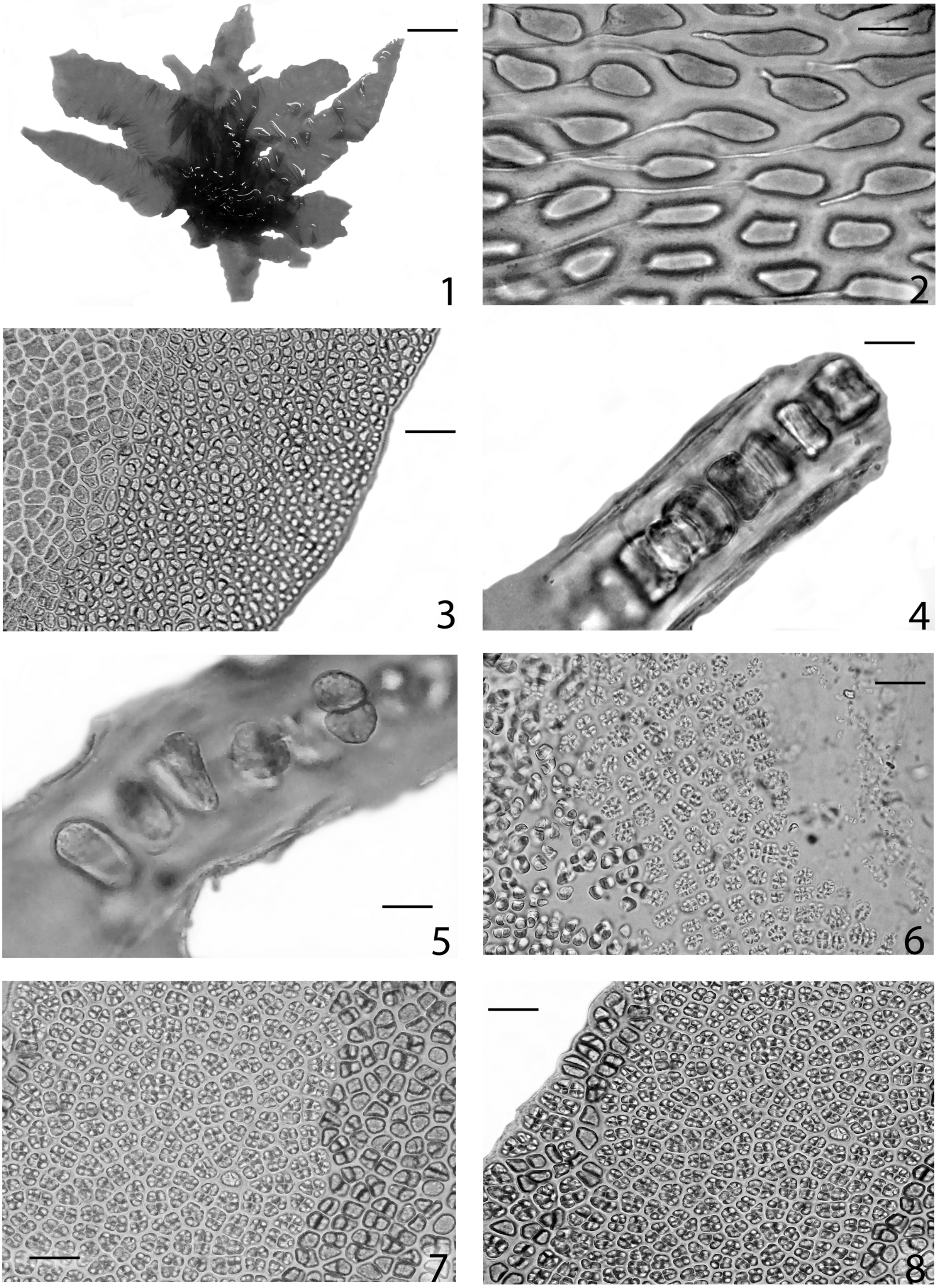
Results

Pyropia raulaguilarii Mateo-Cid, Mendoza-González & Sentíes, *sp. nov.* (Figs 1–8, Table 2)

Gametophytum foliosum, monostromaticum, lanceolatum, basis umbilicalis, margo crispata. Thalli 4 ad 6 cm, adhaerentes perfecte ad papyrum. Thalli steriles usque 40–42 µm crassi. Color rubrum ad aliquando purpureus. Thalli sexuales monoecii spermatangia in viridi inaequalis intermixtam zygotosporangia in rectangulum vel irregularis inaequaliter. Sectiones fertiles (feminei) 50–55 µm crassi. Structurae reproductivae marginales, maculis extensiae intro. Spermatangia in sarcinula 2 × 2 × 4 × 4. Zygotosporangia in sarcinula 2 × 2 × 4.

Type:—MEXICO. Michoacán: Carrizalillo, low deciduous forest, 18°36’00” N, 102°33’03” W , 0 m a.s.l., coll. Mendoza-González and Mateo-Cid, 04-04-2009, *MICH-09-A/01* (holotype: ENCB!, isotype: UAMIZ!, paratype: Michoacán: Caletilla, 18°03’28” N, 102°42’13” W, coll. Mendoza González and Sánchez Heredia, 17-05-2006, *MICH-06-30/01*, ENCB!).

Gametophyte foliose, monostromatic, lanceolate, rarely branched (Fig. 1), often deeply ruffled, margin undulating (Fig. 3). Thalli 4–6 cm high, blades 1–2 cm diameter, red to purple color. Adhering completely to paper. The plants have a well-developed umbilicate base, which is closely adherent to the substratum and attached by rhizoidal cells (Fig. 2). Sterile thalli 40–42 µm thick (Fig. 4), vegetative cells measuring 14–17 µm in diameter and 20–25 µm in length. Vegetative cells with a single stellate plastid with one pyrenoid. Reproductive plants: sexual thalli monoecious, spermatangia in green patches intermixed with zygotosporangia in rectangular or irregular patches in fertile regions of the blades. Fertile female sectors 50–55 µm thick (Fig. 5). Reproductive structures marginal, extending inward in alternating patches. Submarginal tissue paler than the adjacent outer zygotosporangial and inner sterile tissues. Zygotosporangia in packets of 2 × 2 × 4 (Figs 7, 8). Spermatangia in packets of 2 × 2 × 4 × 4 (Fig. 6).



FIGURES 1–8: *Pyropia raulaguilarii sp. nov.* Fig. 1: Habit of the foliose gametophyte. Holotype. El Carrizalillo (ENCB 19 603). Fig. 2: Rhizoidal cells in surface view. Fig. 3: Margin of thallus in surface view. Fig. 4: Transverse section through thallus showing monostromatic blade. Fig. 5: Transverse section of female portion. Fig. 6: Mature spermatangia in surface view. Fig. 7: Surface view of zygotosporangial region of thallus. Fig. 8: Mature marginal zygotosporangias in surface view. Scale bars: Fig. 1: 12 mm; Figs 2, 6: 30 µm; Figs 3, 7, 8: 40 µm; Fig. 4: 15 µm; Fig. 5: 20 µm.

Habitat: —On rocks, intertidal pools.

Etymology: —The species epithet is named in honor of Prof. Raúl Aguilar Rosas, a prominent Mexican phycologist and beloved colleague from Facultad de Ciencias Marinas, Universidad Autónoma de Baja California, Mexico.

Molecular analyses: —Samples of *Pyropia raulaguilarii* from Carrizalillo and Caletilla (Michoacán, Mexico) diverged each other 0.02–0.03%. These samples diverged 3.0 to 3.4% from the GenBank sequence called *Pyropia cf. thuretii* from Mazatlán, Mexico (Table 1). The concatenated phylogenetic data set consisted of 62 taxa (Table 1), including 59 ingroup and three outgroup taxa, with 2767 characters: 1300 from the nrSSU and 1467 from the *rbc*L gene. Bayesian, parsimony (not shown) and distance (not shown) analyses resulted in similar trees. The topology of the Bayesian tree showed eight lineage corresponding to representatives of the following Bangiales genera: *Bangia* Lyngbye, *Boreophyllum, Clymene, Fuscifolium, Lysithea, Porphyra* C.Agardh, *Pyropia* and *Wildemania* (Fig. 9). The Pacific Mexican samples processed in the present study were located in two well-supported terminal *Pyropia* sister clades. The samples collected in Carrizalillo and Caletilla (Michoacán) formed a high supported monophyletic clade with a sister clade called *Pyropia cf. thuretii* from Mazatlán, Mexico (Table 1).

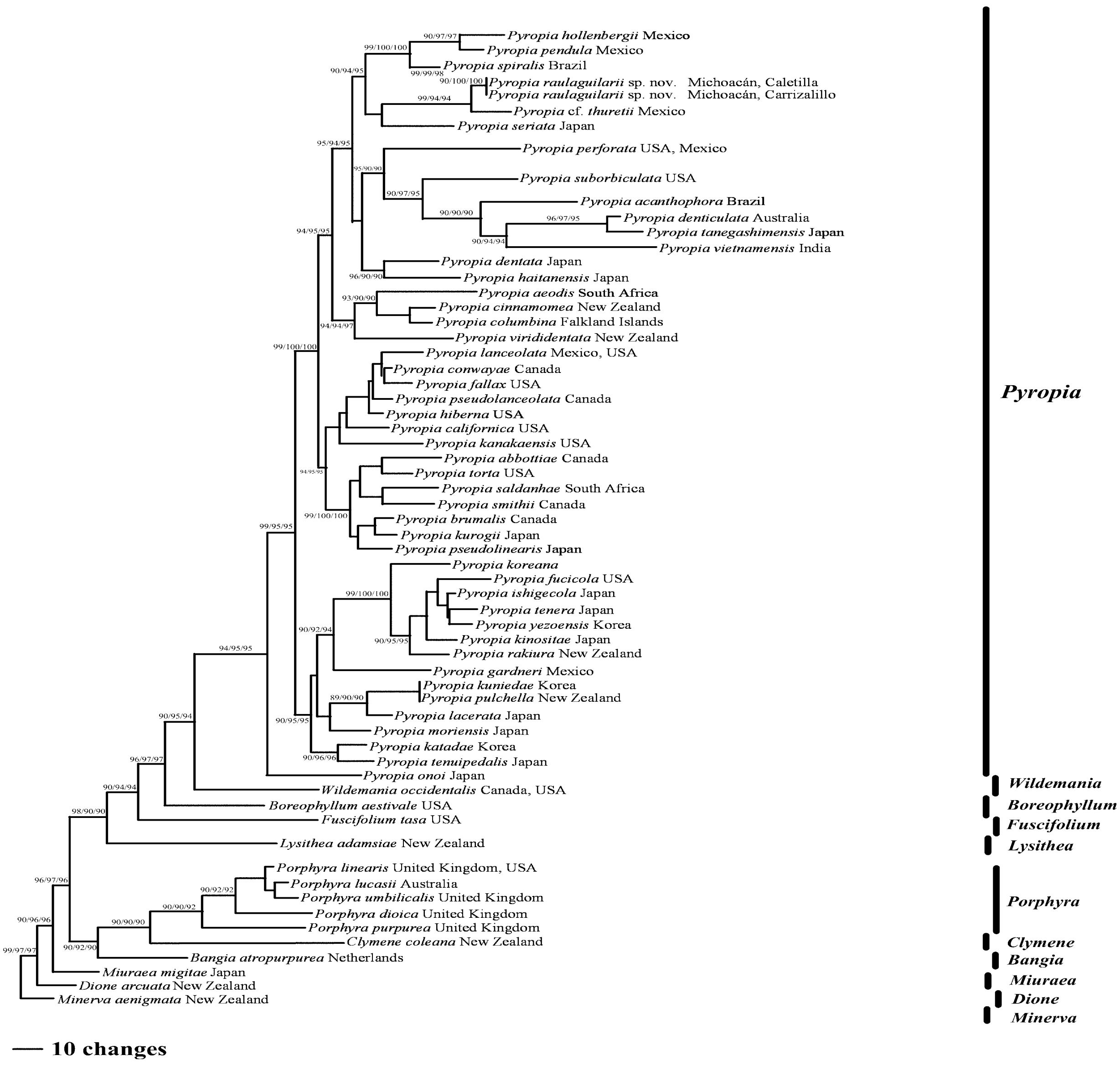


FIGURE 9: Bayesian result for concatenated nuclear SSU ribosomal RNA (nrSSU) and *rbc*L data set. Support values are Bayesian posterior probabilities, and parsimony and distance bootstrap values, respectively. Some internal support values are omitted for clarity.

TABLE 1: Specimen collection information, voucher numbers and GenBank accession numbers of sequences used in phylogenetic analysis.

Taxon	Collection data	GenBank Accession Numbers	
		nrSSU	*rbc*L
Bangia atropurpurea (Roth) C.Agardh	Netherlands, Ysselmeer, Enschede.	AF169341	AF169330
Boreophyllum aestivale (S.C. Lindstr. & Fredericq) S.C.Lindstr.	USA, Alaska, Amaknak Island, Captains Bay, 4 August 2004, UBC A86208 (nrSSU); SCL 1325 (*rbc*L).	GU319836	EU223033
Clymene coleana (W.A.Nelson) W.A.Nelson	New Zealand, North I, Leigh, WELT A22181 (nrSSU); WELT A22181 (*rbc*L).	AF136423	FJ263672
Dione arcuata [b]	New Zealand, South I, Kaikoura, Ohau Stream, WELT A23126 (nrSSU); WELT A23126 (*rbc*L).	AY465354	EU570052
Fuscifolium tasa (Yendo) S.C.Lindstr.	USA, Alaska, Unalaska Island, Spray Cape, 4 June 2005, UBC A86511 (nrSSU); SCL 12178 (*rbc*L).	GU319862	EU223226
Lysithea adamsiae (W.A.Nelson) W.A.Nelson	New Zealand, Antipodes Islands, Orde Lees I, WELT A023233 (nrSSU); WELT A023233 (*rbc*L).	HQ687566	HQ687515
Minerva aenigmata W.A. Nelson [b]	New Zealand, South Island, Westland, Cobden (nrSSU); New Zealand, North I, Puheke, WELT A25775 (*rbc*L).	AY184347	EU570053
Miuraea migitae [b]	Japan, Osaka, Osaka Bay.	EU521642	EU521643
Porphyra dioica J.Brodie & L.M.Irvine	United Kingdom, Sidmouth, WELT A024417 (nrSSU); WELT A024417 (*rbc*L).	HQ687579	HQ687546
Porphyra linearis Grev.	United Kingdom, Aberystwyth, BM000898797 (nrSSU); USA, ME, Kittery, Seapoint, NHA 61204 (*rbc*L).	HQ687580	AF078745
Porphyra lucasii Levring	Australia, WA, Trigg Beach, WELT A23051 (nrSSU); WELT-A23051 (*rbc*L).	AY139685	AY139687
Porphyra purpurea (Roth) C.Agardh	United Kingdom, Litstock, Somerset, BM000898974 (nrSSU); BM000898974 (*rbc*L).	HQ687567	HQ687516
Porphyra umbilicalis Kütz.	United Kingdom, Sidmouth, WELT A024414 (nrSSU); WELT A024414 (*rbc*L).	HQ687584	HQ687559
Pyropia acanthophora (E.C.Oliveira & Coll) M.C.Oliveira *et al.*	Brazil, São Paulo, Ubatuba.	L26197	HQ605695
Pyropia abbottiae (Krishnam) S.C.Lindstr.	Canada, BC, Victoria, Harling Point, 25 April 2005, UBC A85149 (nrSSU); SCL 12567 (*rbc*L).	GU319835	EU223024
Pyropia aeodis (N.J.Griffin *et al.*) J.E. Sutherland	South Africa, Paternoster.	AY292624	GU165843
Pyropia brumalis (Mumford) S.C. Lindstr.	Canada, BC, Vancouver, Stanley Park, 23 February 1998, UBC A84426 (nrSSU); UBC A84426 (*rbc*L).	GU319837	EU223038
Pyropia californica J.Agardh	USA, AK, Northeast Ushagat Island, 25 August 2006, UBC A86168 (nrSSU); SCL 13101 (*rbc*L).	GU319849	EU223117
Pyropia cf. thuretii	Mexico, Sinaloa, Mazatlán, Olas Altas, WELT A024423 (nrSSU); WELT A024423 (*rbc*L).	HQ687587	HQ687519
Pyropia cinnamomea (W.A.Nelson) W.A.Nelson	New Zealand, South I, Otago, Bruce's Rock.	AH008010	EU521637

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TABLE 1 (continued)

Taxon	Collection data	GenBank Accession Numbers	
Pyropia columbina (Mont.) W.A. Nelson	Falkland Islands, East Falkland, Top Island, Port Stanley, February 2001, WELT A023553 (nrSSU); WELT A023553 (*rbc*L).	GU046398	GU046423
Pyropia conwayae (S.C.Lindstr. & K.M.Cole) S.C.Lindstr.	Canada, BC, French Beach, 12 March 2007, UBC A86515 (nrSSU); SCL 13109 (*rbc*L).	GU319838	EU223045
Pyropia dentata (Kjellm.) N.Kikuchi & M.Miyata	Japan, Chiba, Shirahama, WELT A024400 (nrSSU); WELT A024400 (*rbc*L).	HQ687588	HQ687520
Pyropia denticulata (Levring) J.A.Phillips & J.E.Sutherland.	Australia, Queensland, Mooloolaba, WELT A023224 (nrSSU); WELT A023224 (*rbc*L).	HQ687570	HQ687521
Pyropia fallax (S.C.Lindstr. & K.M.Cole) S.C. Lindstr.	USA, Alaska, Avatanak Island, 12 June 2008, UBC A87380 (nrSSU); UBC A87380 (*rbc*L).	GU319840	GU319865
Pyropia fucicola (Krishnam. S.C.Lindstr.	USA, Alaska, Japonski Island, Sitka airport, SW side, 29 end of runway, 19 August 2005, UBC A87689 (nrSSU); SCL 12715 (*rbc*L).	GU319841	EU223088
Pyropia gardneri	Mexico, Baja California, Caminitos, Cabo Punta Banda, CMMEX 4355 (nrSSU); WELT A024420 (*rbc*L).	DQ084423	HQ687522
Pyropia haitanensis	Japan, Ehime, Yuge.	AB013181	AB118585
Pyropia hiberna (S.C.Lindstr. & K.M.Cole) S.C.Lindstr.	USA, CA, Monterey County, Pacific Grove, 31 December 2007, UBC A87474 (nrSSU); UBC A87474 (*rbc*L).	GU319843	GU319866
Pyropia hollenbergii	Mexico, Baja California Sur, Bahía Agua Verde, WELT A023231 (nrSSU).	HQ687589	AY794401
Pyropia ishigecola (A.Miura) N.Kikuchi & M.Miyata	Japan, Chiba, Katsuura, Yoshio, WELT A023199 (nrSSU); WELT A023199 (*rbc*L).	HQ687571	HQ687524
Pyropia kanakaensis (Mumford) S.C.Lindstr.	USA, WA, Olympic Peninsula, between Makah Bay and Sekui, 31 May 2003, UBC A86491 (nrSSU); SCL 10932 (*rbc*L).	GU319844	EU223099
Pyropia katadae (A. Miura) M.S. Hwang *et al.*	Korea, Gyeongsangbukdo, Pohang.	HQ728191	HQ728199
Pyropia koreana (M.S.Hwang & I.K.Lee) M.S.Hwang *et al.*	-	HQ728190	HQ728198
Pyropia kinositae (Yamada & Tak.Tanaka) N.Kikuchi *et al.*	Japan, Hokkaido, Suttu, Arito.	EU521640	EU521641
Pyropia kuniedae (Kurogi) M.S.Hwang & H.G.Choi	Korea, Sachon, Gyeongsangnamdo, Namhae.	HQ728192	HQ728200
Pyropia kurogii (S.C.Lindstr.) S.C.Lindstr.	Japan, Hokkaido, Utoro, WELT A023201 (nrSSU); WELT A023201 (*rbc*L).	HQ687573	HQ687526
Pyropia lacerata (A.Miura) N.Kikuchi & M.Miyata	Japan, Chiba, Shirahama, WELT A024399 (nrSSU); WELT A024399 (*rbc*L).	HQ687574	HQ687527
Pyropia lanceolata	Mexico, Baja California, Punta Popotla (nrSSU); USA, California, Monterey County, Spanish Bay, 1 January 2008, UBC A87475 (*rbc*L).	AY909594	GU319867
Pyropia leucosticta	United Kingdom, Sidmouth, BM000898751 (nrSSU).	HQ687593	DQ191359

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TABLE 1 (continued)

Taxon	Collection data	GenBank Accession Numbers	
Pyropia moriensis (Ohmi) N.Kikuchi & M.Miyata	Japan, Hokkaido, Otaru.	EU521644	EU521645
Pyropia onoi (Ueda) N.Kikuchi & M.Miyata	Japan, Hokkaido, Mori, WELT A023216 (nrSSU); WELT A023216 (*rbc*L).	HQ687575	HQ687529
Pyropia pendula	Mexico, Baja California Sur, Calerita, CMMEX 4437 (nrSSU); CMMEX 4437 (*rbc*L).	DQ084430	HQ687530
Pyropia perforata	USA, California, Del Norte County, Crescent City Lighthouse, 24 May 2002 (nrSSU); Mexico, Baja California, Punta Popotla, 24 February 1999 (*rbc*L).	GU319856	GU046416
Pyropia pseudolanceolata (Krishnam) S.C.Lindstr.	Canada, BC, Victoria, Harling Point, 5 February 2005, UBC A87696 (nrSSU); SCL 12538 (*rbc*L).	GU319857	EU223145
Pyropia pseudolinearis (Ueda) N.Kikuchi *et al.*	Japan, Chiba, Choshi, WELT A024424 (nrSSU); WELT A024424 (*rbc*L).	HQ687590	HQ687531
Pyropia pulchella (Ackland et al.) T.J.Farr & J.E.Sutherland.	New Zealand, North I, Waihau Bay East, WELT A024398 (nrSSU); WELT A024398 (*rbc*L).	HQ687591	HQ687532
Pyropia rakiura (W.A.Nelson) W.A.Nelson	New Zealand, South I, Kaikoura, Ocean View.	AF136425	EU521646
Pyropia saldanhae (Stegenga *et al.*) J.E.Sutherland	South Africa, Kommetjie.	AY292630	GU165838
Pyropia seriata (Kjellm.) N.Kikuchi & M.Miyata	Japan, Kumamoto, Hondo, WELT A023206 (nrSSU); WELT A023206 (*rbc*L).	HQ687576	HQ687533
Pyropia smithii (Hollenberg & I.A.Abbott) S.C.Lindstr.	Canada, BC, Vancouver Island, Barkley Sound, Nudibranch Point, 10 August 2002, UBC A85584 (nrSSU); UBC A85585 (*rbc*L).	GU319861	EU223224
Pyropia spiralis (E.C.Oliveira & Coll) M.C.Oliveira *et al.*	Brazil, Espírito Santo, Vila Velha.	AY766360	HQ605696
Pyropia suborbiculata	USA, NC, Mansonboro Island, 20 May 1996.	HQ728193	AY028523
Pyropia tanegashimensis (Shinmura) N.Kikuchi & E.Fujiyoshi	Japan, Kagoshima, Tanegashima, Iseki, WELT A023236 (nrSSU); WELT A023236 (*rbc*L).	HQ727887	HQ687542
Pyropia tenera (Kjellm.) N.Kikuchi *et al.*	Japan, Kumamoto, Kawaura, CMNH-BA-1354 (nrSSU); CMNH-BA-1354 (*rbc*L).	HQ687577	HQ687543
Pyropia tenuipedalis (A.Miura) N.Kikuchi & M.Miyata	Japan, Chiba, Urayasu.	EU521648	EU521649
Pyropia torta (V.Krishnam) S.C.Lindstr.	USA, WA, San Juan Island, Vista Mar, 22 January 2002, SCL 13289 (nrSSU); SCL 13289 (*rbc*L).	GU319863	EU223236
Pyropia vietnamensis (Takido *et al.*) J.E.Sutherland & Monotilla	India, Kerala, Thangeseri, WELT A024426 (nrSSU); WELT A024426 (*rbc*L).	HQ687578	HQ687544
Pyropia virididentata (W.A.Nelson) W.A.Nelson	New Zealand, Wellington, North I, Lyall Bay.	AF136421	EU521650
Pyropia yezoensis	Korea, Gyeongsangnamdo, Tongyoung, Galmok.	HQ728189	HQ728197
Pyropia raulaguilarii sp. nov. [a]	Mexico, Michoacán, Caletilla, 6 March 2008, ENCB 19524.	JQ684704	JQ684700
Pyropia raulaguilarii sp. nov. [a]	Mexico, Michoacán, Carrizalillo, 4 April 2009, ENCB 19523.	JQ684705	JQ684701

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TABLE 1 (continued)

Taxon	Collection data	GenBank Accession Numbers	
Wildemania occidentalis (Setch. & Hus) S.C.Lindstr.	Canada, Vancouver Island British Columbia, Cape Palmerston, 17 May 2003, UBC A86488 (nrSSU); USA, Washington, Clallam County, Olympic Peninsula, between Makah Bay and Sekiu, 31 May 2003, SCL 10935 (*rbc*L).	GU319851	EU223119

[a]Sequences obtained in this work. [b]Outgroups.

TABLE 2: Comparative vegetative and reproductive characteristics of *Pyropia* species.

Character	*P. hollenbergii*[1]	*P. pendula*[1]	*P. thuretii*[2]	*P. raulaguilarii*
Height (gametophyte thallus)	15–22 cm	25 cm	4–17	4–6 cm
Shape of the blade	Linear-lanceolate	Linear-ligulate	lanceolate	lanceolate
Width of the blade	1–7.5 cm	0.1–2	2–8 cm	1–2 cm
Thalli thickness	45–60 µm	40–54 µm	20–40 µm	40–45 µm
Vegetative cells size Diameter/length	8–14/12–30 µm	6–14/8–25 µm	4–10/8–20 µm	14–17/20–22 µm
Sexuality	Dioecius	Dioecius	Monoecius	Monoecius
Packets of spermatangia	64	128	64	64
Packets of Zygotosporangia	8	8	8	16

[1]Aguilar-Rosas *et al.* (2007). [2]Aguilar-Rosas & Aguilar-Rosas (2003).

Discussion

In the Pacific coast of Mexico there are seven species of *Pyropia* (Aguilar-Rosas *et al.*, 2007, as *Porphyra)*: *Py. gardneri* (G.M.Sm. & Hollenb.) S.C.Lindstr., *Py. hollenbergii* (E.Y.Dawson) J.E.Sutherland *et al.*, *Py. lanceolata* (Setch. & Hus) S.C.Lindstr., *Py. pendula* (E.Y.Dawson) J.E.Sutherland *et al*., *Py. perforata* (J. Agardh) S.C.Lindstr., *Py. suborbiculata* (Kjellm.) J.E.Sutherland *et al.*, and *Py. thuretii* (Setch. & E.Y.Dawson) J.E.Sutherland *et al.* Among them, *Py. hollenbergii* and *Py. pendula* are considered endemic to the Gulf of California, while the remaining five are widely distributed from British Columbia, Canada, to Peru. According to Aguilar-Rosas *et al.* (2007), the thalli of *Py. hollenbergii*, *Py. pendula* and *Py. lanceolata* have a certain morphological and reproductive similarity. However, characters such as the packet number of spermatangia and zygotosporangia, and the length of the thallus, allows to separate these species. Additionally, *Py. thuretii* is widely distributed to British Columbia, Canada to Pacific Northwest coast of Mexico.

Regarding *Py. lanceolata*, it is distributed from the U.S.A. to temperate waters of the Pacific coast of Mexico, it has lanceolate thallus and being over 50 cm length. *Py. perforata* grows in dense groups, up to 1 m length, and wide blades lobed or divided and often located from Alaska to Baja California.

Pyropia raulaguilarii is most closely related with *Py. hollenbergii* and *Py. pendula,* morphological and reproductive differences between these species are shown on Table 2. The most distinctive features of *Pyropia raulaguilarii* are the length of the blade, the surface view in the spermatangial portions and the division formulas of the zygotosporangia (Table 2). In *Py. thuretii* and *Py. hollenbergii* both species exhibit differences in blade thickness and formulas of zygotosporangia division; whereas in *Py. raulaguilarii* there are 16 in *Py. thuretii* there are 32. On the other hand, Aguilar-Rosas & Aguilar-Rosas (2003) and Aguilar-Rosas *et al.* (2007) examined several species of *Porphyra* (*Pyropia*) from the Pacific coast of Mexico. Their examination revealed that there are other *Pyropia* species that may be confused with *Py. raulaguilarii* mentioned here. Table 2 shows the vegetative and reproductive characters of various species of *Pyropia* and in it observed the main differences between species. For example, the gametophyte thallus of *Py. raulaguilarii* reaches a size of 4.0–6.0 cm, while comparatively *Py. hollenbergii* and *Py. pendula* reaches up to 25 cm high and both are dioecious. Furthermore, *Py. raulaguilarii*, is distributed in the Tropical Pacific of Mexico.

The interspecific divergence obtained for the *Pyropia* Pacific Mexican taxa was comparable to the amount of variation observed in other Bangiales taxa. Broom *et al.* (2010) recorded *rbc*L interspecific divergence values between 1.15% and 9.88%, and recorded nrSSU interspecific divergence values between 1.84% and 8.39% for *Porphyra* species recorded in the Falkland Islands. Lindstrom (2008) reported *rbc*L interspecific values between 1% and 4.7% for *Porphyra* species from Pacific region. Brodie *et al*. (2007) obtained a pairwise sequence divergence among *Py. rosengurttii* , *Py. olivii* (Orfanidis *et al.*) J.Brodie & Neefus and *Py. leucosticta* (Thur.) Neefus & J.Brodie in their *rbc*L alignment that ranged from 1% to 11%. Xu *et al.* (2011) mentioned that *Py. yezoensis* (Ueda) M.S.Hwang & H.G.Choi diverged from *Py. haitanensis* (T.J.Chang & B.F.Zheng) N.Kikuchi & M.Miyata by 1% of *rbc*L genetic divergence value. Several studies applying molecular techniques to identify species in *Porphyra* (Stiller & Waaland 1993, Oliveira *et al.* 1995, Broom *et al.* 1999, Kunimoto *et al.* 1999) have revealed considerable interspecies diversity of the nrSSU and *rbc*L regions within this genus and suggest that provides useful characters for species identification.

The molecular phylogeny showed that samples from Carrizalillo and Caletilla (Michoacán) populations are almost identical, confirming that these samples constitute the same taxonomic entity. The phylogenetic analysis also showed that the mentioned Mexican Pacific samples represent a distinctive and well-supported clade segregated from the rest of the *Pyropia* representatives.

The high level of genetic variation observed between the Pacific Mexican samples (from Michoacán and Baja California Sur) and other *Pyropia* species support the recognition of these two taxonomic entities as new taxa within the genus *Pyropia*. Neefus *et al*. (2002) considered that Bangiales is understudied and that the number of species could be greater than those recognized at the present.

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