Endemic Pyropia species (Bangiales, Rhodophyta) from the Gulf of California, Mexico

Article *in* Journal of Applied Phycology · May 2015

DOI: 10.1007/s10811-014-0366-7

5 authors, including:

Juan M. López-Vivas
Autonomous University of Baja California Sur
66 PUBLICATIONS **311** CITATIONS

SEE PROFILE

Raquel Muñiz-Salazar
Autonomous University of Baja California
75 PUBLICATIONS **651** CITATIONS

SEE PROFILE

Isai Pacheco-Ruiz
Autonomous University of Baja California
96 PUBLICATIONS **942** CITATIONS

SEE PROFILE

J Appl Phycol
DOI 10.1007/s10811-014-0366-7

Endemic *Pyropia* species (Bangiales, Rhodophyta) from the Gulf of California, Mexico

Juan Manuel López-Vivas · Raquel Muñiz-Salazar · Rafael Riosmena-Rodríguez · Isaí Pacheco-Ruíz · Charles Yarish

Received: 6 October 2013 / Revised and accepted: 13 June 2014

Abstract The evaluation of geographic distribution of *Pyropia* along the Gulf of California, Mexico, was realized using molecular data from two loci, the plastid *rbc*L gene and the partial 18S ribosomal DNA (rDNA), in conjunction with morphological observations. Dawson described two endemic species for the genus *Pyropia* (*Pyropia hollenbergii* and *Pyropia pendula*) in the Gulf of California based on taxonomic characteristics. In this study, we collected 130 samples from 13 locations from February 1998 to April 2008. Samples showed similar morphologies and growth habits and share a very similar type descriptions and habitat records. Using morphological and anatomical characteristics, we identify individuals as *P. hollenbergii* and *P. pendula*. However, using the molecular data, we identified five identities, which we have classified as *P. hollenbergii*, *P. pendula*, *Pyropia* sp. Gulf of California I (GCI), *Pyropia* sp. GCII, and *Pyropia* sp. GCIII. Phylogenetic trees based on partial 18S rDNA and *rbc*L sequence data showed a deep division in the genus that is not obviously correlated with existing morphological characteristics and indicate that representatives of the Gulf of California flora have undergone long reproductive isolation.

Keywords Endemic species · Gulf of California · *Pyropia hollenbergii* · *Pyropia pendula* · nSSU · *rbc*L

J. M. López-Vivas · R. Riosmena-Rodríguez (✉)
Programa de Investigación en Botánica Marina, Departamento Académico de Biología Marina, Universidad Autónoma de Baja California Sur, Km 5.5 Carretera al Sur, A.P. 19-B, 23080 La Paz, BCS, México
e-mail: riosmena@uabcs.mx

R. Muñiz-Salazar
Escuela de Ciencias de la Salud, Universidad Autónoma de Baja California, Blvd. Zertuche y Blvd. de los Lagos s/n. Fracc. Valle Dorado, 22890 Ensenada, BC, México

I. Pacheco-Ruíz
Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California, Km 107 Carretera Tijuana-Ensenada, A.P. 458, 22860 Ensenada, BC, México

C. Yarish
Ecology and Evolutionary Biology, University of Connecticut, One University Place, Stamford, CT 06901-2315, USA

Introduction

The genus *Pyropia* J. Agardh comprises seven species along the Northern Mexican Pacific (NMP), including the Gulf of California: *Pyropia gardneri* (G. M. Smith et Hollenberg) S. C. Lindstrom, *Pyropia hollenbergii* (E.Y. Dawson) J.E. Sutherland, L.E. Aguilar Rosas et R. Aguilar Rosas, *Pyropia lanceolata* (Setchell et Hus) S.C. Lindstrom, *Pyropia pendula* (E.Y. Dawson) J.E. Sutherland, L.E. Aguilar Rosas et R. Aguilar Rosas, *Pyropia perforata* (J. Agardh) S.C. Lindstrom, *Pyropia suborbiculata* (Kjellmann) J.E. Shutherland, H.G. Choi, M.S. Hwang et W.A. Nelson, and *Pyropia thuretii* (Setchell et E.Y. Dawson) J.E. Sutherland, L.E. Aguilar Rosas et R. Aguilar Rosas (Dawson 1944; 1953b; Gabrielson et al. 2000; Hawkes 1977; 1978; Hollenberg 1943; Norris 1975; Sánchez-Rodríguez and Fajardo-León 1989; Scagel 1957; Smith and Hollenberg 1943).

Three of the seven *Pyropia* species are recorded for the Gulf of California, *P. thuretii*, *P. hollenbergii*, and *P. pendula*; the last two species are endemic of the Gulf of California. *P. thuretii* has been reported from Cabo Colonett, Baja California (BC), to Punta Galeras, Baja California Sur (BCS), within the Gulf of California (Abbott and Hollenberg 1976; Aguilar-Rosas and Aguilar-Rosas 2003; Aguilar-Rosas et al. 2004; Hawkes 1981; Norris 1975; Paul-Chavéz and Riosmena-Rodríguez 2000; Riosmena-Rodríguez and Paul-Chavéz 1997; Rocha-Ramírez and Siqueiros-Beltrones 1991; Rodríguez-Morales and Siqueiros-Beltrones 1999; Scagel 1957). *P. hollenbergii* has been reported at four localities in the Gulf of California, from Bahia Bocochibampo, Sonora (Son), to Isla San Idelfonso, BCS, to Nopolo, BCS (Aguilar-Rosas et al. 2007a; Dawson 1953a;

Published online: 20 August 2014

Espinoza-Ávalos 1993; Mateo-Cid et al. 2000; Norris 1975; Paul-Chavéz and Riosmena-Rodríguez 2000; Riosmena-Rodríguez and Paul-Chavéz 1997). *P. pendula* has been reported at six localities from Isla Patos, Son, to Isla Partida, BC, to Los Planes, BCS (Aguilar-Rosas et al. 2004; Dawson 1953a) (Fig. 1).

The Gulf of California, Mexico, which has an unusual mix of tropical and temperate taxa, shows an extraordinary level of biological diversity, including numerous endemic organisms in both land and coastal marine flora and a variety of habitats and unique ecosystems (Brusca et al. 2005; Phillips and Wentworth-Comus 2000). Espinoza-Ávalos (Espinoza-Ávalos 1993) reported 20 % endemism in seaweeds for this area which are distributed in three main regions: northern, central, and southern. This division corresponds with other studies on fishes (Thomson and Gilligan 1983), invertebrates (Brusca 1980), seagrasses (Muñiz-Salazar et al. 2005), mangroves (Sandoval-Castro et al. 2012), and chlorophyll concentrations (Santamaria Del Angel et al. 1999). The first collection of marine seaweeds along the Gulf of California was at the end of the nineteenth Century and in the early twentieth Century. Most of the seaweeds were documented by Howe (Howe 1911) and Setchell and Gardner (Setchell and Gardner 1924), from expeditions of the Academy of Sciences of California and private collections (Espinoza-Ávalos 1993; Norris 1975). Dawson (1944, 1953b) described

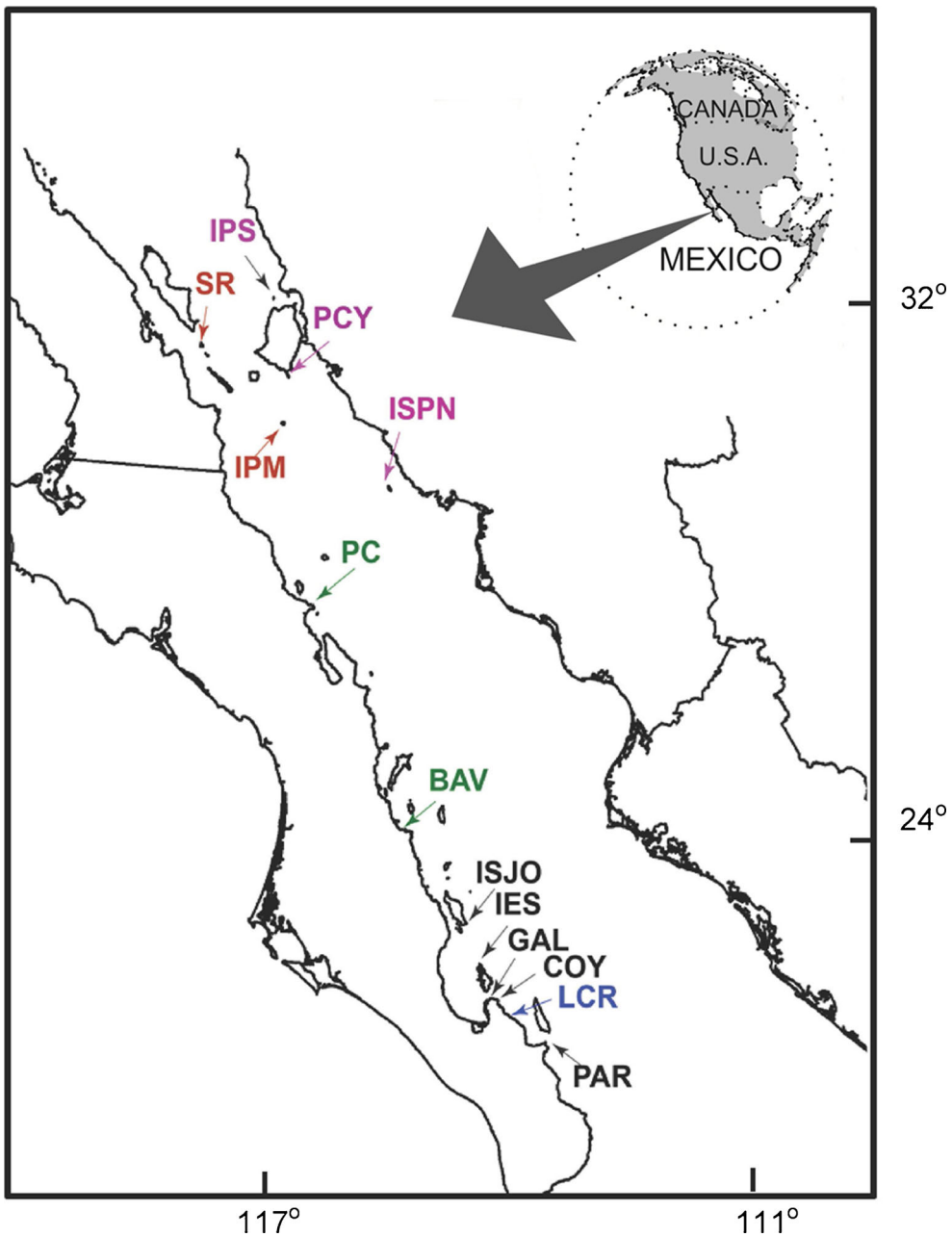

Fig. 1 Distributional map of *Pyropia hollenbergii* (Punta Chivato (PC) and Bahía Agua Verde (BAV)) (lettering in green); *Pyropia pendula* (Isla Partida (SR) and Isla San Pedro Mártir (IPM) (lettering in red); and new entities *Pyropia* sp. GCI (Isla Espíritu Santo (IES), Isla San José (ISJO), Punta Las Galeras (GAL), Punta Coyote (COY) and Punta Arenas (PAR)) (lettering in black); *Pyropia* sp. GCII (Las Cruces (LCR)) (lettering in blue) and *Pyropia* sp. GCIII (Isla Patos (IPS), Isla El Choyero (PCY) and Isla San Pedro Nolasco (ISPN) (lettering in purple); along the Gulf of California

and studied specimens collected by the previous expeditions and carried out 20 expeditions around the Gulf of California. Dawson was the one who contributes more to the knowledge, distribution, and ecology of seaweeds of the Gulf of California, with 15 works published between 1941 and 1966. Since 1970 most of the studies have been by Mexican scientists (Aguilar-Rosas and Aguilar-Rosas 2003; Aguilar-Rosas et al. 2004, 2007a; Dawson 1944, 1953b; Krishnamurthy 1972; Norris 1975; Scagel 1957).

Morphological identification of *Pyropia* taxa has been difficult because of the paucity of morphological characteristics available for species recognition and the high level of plasticity present in response to environmental variables such as temperature and wave exposure. Intensive sampling and molecular studies have revealed extensive cryptic speciation within the genus at the southern hemisphere (Broom et al. 1999, 2004; Jones et al. 2004; Sutherland et al. 2011). The use of molecular sequence data has been a critical tool for taxonomic studies to test hypotheses about species identity, mainly where both phenotypic plasticity and morphological convergence are in evidence at the species level; the molecular data have provided an efficient means of sorting specimens after the initial recognition of morphological, ecological, and distributional forms. The 3′ region of the nuclear small subunit ribosomal RNA (nSSU) ribosomal DNA (rDNA) (Saunders and Kraft 1994) spans approximately 450 bp and includes the V9 variable region of the gene (Neefus et al. 1993) which has been found to be a useful proxy for overall variation in the whole nSSU in *Pyropia* (Broom et al. 1999). Several authors have suggested the use of 18S rDNA exon sequence data for species discrimination in *Pyropia* (Broom et al. 1999; Oliveira et al. 1995). The 18S rDNA is one of the most slowly evolving sequences found through living organisms (Hillis and Dixon 1991). However, a significant amount of variation at this locus has been detected and has been used to discriminate effectively between species of *Pyropia* (Kunimoto et al. 1999; Nelson and Broom 2010; Oliveira et al. 1995).

In this study, we present analyses of *Pyropia* (Bangiales, Rhodophyta) collected along the Gulf of California. The genus had been recorded previously in this region; however, this is the first time using molecular tools. We have used molecular data from two loci—the plastid *rbc*L gene and the 18S ribosomal RNA gene—to support and confirm morphological species groupings and to determine the phylogenetic affinity of these taxa with respect to other Bangiales species. The present study adds to understanding of Gulf of California flora, identifies likely endemics, and adds to our growing understanding of the complexity of *Pyropia* distributions in the Gulf of California.

Materials and methods

Study area

Foliar phases of *Pyropia* were sampled at 13 localities around the coast of the Gulf of California from February 1998 to April 2008. The geographic locations of these localities are presented in Table 1 and Fig. 1. Three to six individuals of *Pyropia* were collected manually from each locality during low tides. Specimens were pressed as herbarium vouchers, and subsamples were dried in desiccant silica gel for subsequent DNA analysis and lodged in the herbarium of the Universidad Autonoma de Baja California Sur (FBCS). Table 1 gives the collection details and voucher numbers of specimens included in the study.

Taxonomic analysis

Plants were identified according to diagnostic criteria using the following characteristics: (1) number of cell layers and chloroplasts, (2) leaf margin, (3) shape of the blade, (4) structure of the basal part, (5) thickness of the blade, (6) color of the blade, and (7) sexuality and other characteristics (Dawson 1944, 1953b; Krishnamurthy 1972; Kurogi 1972; Yoshida 1997).

DNA extraction

Genomic DNA was extracted from approximately 0.02–0.04 g dry weight of dry tissue using the hexadecyltrimethylammonium bromide/polyvinylpyrrolidone (CTAB/PVP) protocol of Stewart and Via (1993), modified by Muñiz-Salazar et al. (2005). Tissue was crushed using a sterile homogenizer and dry ice. The powdered tissue was suspended in approximately 0.85 volumes of CTAB/PVP lysis buffer and incubated at 37 °C for 6 h with protease E and beta-mercaptoethanol. The homogenate was extracted with approximately 0.6 volumes of chloroform/isoamyl alcohol (24:1 *v*/*v*). Samples were centrifuged at 3,300×*g* for 5 min to separate phases. DNA was incubated at −20 °C for 12 h in 0.7 volumes of isopropanol and then concentrated using centrifugation at 15,700×*g* for 20 min. Following rehydration in 1× TE, the DNA was quantified using fluorimetry, diluted to 50 ng μL^{-1} and stored at −20 °C until further analysis.

PCR amplification and sequencing

The 18S ribosomal RNA gene was amplified and sequenced by using primers G06 (Saunders and Kraft 1994) and J04 (Broom et al. 1999; Jones et al. 2004), which amplified a non-coding region (approximately 550 bp) of the 18S rDNA gene. This region does not contain introns (Broom et al. 1999; Saunders and Kraft 1994). The *rbc*L gene was amplified and sequenced from the same specimens by using primers F67 and

Table 1 Collection information, voucher numbers, and GenBank accession numbers for specimens from the Gulf of California, Mexico, in this study

Entity	Code	Location	Lat/Long	Date	Collectors	FBCS no.	*rbc*L	18S
P. pendula	SR	Isla Partida, BC	28° 53′ 33.4″ N, 113° 02′ 45.8″ W	Mar 2006	JMLV, JJHK, GAS, CY, JAZG	12081-123	JQ900553	JN680554
P. pendula	IPM	Isla San Pedro Mártir, Son	28° 22′ 57.90″ N, 112° 19′ 4.55″ W	Mar 2009	JJHK, ANSC	12280a,b	JQ900557	JN680555
Pyropia sp. GCIII	IPS	Isla Patos, Son	29° 16′ 4.32″ N, 112° 27′ 47.36″ W	Feb 2006	JMLV, JJHK, GBG, GAS	12040-49	JQ900554	JX024906
Pyropia sp. GCIII	PCY	Isla El Choyero, Son	28° 44′ 12.43″ N, 112° 18′ 22.33″ W	Feb 2006	JMLV, JJHK, GBG, GAS	12060-65	JQ900556	NR
Pyropia sp. GCIII	ISPN	Isla San Pedro Nolascó, Son	27° 58′ 22.70″ N, 111° 22′ 38.40″ W	Apr 2008	JMLV, RBB, JMGC	12183-99	JQ900555	JN680556
P. hollenbergii	PC	Punta Chivato, BCS	27° 04′ 19.0″ N, 111° 56′ 42.2″ W	Apr 2006	JMLV, JJHK	12132-143	JQ900558	JN680557
P. hollenbergii	BAV	Bahía Agua Verde, BCS	25° 30′ 56.78″ N, 111° 3′ 53.37″ W	Apr 2004	JMLV, JEA	11232-236	AY794401 JQ900559	JN680558
Pyropia sp. GCII	LCR	Las Cruces, BCS	24° 17′ 25.80″ N, 110° 11′ 26.21″ W	Apr 2001	MAAC, EHA, GHA, KLC	1863	JQ900565	JQ900551
Pyropia sp. GCI	IES	Isla Espíritu Santo, BCS	24° 35′ 55.93″ N, 110° 24′ 12.87″ W	Feb 2008	JJHK, JC	12211-13	JQ900562	JQ900548
Pyropia sp. GCI	GAL	Punta Galeras, BCS	24° 21′ 16.35″ N, 110° 16′ 59.97″ W	Apr 2006	JMLV, JJHK	12126-128	JQ900561	JQ900547
Pyropia sp. GCI	COY	Punta Coyote, BCS	24° 20′ 45.47″ N, 110° 14′ 54.72″ W	Mar 2002	JMLV, RMS, EAO	11026-28	JQ900560	JQ900549
Pyropia sp. GCI	PAR	Punta Arenas, BCS	24° 2′ 35.61″ N, 109° 49′ 31.02″ W	Mar 2008	JJHK, JC, CV	12217-220	JQ900563	JQ900552
Pyropia sp. GCI	ISJO	Isla San José, BCS	24° 50′ 33.42″ N, 110° 36′ 21.03″ W	Feb 2008	ASR, ERA, JC, JJHK	12208-10	JQ900564	JQ900550

Collectors' names: *GAS* G. Andrade-Sorcia, *EAO* E. Arroyo-Ortega, *GBG* G. Ballesteros-Grijalva, *RBB* R. Blanco-Betancourt, *JCG* J. Castillo-Guzmán, *JEA* J. Espinoza-Ávalos, *JMGC* J. M. Guzmán-Calderón, *JJHK* J. J. Hernández-Kantún, *GHA* G. Hinojosa-Arango, *EHA* E Holguín-Acosta, *KLC* K. León-Cisneros, *JMLV* J. M. López-Vivas, *RMS* R. Muñiz-Salazar, *ERA* E. Rosas-Alquicira, *ASR* A. Sanchez-Rodríguez, *ANSC* A. N. Suarez-Castillo, *CV* C. Valdez-Navarrete, *CY* C. Yarish, *JAZG* J. A. Zertuche-González

NR not registered

rbc-spc (Teasdale et al. 2002). PCR amplification for 18S and *rbc*L were performed on 25 μL containing a final concentration of 0.1–0.2 μg DNA template, PCR buffer (1 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.02 mM each dNTP), 10.0 pmol of each unlabeled forward and reverse primer, 0.3 units of *Taq* polymerase (Applied Biosystems), and 2 μg μL^{-1} of bovine serum albumin. The thermocycling profile for 18S consisted of a 4-min denaturation at 94 °C, followed by 33 cycles of 30 s at 94 °C, 45 s at 55 °C, and 2 min at 72 °C, and 6 min at 72 °C of extension. Thermal profile for *rbc*L was 3-min denaturation at 94 °C, followed by 28 cycles of 15 s at 94 °C, 30 s at 55 °C, and 1 min at 72 °C, and 10 min at 72 °C of extension. Each PCR product for both genes was electrophoresed in a 1.5 % agarose gel stained with GelStar. Electrophoresis was done at 100 V for 40 min, and the gels were visualized under UV light. Amplification products were purified and then sequenced in forward and reverse directions, using these same primers. DNA sequencing was performed on Applied Biosystems 377 sequencers (Applied Biosystems, USA). For quality control purposes, one individual representing each population was reextracted and reanalyzed.

Molecular data analysis

Sequence electropherograms were examined for signal quality and edited using CodonCode Aligner (CodonCode Corporation (2006)) before alignment. Sequences were compared with existing *Porphyra* and *Pyropia* sequences from GenBank (Sutherland et al. 2011) using BLAST (Altschul et al. 1990) and were aligned with CLUSTALX (Thompson et al. 1997) implemented in the MEGA 5.0 (Tamura et al. 2011). Phylogenetic analyses were performed using three data sets: the *rbc*L, the 18S, and a combined data set with the genes concatenated. For maximum likelihood (ML) analyses, the evolutionary model selected was the TrN+I+G using the Akaike information criterion (Posada and Buckley 2004) inferred from the program Modeltest v.3.06. ML trees were individually obtained for each data set using ML heuristic searches in PAUP* 4.0b10 (Swofford 2002) using the tree bisection-reconnection (TBR) branch-swapping algorithm. Bootstrap support values were obtained by ML analyses of 100 pseudoreplicates of each data set. MrBayes 3.2

(Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) was used to conduct Bayesian analyses using the substitution models chosen by Modeltest 3.7. We ran two independent simultaneous Markov Chain Monte Carlo runs with four chains each for 5 million generations and sampled trees every 100 generations. Convergence of the MCMC runs was determined by visually examining the cumulative posterior and between-run variation in split frequencies (Nylander et al. 2008) using the online tool AWTY (Wilgenbusch et al. 2004). Parameters and corresponding trees were summarized after discarding the initial 25 % of each chain as burn in. GenBank accession numbers for the *rbc*L and 18S sequences for each locality analyzed in this study are listed in Table 1.

Results

Taxonomic analysis

When plants reproduce by means of zygotosporangia, they tend to exhibit colors ranging from light pink to red and to present a variety of shapes: ovate and sometimes lanceolate with linear or partial branching, undulating to undulating heavily, and even sometimes smooth (Figs. 2 and 3). The division formula of zygotosporangia in all specimens is 8 (a/2, b/2, c/2), the division of spermatangia is 128 (a/4, b/4, c/8), and all of them are dioecious. Isotypes of *P. hollenbergii* (UC 925752) and *P. pendula* (UC 925750) were examined, the specimens housed in the Herbarium of the University of California at Berkeley where no molecular material was obtained. Specimens of both *P. hollenbergii* and *P. pendula* were collected at the type localities. Its morphological characteristics are consistent with the descriptions and isotypes that were reviewed. The collection process, as described by Dawson (Dawson 1944) for *P. hollenbergii* and *P. pendula*, was undertaken during February 1940 and 1946, whereas our samples were collected in different months: *P. hollenbergii* in January, February, March, and April and *P. pendula* only collected in March. Table 2 shows distribution, habitat, and morphology of the 13 sampled localities.

Molecular analysis

Partial sequences of the *rbcL* loci (880 bp) and 18S (478 bp) were obtained from 31 samples of 13 localities along the Gulf of California. The phylogenetic trees of *rbc*L and 18S genes (data not shown) and concatenated sequence gene trees (Fig. 4) showed a similar topology. On the basis of this sequence data, the samples were identified as *P. pendula*, *P. hollenbergii*, and three new identities. Samples from Isla Partida (SR) and IPM, located in the northern part of the Gulf of California along the Baja California peninsula, were identified as *P. pendula*. Along the mainland coast, samples from IPS, ISPN, and PCY were identified as a new identity, which we have named *Pyropia* sp. GCIII. At the central region of the Gulf of California (BAV and PC), the samples were identified as *P. hollenbergii*. These samples matched 100 % with the earlier *rbc*L gene sequence (GenBank accession AY794401) acquired at BAV (Lynch et al. 2008; López-Vivas et al. 2011), which is the type locality for *P. hollenbergii* (Dawson 1944, 1953a, b). Therefore, it was considered that the samples in this group corresponded to *P. hollenbergii*.

At the southern Gulf of California, we found two new identities: *Pyropia* sp. GCII, found in one locality only (LCR), and *Pyropia* sp. GCI, distributed in five localities (IES, GAL, COY, PAR, and ISJO). Interestingly, an earlier 18S sequence registered as *P. pendula* in GenBank (DQ084430; Nelson et al. 2006) matched 100 % with samples of *Pyropia* sp. GCI. Since SR is the type locality for *P. pendula* according to Dawson (1944), we suggest that the 18S sequence DQ084430 has been misclassified.

The interspecific sequence divergence between samples identified as *P. hollenbergii* and *P. pendula* was 2.6 % for 18S gene. The three new identities (*Pyropia* sp. GCI, *Pyropia* sp. GCII, and *Pyropia* sp. GCIII) versus *P. hollenbergii* and *P. pendula* ranged from 0 % (*Pyropia* sp. GCIII vs *P. pendula*) to 3.2 % (*Pyropia* sp. GCIII vs *P. pendula* and *Pyropia* sp. GCIII vs *Pyropia* sp. GCI) (Table 3). On the other hand, the interspecific sequence divergence between samples identified as *P. hollenbergii* and *P. pendula* was 6.2 % for *rbc*L gene. Though, the interspecific sequence divergence among the three new identities versus *P. hollenbergii and P. pendula* ranged from 1.8 % (*Pyropia* sp. GCIII vs *P. pendula*) to 6.0 % (*Pyropia* sp. GCIII vs *P. hollenbergii*) (Table 4).

Discussion

This study redefines the limits of geographical distribution of endemic species of *P. hollenbergii* and *P. pendula* in the Gulf of California using molecular and morphological markers. Dawson (1944) recorded two endemic species of *Pyropia*, *P. hollenbergii*, and *P. pendula* in the Gulf of California; however, our molecular data confirms the presence of three possible new identities of *Pyropia* GCI, *Pyropia* sp. GCII, and *Pyropia* sp. GCIII. Considering previous work of *Pyropia* based on *rbc*L and 18S sequences, we consider that the genetic distances among the three new identities of *Pyropia* and *P. hollenbergii* and *P. pendula* are large enough to indicate that they should be considered separate taxons. For example, new identities have been proposed as new species of *Pyropia* for the Falkland Islands, which displayed values of

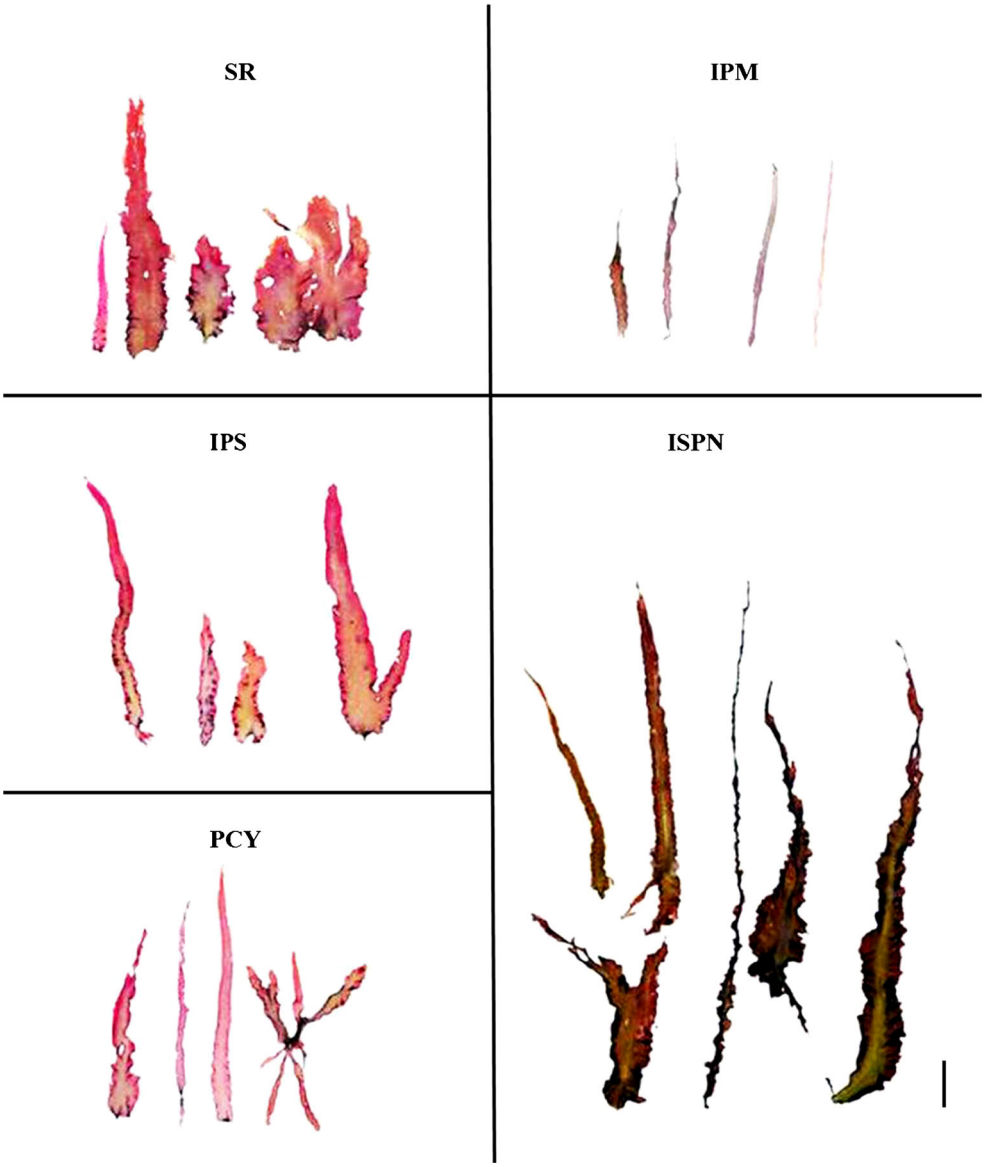

Fig. 2 Representative specimens with morphological characteristics such as color and shape of *Pyropia pendula* (SR and IPM) and *Pyropia* sp. (IPS, PCY and ISPN)

interspecific sequence divergence from 0.35 to 3.68 % (Broom et al. 2010). Similarly, the interspecific sequence divergence between *Boreophyllum birdiae* and *Boreophyllum aestivales* is 0.8 % for the *rbc*L gene (Lindstrom and Fredericq 2003; Neefus et al. 2002). In conserved loci such as SSU rDNA, significant divergence between taxa sequences indicates a long reproductive separation; though, when the specimens only show few substitutions, it is not clear how they can be registered. Results suggest that nSSU sequence differences of only a few substitutions should be considered possible signals of species boundaries in this genus and that in *Porphyra* and *Pyropia*, *rbc*L sequences are more variable than nSSU (Broom et al. 1999, 2002, 2010), which is very similar to our results. Besides, it is important to highlight that the 18S region amplified in this study does not have introns, which are inherently more variable than the exons; consequently, the low interspecific sequence divergence registered among entities for 18S gene can be the result of low variability of this region.

In this study, we did not identify morphological differences between thalli samples of *P. hollenbergii*, *P. pendula*, and *Pyropia* sp. GCI, GCII, and GCIII. Reproductive differences between these species are shown on Table 2. Morphological and reproductive evidence suggests a possible close relationship. All thalli are membranaceous, monostromatic, and showed a single chloroplast. In addition, a base is formed by disk-shaped cells with a hyaline filament. Furthermore, the blades have different morphologies, lanceolate, linear, obovate, or linguada. The blades can be simple or split from a common basal area. All are dioecious. Male gametophytes are 128 spermatia formed in the spermatangia having a division

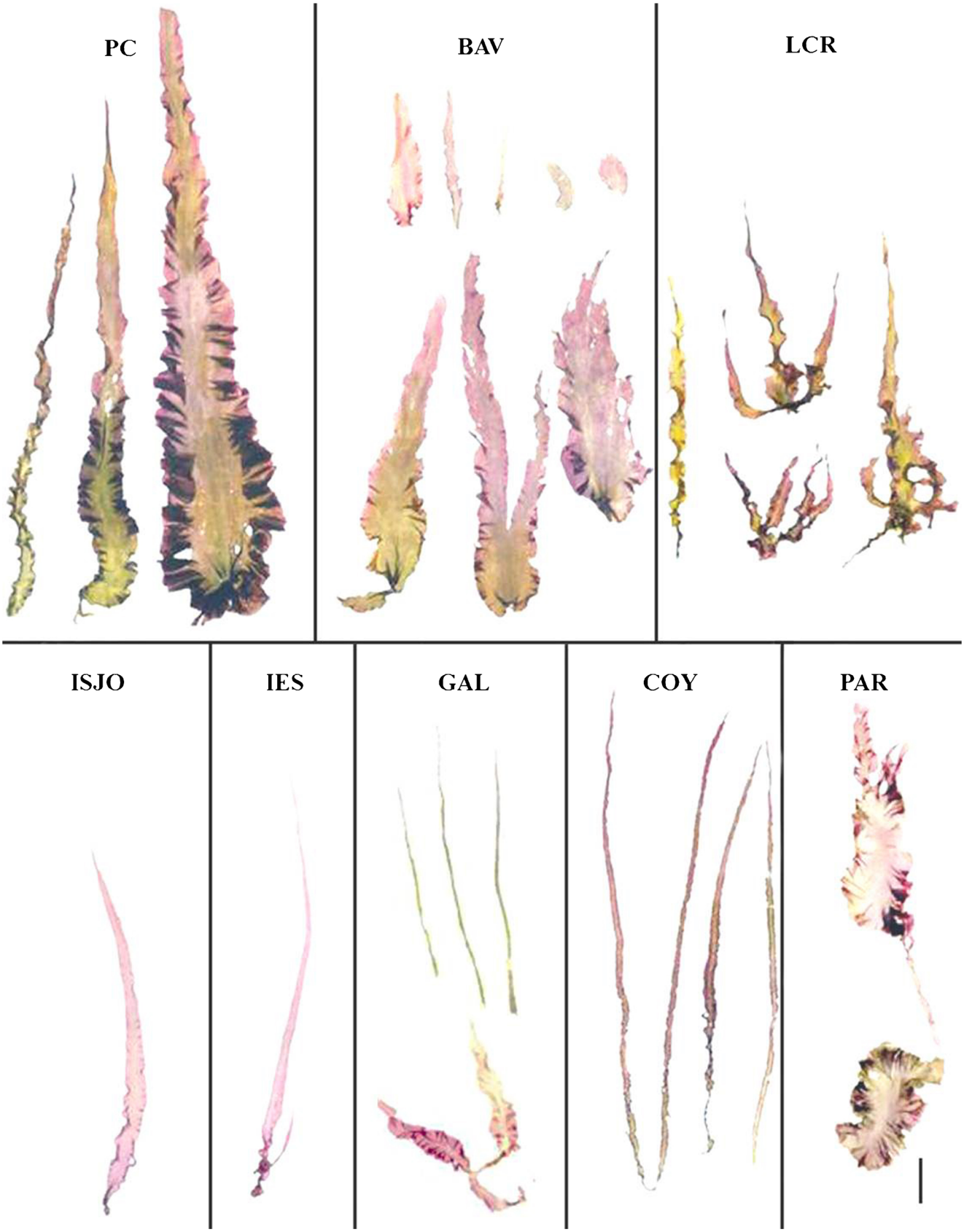

Fig. 3 Representative specimens with morphological characteristics such as color and shape of *Pyropia hollenbergii* (PC and BAV), *Pyropia* sp. GCI (IES, COY, GAL, PAR, and ISJO) and *Pyropia* sp. GCII (LCR)

Table 2 Morphological features of five species of *Pyropia* from the Gulf of California Mexico

	P. hollenbergii	*P. pendula*	*Pyropia* sp. GCI	*Pyropia* sp. GCII	*Pyropia* sp. GCIII
Seasonality	Winter–spring	Winter–spring	Winter–spring	Winter–spring	Winter–spring
Substrate relationship	Epilithic	Epilithic	Epilithic	Epilithic	Epilithic
Vertical distribution	Mid- to High-Intertidal	Mid- to High-Intertidal	Mid- to High-Intertidal	Mid- to High-Intertidal	Mid- to High-Intertidal
Geographic distribution	Gulf of California Endemic, Punta Chivato, and Bahía Agua Verde, B.C.S., México	Gulf of California Endemic, Isla Partida, B.C.; Isla San Pedro Martir, Son., México	Gulf of California Endemic, Isla San José, Isla Espiritu Santo, Punta Galeras; Punta Coyote and Punta Arenas, B.C.S, México	Gulf of California Endemic, Las Cruces, B.C.S., México	Gulf of California Endemic, Isla Patos, Isla el Choyero; and Isla San Pedro Nolasco Son., México
Locality type	Bahía Agua Verde, BCS, México	Isla Partida, BC, México	Punta Coyote, BCS, México	Las Cruces, BCS, México	Isla Patos, BCS, México
Thalli color	Pink pale/red brilliant/green olive	Pink pale/red brilliant	Pink pale/red brilliant/green olive	Pink pale/red brilliant/green olive	Pink pale/red brilliant/green olive
Margin	Lobulate/entire	Lobulate/entire	Lobulate/entire	Lobulate/entire	Lobulate/entire
Texture	Membranous	Membranous	Membranous	Membranous	Membranous
Holdfast	Discoid/hyaline filament	Discoid/hyaline filament	Discoid/hyaline filament	Discoid/hyaline filament	Discoid/hyaline filament
Thalli construction	Monostromatic	Monostromatic	Monostromatic	Monostromatic	Monostromatic
Thalli form	Lanceolate/linear/obovate	Lanceolate/linear/obovate/linguada	Lanceolate/linear/linguada	Lanceolate/linear/obovate	Lanceolate/linear/obovate
Number of chloroplast	1	1	1	1	1
Form of the cells	Polygonal/irregular	Irregular	Polygonal/irregular	Polygonal/irregular	Polygonal/irregular
Chromosome number	2	2	ND	ND	ND
Spermatangia color	Yellow	Yellow	Yellow	Yellow	Yellow
Zygotosporangia color	Pink/pale/purple red	Pink /brilliant/purple red	Pink/pale/brilliant red/purple	Pink/pale/brilliant red/purple	Pink/pale/brilliant red/purple
Distribution pattern of the reproductive cells	Continuous in the margin, separate blades	Continuous in the margin separate blades	Continuous in the margin, separate blades	Continuous in the margin, separate blades	Continuous in the margin, separate blades
Spermatangia arrangement	64/128 (4/a, 4/b, 4/8/c)	128 (4/a, 4/b, 8/c)	128 (4/a, 4/b, 4/8/c)	128 (4/a, 4/b, 4/8/c)	128 (4/a, 4/b, 4/8/c)
Zygotosporangia arrangement	8 (2/a, 2/b, 2/c)	8 (2/a, 2/b, 2/c)	8 (2/a, 2/b, 2/c)	8 (2/a, 2/b, 2/c)	8 (2/a, 2/b, 2/c)
Asexual reproduction	Conchocelis	Conchocelis	Conchocelis	Conchocelis	Conchocelis
Sexuality	Dioecious	Dioecious	Dioecious	Dioecious	Dioecious
Thalli length	60–150 mm	250 mm	15–170 mm	12–350 mm	10–250 mm
Thalli width	10–75 mm	10–20	3–54 mm	2–28	1–90 mm
Thalli height	45–60 μm	45–50 μm	40–55 μm vegetative	40–50 μm vegetative	40–55 μm vegetative
Length, height, and width of vegetative cells' surface view	12–30 μm	8–25 μm	20–23 μm height/22–25 μm width	10–20	8–25 μm
Length, height, and width of vegetative cells' cross view	25–40 μm	7–11 μm height/22–25 μm width	20–40	10–15/20–25	20–40
Description	Dawson (1953a, b)	Dawson (1953a, b)	This work	This work	This work
References	(Abbott and Hollenberg 1976); (Aguilar-Rosas and Aguilar-Rosas 2003); (Aguilar-Rosas et al. 2004), (2007a), (2007b	(Aguilar-Rosas and Aguilar-Rosas 2003); (Aguilar-Rosas et al. 2004), (2007a), (2007b)			

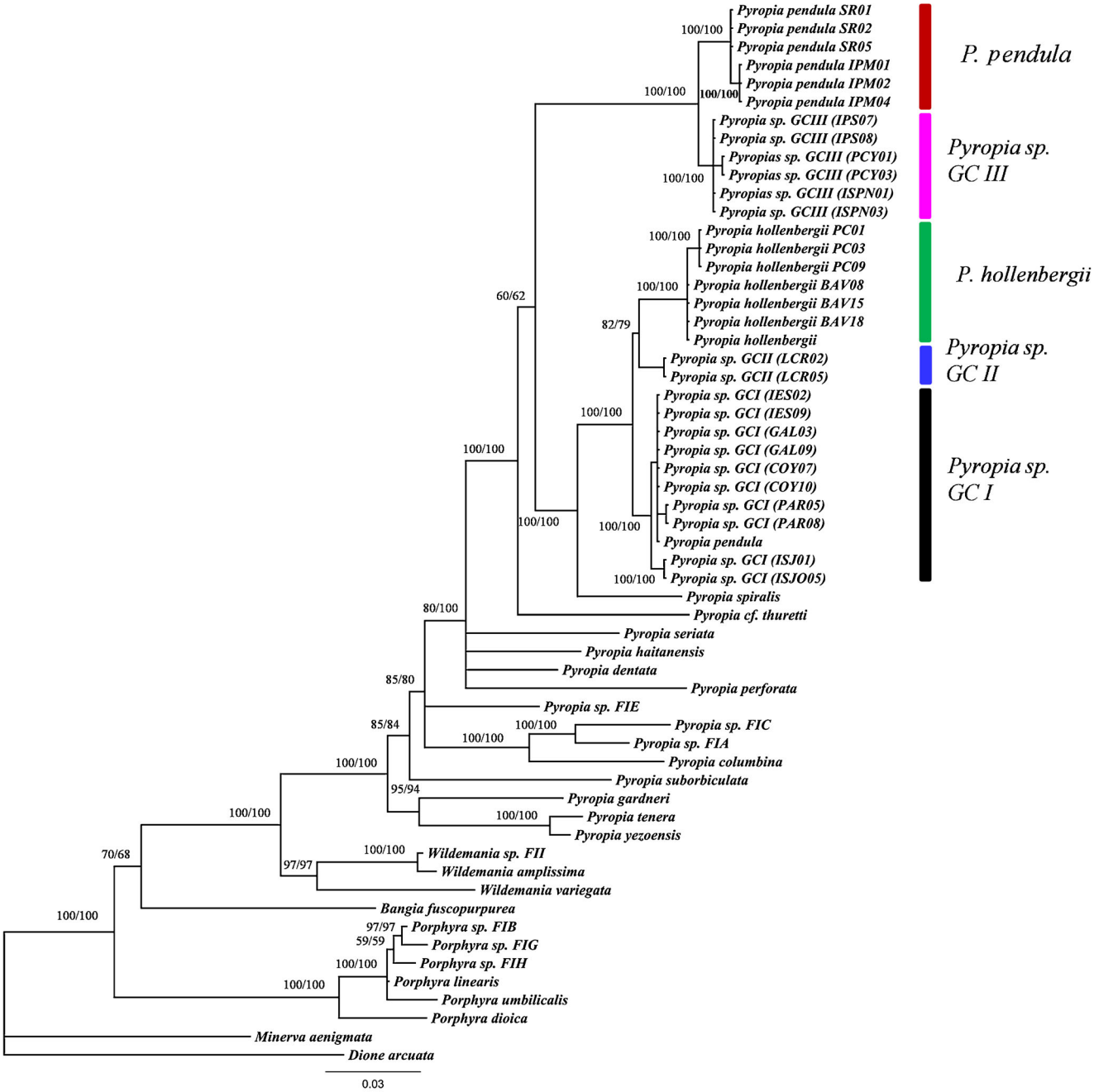

Fig. 4 Maximum likelihood and Bayesian phylogram constructed from concatenated nSSU and *rbc*L sequences of Bangiales taxa, including *Pyropia hollenbergii*, *Pyropia pendula*, and the three new entities, *Pyropia* sp. GCI, *Pyropia* sp. GCII and *Pyropia* GCIII. ML bootstrap values (*left*) and Bayesian PP values (*right*)

formula of a/4, b/4, and c/4, and they are yellowish. While eight zygotospores form in the zygotosporangia, the division formula is a/2, b/2, and c/2, and they are reddish. All entities have sexually dimorphic differences in the length and wide of the thalli, different coloration between the male and female and have different number of spores in the sporangia. The margin is slightly ruffled in males whereas it is deeply ruffled in the female (Aguilar-Rosas et al. 2004, 2007b; López-Vivas 2000, 2003). The coloration of the thallus has to do with the maturation of sexual reproductive structures and the development of reproductive packages. According to Kurogi (1972), the expression of the peculiar shades of colors for each species makes them difficult to identify. Most species of *Pyropia* have two or three characteristic colors. The number of spermatangia also profusely undulates the margins of the plates, which was the characteristic that mainly helped separate *P. hollenbergii* from *P. pendula*. According to some authors, *P. hollenbergii* differs from *P. pendula* mainly in the

Table 3 Absolute sequence differences (below diagonal) and percentage sequence divergence (above diagonal) of 18S sequences from the Gulf of California *Pyropia* entities identified in this study

		Pyropia pendula		*Pyropia* sp. GCIII			*Pyropia hollenbergii*			*Pyropia* sp. GCII	*Pyropia* sp. GCI					
		SR	IPM	IPS	PCY	ISPN	PC	BAV	HQ687589	LCR	IES	GAL	COY	PAR	ISJO	DQ084430
Pyropia pendula	SR		0.0	0.0	0.0	0.0	2.7	2.9	2.9	3.1	3.3	3.3	3.3	3.8	3.1	3.3
	IPM	0		0.0	0.0	0.0	2.7	2.9	2.9	3.1	3.3	3.3	3.3	3.8	3.1	3.3
Pyropia sp. GCIII	IPS	0	0		0.0	0.0	2.7	2.9	2.9	3.1	3.3	3.3	3.3	3.8	3.1	3.3
	PCY	0	0	0		0.0	2.7	2.9	2.9	3.1	3.3	3.3	3.3	3.8	3.1	3.3
	ISPN	0	0	0	0		2.7	2.9	2.9	3.1	3.3	3.3	3.3	3.8	3.1	3.3
Pyropia hollenbergii	PC	12	12	12	12	12		0.2	0.2	1.3	1.1	1.1	1.1	1.6	1.3	1.1
	BAV	13	13	13	13	13	1		0.0	1.1	0.9	0.9	0.9	1.3	1.1	0.9
	HQ687589	13	13	13	13	13	1	0		1.1	0.9	0.9	0.9	1.3	1.1	0.9
Pyropia sp.GCII	LCR	14	14	14	14	14	6	5	5		0.2	0.0	0.2	0.7	0.9	0.2
Pyropia sp. GCI	IES	15	15	15	15	15	5	4	4	1		0.2	0.0	0.4	0.7	0.0
	GAL	15	15	15	15	15	5	4	4	0	1		0.0	0.4	0.7	0.0
	COY	15	15	15	15	15	5	4	4	1	0	0		0.4	0.7	0.0
	PAR	17	17	17	17	17	7	6	6	3	2	2	2		1.1	0.4
	ISJO	14	14	14	14	14	6	5	5	4	3	3	3	5		0.7
	DQ084430	15	15	15	15	15	5	4	4	1	0	0	0	2	3	

number of spermatangia (64), compared with that presented by *P. pendula* (128) (Aguilar-Rosas et al. 2004; Dawson 1944, 1953a). Recent studies have demonstrated that there is a large genetic diversity in the Bangiales which is not reflected in morphological diversity (Niwa et al. 2010, 2014; Sutherland et al. 2011).

During the review conducted for this study, it was observed that all species have 128 spermatangia packages. López-Vivas (2003) and Aguilar-Rosas et al. (2007b) confirmed the presence of 128 packages for *P. hollenbergii* spermatangia. With regard to the comparison of both species margins, the margins share the same characteristic, profusely undulated, also confirmed by Aguilar Rosas et al. (2004, 2007b). Although the original and subsequent descriptions by Krishnamurthy (Krishnamurthy 1972) mentioned that *P. hollenbergii* has a deeply ruffled margin, *P. pendula* has a prominently ruffled

Table 4 Absolute sequence differences (below diagonal) and percentage sequence divergence (above diagonal) of *rbcL* sequences from the Gulf of California *Pyropia* entities identified in this study

		Pyropia pendula					*Pyropia hollenbergii*			*Pyropia* sp. GCI						
		SR	IPM	IPS	PCY	ISPN	PC	BAV	HQ687523	LCR	IES	GAL	COY	PAR	ISJO	HQ687530
Pyropia pendula	SR		0.2	1.4	1.6	1.4	6.5	6.3	6.3	5.8	6.1	6.1	6.1	6.1	5.9	6.1
	IPM	2		1.6	1.9	1.6	6.3	6.1	6.1	5.6	5.8	5.8	5.8	5.8	5.7	5.8
	IPS	12	14		0.2	0.0	6.3	6.1	6.1	5.9	5.9	5.9	5.9	5.9	5.8	5.9
	PCY	14	16	2		0.2	6.3	6.1	6.1	5.9	5.9	5.9	5.9	5.9	5.8	5.9
	ISPN	12	14	0	2		6.3	6.1	6.1	5.9	5.9	5.9	5.9	5.9	5.8	5.9
Pyropia hollenbergii	PC	56	54	54	54	54		0.2	0.2	2.0	2.0	2.0	2.0	2.0	1.9	2.0
	BAV	54	52	52	52	52	2		0.0	1.7	1.7	1.7	1.7	1.7	1.6	1.7
	HQ687523	54	52	52	52	52	2	0		1.7	1.7	1.7	1.7	1.7	1.6	1.7
Pyropia sp. GCI	LCR	50	48	51	51	51	17	15	15		1.4	1.4	1.4	1.4	1.3	1.4
	IES	52	50	51	51	51	17	15	15	12		0.0	0.0	0.0	0.1	0.0
	GAL	52	50	51	51	51	17	15	15	12	0		0.0	0.0	0.1	0.0
	COY	52	50	51	51	51	17	15	15	12	0	0		0.0	0.1	0.0
	PAR	52	50	51	51	51	17	15	15	12	0	0	0		0.1	0.0
	ISJO	51	49	50	50	50	16	14	14	11	1	1	1	1		1
	HQ687530	52	50	51	51	51	17	15	15	12	0	0	0	0	0.1	

margin (Dawson 1953a; Krishnamurthy 1972). The main problem is that morphological characteristics are subjective, since they do not have a unit of measurement. Pedroche and Sentíes (2003) mention that although algae are very simple organisms, with easily recognizable levels of organization, a small number of characteristics for the description and characterization of species are often used, making it difficult to distinguish or differentiate species. Algae already exhibit great morphological plasticity that responds to different environmental conditions.

Most of the morphological, anatomical, and reproductive characteristics of these species are very similar to each other, such as color variations, shape, and size. According to Neefus et al. (2002), many of the characteristics are similar between species or between closely related individuals; however, the differences represent phenotypic plasticity and, in some cases, are evidence of the formation of new species. Recent studies combining biochemical, molecular, morphological, and ecological features have shown that several species of *Porphyra* form "species" or "complexes," which maintain morphological similarities but are of different taxa (Bray et al. 2006, 2007; Neefus et al. 2002).

Before this work, only extreme populations of *P. hollenbergii* had been found exclusively around the islands in the Gulf of California (Aguilar-Rosas et al. 2004; Espinoza-Ávalos 1993). In this study, *P. hollenbergii* was not found in any locality along the continental coast of Sonora (Aguilar-Rosas et al. 2004) and Sinaloa; furthermore, it has only been recorded along the southern coast of Baja California (PC and BAV). This shows that the name of *P. hollenbergii* has been widely and probably incorrectly applied in many localities of the Gulf of California. Individuals designated as *Pyropia* sp. GCI and GCII were registered only at the southern Gulf of California. The interspecific sequence divergence between *Pyropia* sp. GCI and *Pyropia* sp. GCII is 1.3 and 0.4 % for *rbc*L and 18S, respectively. However, *Pyropia* sp. GCII is distributed only in one location (LCR), which is in the middle of the distribution of the five locations of *Pyropia* sp. GCI (IES, GAL, COY, and ISJO). The geological features of LCR have played an important role so that individuals of this site have diverged from the rest of the individuals of *Pyropia* sp. GCI. LCR is located halfway through an alluvial fan of considerable dimensions, while IES, GAL, COY, and ISJO sites are not influenced by an alluvial fan of comparable importance (Antinao and McDonald 2009). In addition to the discussions above, the watershed for LCR is much greater than that of the nearby sites; hence, the flows are very different and thus the size of the alluvial fans. The presence of this alluvial fan goes back to a long period of time (Antinao and McDonald 2009) associated with the geomorphological features of the opening of the Gulf of California (Ledesma-Vázquez and Carreño 2010). Sea level change has change and drop several times in the past 1 million years (Compton 2011) while the flow of fresh water to the coast has been "persistent" in the area, extending the extent of the alluvial fan toward the ocean, on a ratio up to 1:60 (Dean 1990). Because the plants from LCR have been exposed to low salinity conditions for long time, plants must have adapted to this micro-environment which is reflected in the genetic divergence with individuals of *Pyropia* sp. GCII. Some studies have shown that the salinity is an important factor for maturation of conchocelis phase in some *Porphyra* species, indicating that the highest growth is under conditions of 25–35 ppt, while the lowest growth rate has been observed at lower (10–20 ppt) salinities (Conitz et al. 2001; Ogata and Schramm 1971; Ruangchuay and Notoya 2003). This also demonstrates the great morphological plasticity of *Pyropia* in the Gulf of California, which leads to the fact that identification of these species using morphological characteristics is not enough; therefore, it is important to integrate molecular markers to analyze seaweed distribution.

Acknowledgments JMLV acknowledges the support of CONACYT scholarship (157780) for PhD studies. This study was supported by the Mexican Council for the Sciences and Technology (CONACYT-50173Q) and the Autonomous University of Baja California (UABC-0572). We thank the support of the Connecticut Sea Grant College Program, the National Marine Aquaculture Initiative (NOAA, US DOC), and USAID TIES program between the Universidad Autónoma de Baja California and the University of Connecticut (UABC-UCONN). Thanks to Alberto Gálvez T. and Biology students at UABC and personnel from UABCS herbarium and UCONN Laboratory for their technical assistance and support during the field and laboratory work. We thank Jorge Ledesma for his helpful discussions.

References

Abbott IA, Hollenberg GJ (1976) Marine algae of California. Stanford University Press, Stanford

Aguilar-Rosas LE, Aguilar-Rosas R (2003) El género *Porphyra* (Bangiaceae, Rhodophyta) en la costa Pacífico de México. II Porphyra thuretii Setchell et Dawson. Hidrobiológica 13:159–164

Aguilar-Rosas LE, Aguilar-Rosas R, Sánchez-Rodríguez I, Broom JE, Nelson WA (2004) El género *Porphyra* (Bangiaceae, Rhodophyta) en la costa del Pacífico de México. IV. *Porphyra pendula* E.Y. Dawson. Hidrobiológica 14:121–126

Aguilar-Rosas R, Aguilar-Rosas LE, Mateo-Cid LE, Mendoza-González C (2007a) New records of marine rhodophyta from the Pacific coast of Mexico. Algae 22:153–157

Aguilar-Rosas R, Aguilar-Rosas LE, Sanchez-Rodríguez I, Broom JE, Nelson WA (2007b) Morfología y distribución de *Porphyra hollenbergii* (Bangiaceae, Rhodophyta) en la costa Pacífico de México. Rev Mex Biodivers 78:351–357

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Antinao J, McDonald E (2009) Late Quaternary alluvial fans of southern Baja California, Mexico: relation to Eastern Pacific tropical cyclones. In: American Geophysical Union, Fall Meeting 2009, abstract #T31A-1780

Bray TL, Neefus CD, Mathieson AC (2006) Morphological and molecular variability of *Porphyra purpurea* (Roth) C. Agardh

(Rhodophyta, Bangiales) from the Northwest Atlantic. Nova Hedwigia 82:1–22

Bray TL, Neefus CD, Mathieson AC (2007) A morphological and molecular investigation of the *Porphyra purpurea* (Bangiales, Rhodophyta) complex in the Northwest Atlantic. Nova Hedwigia 84:277–298

Broom JES, Jones WA, Hill DF, Knight GA, Nelson WA (1999) Species recognition in New Zealand *Porphyra* using 18S rDNA sequencing. J Appl Phycol 11:421–428

Broom JES, Nelson WA, Yarish C, Jones WA, Aguilar-Rosas R, Aguilar-Rosas LE (2002) A reassessment of the taxonomic status of *Porphyra suborbiculata*, *Porphyra carolinensis* and *Porphyra lilliputiana* (Bangiales, Rhodophyta) based on molecular and morphological data. Eur J Phycol 37:227–235

Broom JES, Farr TJ, Nelson WA (2004) Phylogeny of the *Bangia* flora of New Zealand suggests a southern origin for *Porphyra* and *Bangia* (Bangiales, Rhodophyta). Mol Phylogenet Evol 31: 1197–1207

Broom JES, Nelson WA, Farr TJ, Phillips LE, Clayton M (2010) Relationships of the *Porphyra* (Bangiales, Rhodophyta) flora of the Falkland Islands: a molecular survey using *rbc*L and nSSU sequence data. Aust Syst Bot 23:27–37

Brusca RC (1980) Common intertidal invertebrates of the Gulf of California, 2nd edn. University of Arizona Press, Tucson, AZ

Brusca RC, Findley LT, Hastings PA, Hendricks ME, Torre-Cosio J, van der Heiden AM (2005) Macrofaunal diversity in the Gulf of California. In: Cartron JLE, Ceballos G, Felger RS (eds) Biodiversity, ecosystems and conservation in northern Mexico. Oxford University Press, Oxford, pp 179–202

Codon Code Corporation (2006) Codon Code Aligner. http://www.codoncode.com/index.htm. Consulted on September 20, 2013

Compton JS (2011) Pleistocene sea-level fluctuations and human evolution on the southern coastal plain of South Africa. Q Sci Rev 30: 506–527

Conitz J, Fagen R, Lindstrom S, Gerald Plumley F, Stekoll M (2001) Growth and pigmentation of juvenile *Porphyra torta* (Rhodophyta) gametophytes in response to nitrate, salinity and inorganic carbon. J Appl Phycol 13:423–431

Dawson EY (1944) A new parasitic red algae from Southern California. Bull Torrey Bot Club 71:655–657

Dawson EY (1953a) Marine red algae of Pacific Mexico vol 17. Allan Hancock Pacific Expeditions, Los Angeles, California

Dawson EY (1953b) On the occurrence of *Gracilariopsis* in the Atlantic and Caribbean. Bull Torrey Bot Club 80:314–316

Dean R (1990) Beach response to sea level change. In: Mehaute L, Hanes B (eds) Ocean Engineering Science, vol 9. Wiley, New York, pp 869–887

Espinoza-Ávalos J (1993) Macroalgas marinas del Golfo de California. In: Salazar-Vallejo S, González N (eds) Biodiversidad Marina y Costera de México. Comisión Nacional Biodiversidad y CIQRO, Mexico, p 865

Gabrielson PW,Widdowson TB, Lindstrom CS, Hawkes MW, Scagel RF (2000) Keys to the benthic Marine Algae and Seagrasses of BritishColumbia, Southeast Alaska, Washington and Oregon. Departament of Botany. University of British Columbia, Vancouver Canada, pp 189

Hawkes MW (1977) A field, culture and cytological study of *Porphyra gardneri* (Smith & Hollenberg) comb. nov., (*Porphyrella gardneri* Smith & Hollenberg), (Bangiales, Rhodophyta). Phycologia 16: 457–469

Hawkes MW (1978) Sexual reproduction in *Porphyra gardneri* (Smith et Hollenberg) Hawkes (Bangiales, Rhodophyta). Phycologia 17:329–353

Hawkes MW (1981) *Porphyra nereocystis* and *P. thuretii* (Rhodophyta): gametophyte morphology, distribution, and occurrence. *Syesis* 14: 97–108

Hillis DM, Dixon MT (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. Q Rev Biol 66:411–453

Hollenberg GJ (1943) New marine algae from Southern California. II. Am J Bot 30:571–579

Howe MA (1911) Phycological studies V, Some marine algae of lower California, Mexico. Bull Torrey Bot Club 38:489–514

Huelsenbeck JP, Ronquist F (2001) Mr Bayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755

Jones WA, Griffin NJ, Jones DT, Nelson WA, Farr TJ, Broom JE (2004) Phylogenetic diversity in South African *Porphyra* (Bangiales, Rhodophyta) determined by nuclear SSU sequence analyses. Eur J Phycol 39:197–211

Krishnamurthy V (1972) A revision of the species of the algal Genus *Porphyra* occurring on the Pacific Coast of North America. Pac Sci 26:24–49

Kunimoto M, Kito H, Kaminishi Y, Mizukami Y, Murase N (1999) Molecular divergence of the ssu rRNA gene and internal transcribed spacer 1 in *Porphyra yezoensis* (Rhodophyta). J Appl Phycol 11: 211–216

Kurogi M (1972) Systematics of *Porphyra* in Japan. In: Abbott IA, Kurogi M (eds) Contributions to the systematics of benthic marine algae of the North Pacific. Japanese Society of Phycology, Japan, pp 167–185

Ledesma-Vázquez J, Carreño AL (2010) Origin, age, and geological evolution of the Gulf of California. In: Brusca RC (ed) The Gulf of California: biodiversity and conservation. The University of Arizona Press, Tucson, pp 7–23

Lindstrom SC, Fredericq S (2003) *rbc*L gene sequences reveal relationships among north-east Pacific species of *Porphyra* (Bangiales, Rhodophyta) and a new species, *P. aestivalis*. Phycol Res 51:211–224

López-Vivas JM (2000) Fenología de *Porphyra pendula* (Bangiales; Rhodophyta), en Punta Coyote, Baja California Sur, México. Bachelor Thesis, Universidad Autónoma de Baja California Sur

López-Vivas JM (2003) Biogeografía y taxonomía de *Porphyra* (Rhodophyta), en el Pacífico Noroccidental Mexicano. Master Science Thesis, Universidad Autónoma de Baja California Sur

López-Vivas JM, Pacheco-Ruíz I, Riosmena-Rodriguez R, Yarish C (2011) Life history of Porphyra hollenbergii Dawson (Bangiales, Rhodophyta) from the Gulf of California. México Phycologia 50: 520–529

Lynch MDJ, Sheath RG, Müller KM (2008) Phylogenetic position and ISSR-estimated intraspecific genetic variation of Bangia maxima (Bangiales, Rhodophyta) Phycologia 47:599–613

Mateo-Cid LE, Mendoza-González AC, Galicia-García C, Huerta-Múzquiz L (2000) Contribución al estudio de las algas marinas bentónicas de Punta Arena y Cabo Pulmo, Baja California Sur, México. Acta Bot Mex 52:55–73

Muñiz-Salazar R, Talbot SL, Sage GK, Ward DH, Cabello-Pasini A (2005) Population genetic structure of annual and perennial populations of *Zostera marina* L. along the Pacific coast of Baja California and the Gulf of California. Mol Ecol 14: 711–722

Neefus CD, Allen BP, Baldwin HP, Mathieson AC, Eckert RT, Yarish C, Miller MA (1993) An examination of the population 350 genetics of *Laminaria* and other brown algae in the Laminariales using starch gel electrophoresis. Hydrobiologia 260/261:67-79

Neefus CD, Mathieson AC, Klein AS, Teasdale B, Bray T, Yarish C (2002) *Porphyra birdiae* sp. nov. (Bangiales, Rhodophyta): a new species from the northwest atlatic. Algae 17:203–216

Nelson WA, Broom JE (2010) The identity of *Porphyra columbina* (Bangiales, Rhodophyta) originally described from the New Zealand subantarctic islands. Aust Syst Bot 23:16–26

Nelson WA, Farr TJ, Broom JES (2006) Phylogenetic relationships and generic concepts in the red order Bangiales: challeges ahead. Phycologia 45:249–259

Niwa K, Kobiyama A, Sakamoto T (2010) Interspecific hybridization in the haploid blade-forming marine crop Porphyra (Bangiales, Rhodophyta): occurrence of allodiploidy in surviving F1 gametophytic blades. J Phycol 46:693–702

Niwa K, Kikuchi N, Hwang MS, Choi H-G, Aruga Y (2014) Cryptic species in the *Pyropia yezoensis* complex (Bangiales, Rhodophyta): sympatric occurrence of two cryptic species even on same rocks. Phycol Res 62:36–43

Norris JN (1975) Marine algae from the northern Gulf of California. Ph.D. Dissertation, University of California

Nylander JAA, Wilgenbusch JC, Warren DL, Swofford DL (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24: 581–583

Ogata E, Schramm W (1971) Some observations on the influence of salinity on growth and photosynthesis in *Porphyra umbilicalis*. Mar Biol 10:70–76

Oliveira MC et al (1995) A preliminary investigation of the order Bangiales (Bangiophycidae, Rhodophyta) based on sequences of nuclear small-subunit ribosomal RNA genes. Phycol Res 43:71–79

Paul-Chavéz L, Riosmena-Rodríguez R (2000) Floristic and biogeographical trends in seaweed assemblages from a subtropical insular Island complex in the Gulf of California. Pac Sci 2:137–147

Pedroche FF, Sentíes AG (2003) Ficología marina mexicana. Diversidad y problemática actual. Hidrobiológica 13:23–32

Phillips SJ, Wentworth-Comus P (2000) A natural history of the Sonoran Desert. Arizona–Sonora desert museum. University of California Press, Berkeley, California

Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: advantages of Akaike Information Criterion and Bayesian Approaches over likelihood ratio tests. Syst Biol 53: 793–808

Riosmena-Rodríguez R, Paul-Chavéz L (1997) Sistemática y biogeografía de las macroalgas de la Bahía de La Paz, B.C.S. In: Urbán RJ, Ramírez MR (eds) La Bahía de La Paz, investigación y conservación. UABCS-CICIMAR-SCRIPPS, México, pp 59–82

Rocha-Ramírez V, Siqueiros-Beltrones DA (1991) El herbario ficológico de la U.A.B..C.S.: elenco floristico de macroalgas para Balandra en La Bahía de La Paz, B.C.S., México. Rev Inv Cient 2:13–34

Rodríguez-Morales EO, Siqueiros-Beltrones DA (1999) Time variations in a subtropical macroalgal assemblage from the Mexican Pacific. Oceanides 13–14:11–24

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Ruangchuay R, Notoya M (2003) Physiological responses of blade and conchocelis of *Porphyra vietnamensis* Tanaka et Pham-Hoang Ho (Bangiales, Rhodophyta) from Thailand in culture. Algae 18:21–28

Sánchez-Rodríguez I, Fajardo-León MC (1989) Estudio floristico estacional de las algas en Bahía Magdalena, B.C.S., México. Inv Mar CICIMAR 4:35–48

Sandoval-Castro E, Muñiz-Salazar R, Enriquez-Paredes L, Riosmena-Rodríguez R, Tovilla-Hernández C, Arredondo-García M (2012) Genetic population structure of red mangrove (*Rhizophora mangle* L.) along the Northwestern Mexico. Aquat Bot 99:20–26

Santamaria Del Angel E, Alvarez-Borrego S, Muller Kager FE (1999) Regiones biogeográficas del Golfo de California basadas en las imágenes del Coastal Zone Color Scanner. In: González Farias F, de la Rosa-Vélez J (eds) Temas de oceanografía biológica en México, vol II. Universidad Autónoma de Baja California, pp 63-83

Saunders GW, Kraft GT (1994) Small-subunit rRNA gene sequences from representatives of selected families of the Gigartinales and Rhodymeniales (Rhodophyta). 1. Evidence for the Plocamiales ord.nov. Can J Bot 72:1250–1263

Scagel RF (1957) An annotated list of the marine algae of British Columbia and Northern Washington. (Including keys to genera). Biological Series No. 52. Queen's printer and Controller of Stationery, Ottawa, Canada

Setchell W, Gardner N (1924) New marine algae from the Gulf of California. Proc Calif Acad Sci Ser 4(12):695–949, 77 plates

Smith GM, Hollenberg GJ (1943) On some Rhodophyceae from the Monterey Peninsula, California. Am J Bot 30:211–222

Stewart NC, Via LE (1993) A rapid CTAB DNA isolation technique useful for RAPD fingerprint and other PCR applications. Biotechniques 14:748–749

Sutherland JE, Lindstrom SC, Nelson WA, Brodie J, Lynch MDJ, Hwang MS, Choi H-G, Miyata M, Kikuchi N, Oliveira MC, Farr T, Neefus C, Mols-Mortensen A, Milstein D, Müller KM (2011) A new look at an ancient order: generic revision of the Bangiales (Rhodophyta). J Phycol 47:1131–1151

Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (*and other methods), 4th edn. Sinauer Associates, Sunderland, MA

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Teasdale B, West AL, Taylor H, Klein AS (2002) A simple restriction fragment length polymorphism (RFLP) assay to discriminate common *Porphyra* (Bangiophyceae, Rhodophyta) taxa from the Northwest Atlantic. J Appl Phycol 14:293–298

Thompson JD, Gibson TJ, Plewnial F, Jeanmougin F, Higgins DG (1997) The CLUSTAL-Windows interface: flexible strategies for multiple alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876–4882

Thomson D, Gilligan M (1983) The rocky-shore fishes. In: Case T, Cody M (eds) Biogeography in the Sea of Cortez. University of Berkeley Press, Berkeley, CA, pp 98–129

Wilgenbusch J, Warren D, Swofford D (2004) AWTY: a system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. http://ceb.csit.fsu.edu/awty

Yoshida T (1997) The history and future prospects of systematics of Bangiaceae, Rhodophyta. Nat Hist Res Special Issue 3:1–4