diversity

MDPI

Article

Molecular Assisted Identification Reveals Hidden Red Algae Diversity from the Burica Peninsula, Pacific Panama

David Wilson Freshwater [1,*], Jennifer N. Idol [1], Seth L. Parham [1], Cindy Fernández-García [2], Noemi León [3], Paul W. Gabrielson [4] and Brian L. Wysor [5]

1 Center for Marine Science, University of North Carolina at Wilmington, Wilmington, NC 28409, USA; Jennifer.Idol@jax.org (J.N.I.); sethlparham@gmail.com (S.L.P.)
2 Centro de Investigación en Ciencias del Mar y Limnlogía y Escuela de Biología, Universidad de Costa Rica, San José 2060, Costa Rica; cindy.fernandezgarcia@ucr.ac.cr
3 Departmento de Botánica, Universidad de Panamá, Panamá 4, Panamá; leon.noemi@gmail.com
4 NCU Herbarium, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3280, USA; drseaweed@hotmail.com
5 Department of Biology, Marine Biology & Environmental Science, Roger Williams University, Bristol, RI 02809, USA; bwysor@rwu.edu
* Correspondence: freshwaterw@uncw.edu; Tel.: +1-910-962-2375

Academic Editor: Rupert Ormond
Received: 31 January 2017; Accepted: 8 April 2017; Published: 14 April 2017

Abstract: The marine flora of Panama harbors a rich diversity of green, red and brown algae, and despite chronic understudy, it is reported as the second most diverse marine flora along the Pacific Central American coast, with 174 macroalgal species. Extensive new collections and molecular assisted identification (MAI) by an international team of researchers has revealed an even greater diversity for this country. Here, the intertidal and shallow subtidal marine flora of the remote Burica Peninsula is introduced. This area is characterized by an uplifted extensive intertidal flat composed of firm, sedimentary benthos known as mudrock, on which abundant algal communities thrive, even during extended periods of exposure. A collection of nearly 200 brown, green and red macroalgae specimens representing the first marine floristic inventory of this region was made in January 2011, and results of analyses of 45 foliose red algae specimens are presented. DNA sequence data for several loci (*rbc*L-3P; COI-5P; UPA) have been generated for molecular assisted identification and to guide morphological assessments. Twenty-six species were identified among the specimens including 21 new Pacific Panama records, as well as previously unrealized transisthmian distributions, and two new species, *Neorubra parvolacertoides* sp. nov. and *Grateloupia irregularis* sp. nov.

Keywords: COI-5P; DNA barcoding; marine algae; marine floristics; Pacific Panama; *rbc*L; UPA

1. Introduction

> *" . . . our surroundings seemed ideal for successful work with the marine flora—except for the one unhappy fact, which gradually became apparent, that a marine flora, in the ordinary sense, was in that region almost non-existent."*
>
> —Marshall Avery Howe [1]

The study of marine algae from the Pacific coast of Panama had an inauspicious start. An extreme tidal amplitude and often hot and dry conditions make the intertidal environment of Pacific Panama inhospitable for most marine algae and the subtidal region has only rarely been explored. This is especially true for the Gulf of Chiriqui. Taylor [2] made limited, collections of marine algae from the

middle and eastern regions of the Gulf during the 1934 and 1939 Allan Hancock expeditions to the Galapagos Islands. Only a few subsequent efforts followed in this region and substantial marine algal collections had only been made around the Coiba Island world heritage site [3,4].

The Burica Peninsula is a phycologically unexplored region in the western Gulf of Chiriqui, and Punta Burica, the southernmost point of the peninsula, is a relatively remote area accessible by motorized vehicle only at low tide (Figure 1). There are no known collecting sites or reports of marine macroalgae from this area [5]. In contrast, multiple collections had been made along the immediately adjacent southeastern coast of Costa Rica [2,6], however, these have all been from sites within Golfo Dulce and not along the western coast of the Burica Peninsula.

The Punta Burica intertidal zone is distinctive from other sites throughout the region, in being composed of compacted, fine-grained, layered sedimentary rock known as mudstone. This layered substratum has a block-in-matrix texture at various scales produced by sedimentary processes, and has been thrust at an angle to the ground (Figure 1B,C) owing to tectonic activity dating back 30–60 Ka [7,8]. It is the product of thrust sheets of the NW margin of the Caribbean plate being forced over the subducting Nazca plate, along the Cocos Ridge. The differential integrity of these tilted marine sediments, thought to be of Charcoal Azul or Armuelles formations [7], results in layers of intact benthos, separated by eroded channels that form labyrinths of interconnected channels and pools on the ebb tide (Figure 1C). The compacted sediments can be easily broken by a light hammer, but are also not compromised under the weight of a small truck. These traits appear to make the Burica intertidal an adequate habitat for the colonization of marine algae, characterized by turfs and biofilms of microfilamentous and encrusting algae that occupy exposed substrates at low tide (Figure 1D). Colonization of this habitat may be facilitated by the ability of turf algae to penetrate a porous and laminated surface that also retains moisture. In addition, the entrainment of water in small intertidal pools supports the development of small, foliose algal communities (Figure 1E).

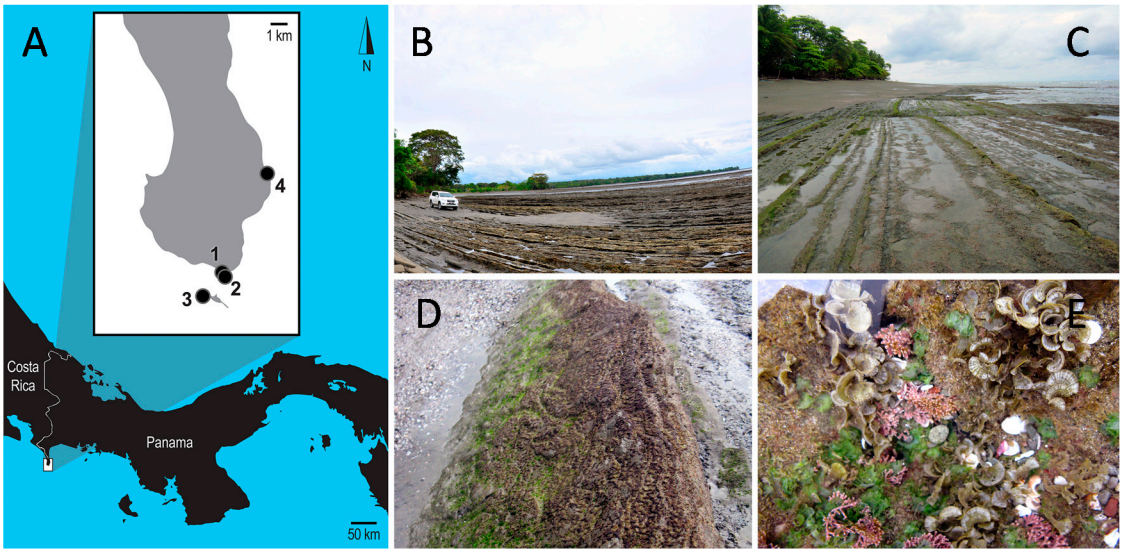

Figure 1. (**A**) Map showing the location of Punta Burica along the Pacific coast of Panama. Collection sites are shown as dots on inset: (1) intertidal mudstone substrate and tide pools, near Mono Feliz, Punta Burica; (2) shallow subtidal mixed sediment-rock substrate just beyond the surf zone, near Mono Feliz, Punta Burica; (3) subtidal rock and bolder substrate southwest of Isla Burica; (4) high intertidal rock, east side of Burica peninsula; (**B**) Vehicle on the road to Punta Burica with extensive intertidal mudstone layers exposed at low tide; (**C**) Punta Burica intertidal and shallow subtidal habitat: long, shallow tide pools formed by the erosion of intertidal mudstone layers thrust on angles and deeper channels to seaward; (**D**) Turf algal community growing on intertidal mudstone substrate; (**E**) Tide pool algal community.

Molecular Assisted Identification (MAI) uses relatively short DNA sequences to cluster specimens into species for further phylogenetic and morphological analyses [9]. Where appropriate baseline data is available, DNA sequences may be used as DNA barcodes to provide objective species identifications of specimens [10]. Saunders [11] and Robba et al. [12] introduced the five prime end of the mitochondrial marker cytochrome c oxidase subunit 1 (COI-5P) for DNA barcoding in red algae and many subsequent studies have demonstrated its utility for species delimitation and identification e.g., [13–15]. Domain V of the plastid-encoded large ribosomal subunit gene, which is also referred to as the Universal Plastid Amplicon (UPA) and the RuBisCO large subunit gene (*rbc*L), specifically the 3′ end of this locus (*rbc*L-3P) are other DNA sequences that have been used to delimit and identify red macroalgae e.g., [16–18].

Here MAI results based on three DNA loci (*rbc*L-3P, COI-5P and UPA) and morphological characters for foliose marine red macroalgae collected from Punta Burica, Panama are presented, and when suitable background data is available, their phylogenetic relationships explored. An unrealized diversity of species including many new records for Pacific Panama and the eastern tropical Pacific are revealed. Two new species are described and others identified that may represent new species, but require additional study. That 26 species are documented among 45 specimens, from a preliminary investigation spanning only 3 days of field work, foreshadows a likely productivity of further field investigations coupled with MAI methods, and contradicts the apparent "*non-existent*" diversity mentioned by Howe [1].

2. Materials and Methods

Marine macroalgae were collected at four sites around Punta Burica, Panama during 8–10 January 2011 (Figure 1A). Specimens were sorted in the field, a herbarium specimen made and a portion of the thallus dried with silica gel desiccant [19]. When specimens were small and possibly included a mixture of species, the entire collection was silica gel dried, brought back to the lab, and sorted after rewetting. Vouchers (herbarium specimens and/or permanent slides) for all collections were accessioned in the David J. Seiren Herbarium at the University of North Carolina Wilmington (WNC), and duplicates deposited in the herbaria of the University of Panama (UPAN), the University of Costa Rica (USJ), and Roger Williams University. Specimen collection data is publically available at doi: www.dx.doi.org/10.5883/DS-RAPBPAN.

Silica gel dried specimens were rewet with seawater and sorted to insure monospecificity of samples. Total genomic DNA was extracted from these samples following the protocol of Hughey et al. [20] or using the illustra Nucleon Phytopure Genomic DNA Extraction Kit (GE Healthcare, Pittsburgh, PA, USA). Three different loci UPA, COI-5P and *rbc*L-3P were targeted for amplification and sequencing in this study. PCR amplifications were set up and run as described in Freshwater et al. [21] and Mamoozadeh and Freshwater [9]. Successful amplifications were cleaned using a StrataPrep PCR Purification Kit (Stratagene, La Jolla, CA, USA) and used as templates in BigDye Terminator v.3 (ThermoFisher Scientific, Foster City, CA, USA) sequencing reactions. Sequence reactions were cleaned with a Zymo DNA Sequencing Clean up Kit (Zymo Research, Irvine, CA, USA) and run on an ABI 3130*xl* Genetic Analyzer (ThermoFisher Scientific). Primers used in amplification and sequencing reactions are shown in Table 1. Amplifications with the *rbc*L-3P primer pair also spanned the *rbc*L-*rbc*S spacer region, but this locus was specifically analyzed for only the collected *Asparagopsis* specimens.

Table 1. Oligonucleotide primers used in the amplification and sequencing of three loci from marine red algae of Punta Burica, Panama.

Locus	Primer	Sequence	Citation
COI-5P	GHalF	TCAACAAATCATAAAGATATYGG	[22]
	GazR1	ACTTCTGGATGTCCAAAAAAYCA	[11]
	GWSFn	TCAACAAAYCAYAAAGATATYGG	[13]
	GWSRx	ACTTCTGGRTGICCRAARAAYCA	[23]
*rbc*L-3P	F753	GGAAGATATGTATGAAAGAGC	[24]
	RrbcSstart	GTTCCTTGTGTTAATCTCAC	Modified from [24]

Table 1. *Cont.*

Locus	Primer	Sequence	Citation
5P-*rbc*L [1]	F57	GTAATTCCATATGCTAAAATGGG	[24]
	R893	GAATAAGTTGARTTWCCIGCAC	[25]
UPA	p23SrV-f1	GGACAGAAAGACCCTATGAA	[16]
	p23SrV-r1	TCAGCCTGTTATCCCTAGAG	[16]

[1] Used to generate the larger *rbc*L segments for *Polysiphonia sensu lato* species.

Individual sequence reactions were compiled and edited using Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA) and final sequences accessioned in the BOLD and GenBank databases. Accession numbers and sequences are available at doi: dx.doi.org/10.5883/DS-RAPBPAN. Alignments of the different loci were constructed using MUSCLE [26] as implemented in the Geneious software package (Biomatters Limited, Aukland, New Zealand) and adjusted by eye. UPGMA cluster diagrams were generated from alignments of the three loci with the Geneious tree builder option and Jukes-Cantor distances. These analyses were done exclusively to cluster sequences by similarity for genetic species definition, and UPGMA was used explicitly to indicate that phylogenetic relationships were not inferred. Closest taxonomic affinities of the cluster-analyses defined genetic species were determined by BLAST [27] queries. DNA sequence data sets to examine the phylogenetic relationships of individual species were compiled from GenBank, aligned using MUSCLE and analyzed with RAxML [28] and MrBayes [29] as implemented in Geneious. Data matrix dimensions and specific methodologies for these analyses are included in Supplementary Material Table S1.

3. Results

3.1. Overall "Barcoding" Cluster Analyses

Forty-five samples of foliose red algae were included in this study, and sequence data for at least two of the three targeted loci were generated for all but two of the samples (Table 2). Cluster analyses of the UPA data resulted in 24 clusters or individual sequences at least 1.1% different from the next closest cluster/sequence (Figure 2A). These clusters and individual sequences were considered to represent sequence-based species. Sequence variation within clusters of multiple specimens ranged from 0–0.5%. The maximum intra-cluster value was for the *Gelidium* specimens that *rbc*L-3P and COI-5P analyses indicated were different species and the next highest intra-cluster sequence variation was 0.3%. Cluster analysis of *rbc*L-3P also revealed 24 sequenced-based species (Figure 2B). Intra-cluster sequence variation ranged from 0–0.5%, and clusters or individual sequences were at least 0.9% different. As with the UPA data, the maximum intra-cluster variation value was between specimens of *Ceratodictyon* that formed distinct clusters in UPA and COI-5P analyses. The next highest *rbc*L-3P intra-cluster variation value was only 0.1%. The species identified by cluster analyses of these two data sets were identical except that the UPA cluster diagram did not clearly differentiate the two *Gelidium* species collected, and the *rbc*L-3P cluster diagram did not clearly differentiate the two *Ceratodoctyon* species collected. The analysis of the COI-5P data clearly differentiated 26 sequenced-based species (Figure 2C) including all those in the UPA and *rbc*L-3P data. Intra-cluster variation ranged from 0–0.3%, and clusters or individual sequences were at least 6.5% different.

Initial assessments by BLAST searches provided identifications at the genus level for all sequenced specimens (Table 2), but in some cases investigation of the current generic nomenclature was required because of changes that had occured since the submission of sequences into GenBank. Examples of this were the specimens of *Hommersandiophycus* and *Ceratodictyon*. Positive species identifications based on BLAST results were also possible for some specimens. The study of *Centroceras* by Won et al. [30] populated GenBank with *rbc*L sequence data for multiple species and provided the contex by which positive identifications of PHYKOS-4594 and PHYKOS-4600 as *C. gasparrini* were made. Similarly, Grusz and Freshwater [31] and Boo et al. [32] deposited *rbc*L and COI sequences for *Gelidium sclerophyllum* type specimens in GenBank and this allowed the positive identification of PHYKOS-4514, 4629, and 4645 as this species.

Table 2. MAI Identification, PHYKOS database collection number and closest BLAST result for 45 red algal collections from Punta Burica, Panama. Values in parentheses represent BLAST maximum identity scores. [1] First report from Pacific Panama; [2] First report from the tropical East Pacific. " = BLAST result is identical to that of the previous PHYKOS number.

	PHYKOS	*rbc*L-3P	COI-5P	UPA
MAI ID	**Coll. No.**	**BLAST**	**BLAST**	**BLAST**
[1] *Tricleocarpa cylindrica* (J. Ellis and Solander) Huisman and Borowitzka	4617	*Tricleocarpa cylindrica* (98%)	-	*Dichotomaria marginata* and *Galaxaura rugosa* (97%)
	4634	„	*Tricleocarpa cylindrica* (96%)	„
[1,2] *Hommersandiophycus borowitzkae* (Huisman) S.-M. Lin and Huisman	4618	*Hommersandiophycus borowitzkae* (99%)	*Ganonema yoshizakii* (91%)	*Ganonema yoshizaki* and *Hommersandiophycus borowitzkae* (99%)
[1] *Izziella* sp.	4576	*Izziella formosana* (98%)	*Izziella orientalis* (95%)	*Izziella formosana* (100%)
	4599	-	„	„
[1] *Liagora ceranoides* J.V. Lamouroux	4614	*Liagora ceranoides* (100%)	*Liagora* sp. (96%)	*Liagora ceranoides* (99%)
[1] *Neoizziella asiatica* S.-M. Lin, S.-Y. Yang and Huisman	4619	*Neoizziella asiatica* (100%)	*Neoizziella divaricata* (99%)	*Neoizziella asiatica* (100%)
[1] *Gelidium sclerophyllum* W.R. Taylor	4514	-	*Gelidium sclerophyllum* (98%)	*Gelidium sclerophyllum* (100%)
	4629	*Gelidium sclerophyllum* (100%)	-	„
	4645	„	„	„
[1] *Gelidium* sp.	4564	*Gelidium floridanum* and *G. sclerophyllum* (99%)	*Gelidium floridanum* and *Gelidium* sp. (94%)	*Gelidium sclerophyllum* (99%)
	4566	„	„	„
	4597	„	„	„
Millerella sp.	4570	*Millerella myriocladus*, *M. tinerfensis* and *M. felicinii* (93%)	*Milerella myriocladus* (86%)	*Millerella* sp. (97%)
Asparagopsis sp.	4636	*Asparagopsis taxiformis* (97%)	*Asparagopsis taxiformis* (100%)	*Asparagopsis taxiformis* (100%)
	4646	-	-	„
[1] *Plocamium sp.*	4643	*Plocamium pacificum* [as *P. cartilagineum*] (99%)	*Plocamium pacificum* (95%)	*Plocamium cartilagineum* and *P. telfairiae* (99%)
[1,2] *Hypnea flava* Nauer, Cassano and M.C. Oliveira	4574	*Hypnea flava* (99%)	*Hypnea 'spinella'* (96%)	*Hypnea 'spinella'* (99%)
Hypnea pannosa J. Agardh	4631	*Hypnea pannosa* (100%)	*Hypnea panosa* (99%)	*Hypnea nudifica* + 4 others (99%)
[1,2] *Neorubra parvolacertoides* sp. nov.	4528	*Neorubra decipiens* (96%)	*Grateloupia angusta*, *G. taiwanensis* (91%)	*Grateloupia phuquocensis* + 6 others (98%)
	4589	„	„	„

Table 2. *Cont.*

	PHYKOS	*rbc*L-3P	COI-5P	UPA
MAI ID	**Coll. No.**	**BLAST**	**BLAST**	**BLAST**
[1,2] *Grateloupia irregularis* sp. nov.	4522	*Grateloupia dichotoma* and *G. filicina* (97%)	*Prionitis filiformis* (92%)	*Grateloupia phuquocensis* (99%)
	4556	„	„	„
	4620	„	„	„
[1] *Ceratodictyon repens* (Kützing) R.E. Norris	4627	-	*Ceratodictyon scoparia* (94%)	*Ceratodictyon scoparia* (99%)
	4632	*Ceratodictyon repens* (100%)	„	„
	4635	„	„	„
[1,2] *Ceratodictyon scoparium* (Montagne and Millardet) R.E. Norris	4590	*Ceratodictyon* sp. 'Calerita' (100%)	*Ceratodictyon scoparium* (99%)	*Ceratodictyon scoparium* (100%)
[1] *Gracilaria* sp.1	4640	*Gracilaria tikvahiae*, *G. cuneifolia* and *G. isabellana* (97%)	*Gracilaria incurvata* (94%)	*Gracilaria parvispora* + 6 others (99%)
[1] *Gracilaria* sp.2	4519	-	*Gracilaria galetensis* (92%)	*Gracilaria galetensis* (98%)
	4541	*Gracilaria galetensis* (97%)	„	„
	4630	„	„	„
[1] *Gracilaria* sp.3	4548	*Gracilaria damaecornis*, *G. isabellana*, *G. chouae* and *G. parvispora* (98%)	*Gracilaria isabellana*, *G. caudata* (94%)	*Gracilaria parvispora* + 7 others (99%)
[1,2] *Aglaothamnion* sp.	4553	*Aglaothamnion halliae* and *A. hookeri* (96%)	*Callithamnion tetragonum* and *Aglaothamnion* sp. (91%)	*Callithamnion corymbosum* and *Aglaothamnion* spp. (96%)
Centroceras gasparrinii (Meneghini) Kützing	4594	*C. gasparrini* (100%)	*Centroceras clavulatum* (94%)	*Centroceras* sp. (99%)
	4600	„	„	„
[1,2] *Spyridia* sp.	4584	-	*Spyridia filamentosa* (95%)	-
[1] *Melanothamnus* sp.	4568	*Melanothamnus pseudovillum* (98%)	*Melanothamnus pseudovillum* (97%)	*Melanothamnus* spp. and *'Polysiphonia'* spp. (98%)
[1,2] *'Polysiphonia' binneyi* Harvey	4534	*'Polysiphonia' binneyi* (99%)	*'Polysiphonia' echinata* (93%)	*'Polysiphonia'* sp. (97%)
[1,2] *'Polysiphonia'* sp.	4515	*'Polysiphonia'* sp. (99%)	*'Polysiphonia'* sp. (99%)	*Herposiphonia* sp., *Symphyocladia latiuscula* (97%)
	4525	„	„	„
	4526	„	„	„
	4552	„	-	„
Wilsonosiphonia howei (Hollenberg) D. Bustamante, Won and T.O. Cho	4657	*Wilsonosiphonia howei* (99%)	-	*Wilsonosiphonia howei* (99%)
	4663	„	*Wilsonosiphonia howei* (97%)	-

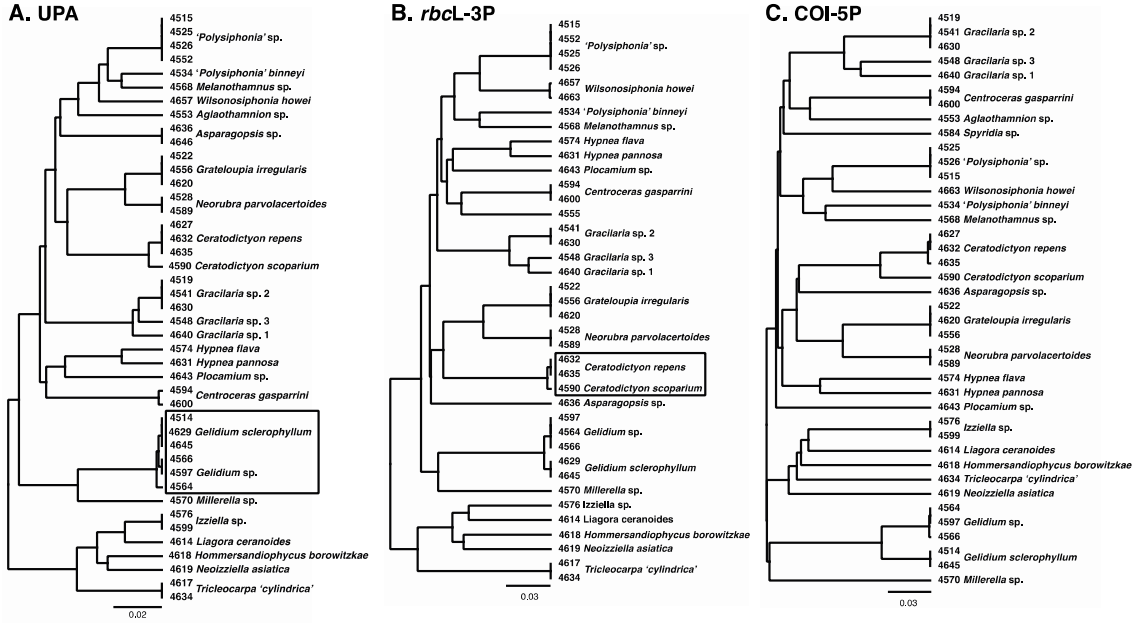

Figure 2. UPGMA cluster diagrams for three sequenced loci: (**A**) UPA, (**B**) *rbc*L-3P, (**C**) COI-5P. Boxed species were not clearly separated by the analysis.

3.2. Species Treatments

3.2.1. *Tricleocarpa cylindrica* (J. Ellis and Solander) Huisman and Borowitska

Sequences from PHYKOS-4617 and PHYKOS-4634 were found to be closest matches to *Tricleocarpa* species, with a closest homology to *rbc*L-3P and COI-5P sequences of specimens identified as *Tricleocarpa cylindrica* (Table 2). Phylogenetic analyses of *Tricleocarpa* species *rbc*L-3P sequences resolved the Punta Burica specimens within a *T. cylindrica* clade that includes specimens from the Indo-Pacific, Hawaiian Islands and Caribbean (Figure 3A). The Punta Burica specimens were weakly grouped (maximum likelihood bootstrap [ML] = 76%; Bayesian posterior probabilities [PP] = 0.84) with specimens from Thailand, Indonesia and Guadeloupe within this clade. COI-5P data is available for most of these same specimens and analyses of this locus more strongly supports (ML = 96; PP = 0.91) this relationship (Figure 3B). Percent divergences among *T. cylindrica rbc*L-3P and COI-5P sequences ranged from 0.0–2.0% and 0.0–7.3%, respectively. Pairwise divergences between the Punta Burica specimens and the most closely allied specimens in the phylogenetic analyses were 1.5–2.0% for *rbc*L-3P and 1.6–4.7% for COI-5P.

The specimens were collected from two different habitats. PHYKOS-4617 was growing on mudstone substrate in tide pools along the Punta Burica shoreline, while PHYKOS-4634 was collected from subtidal boulders at 3–5 m depth off the southwest point of Isla Burica (Figure 1). The specimens were heavily calcified except at the non-calcified joints, and had dichotomously branched cylindrical axes that were constricted at branch points (Figure 4A,B). They had relatively few, longitudinally oriented medullary cell filaments; inflated subsurface cells, and fused, polygonal surface cells (Figure 4C).

Remarks: Previous studies [33,34] found that specimens of *Tricleocarpa* from a wide geographic range matched the morphology described for *T. cylindrica* [35], but varied greatly in their *rbc*L and COI sequences, suggesting the presence of cryptic species. Both PHYKOS-4617 and PHYKOS-4634 matched the morphological concept of *T. cylindrica*, and they are resolved with varying levels of support in a clade that includes specimens from the Caribbean where the type was collected. However, the level of sequence variation between the Punta Burica specimens and other specimens in this clade are greater than the intraspecific values that have been found in many studies i.e., [36,37], and although

T. cylindrica is assigned to the Punta Burica specimens here, a world-wide reassessment of the species is needed to determine its true distribution. *Tricleocarpa cylindrica* has been recorded previously from both the Caribbean and Pacific coasts of Central America e.g., [5,38,39]. This is the first report of the species from Pacific Panama.

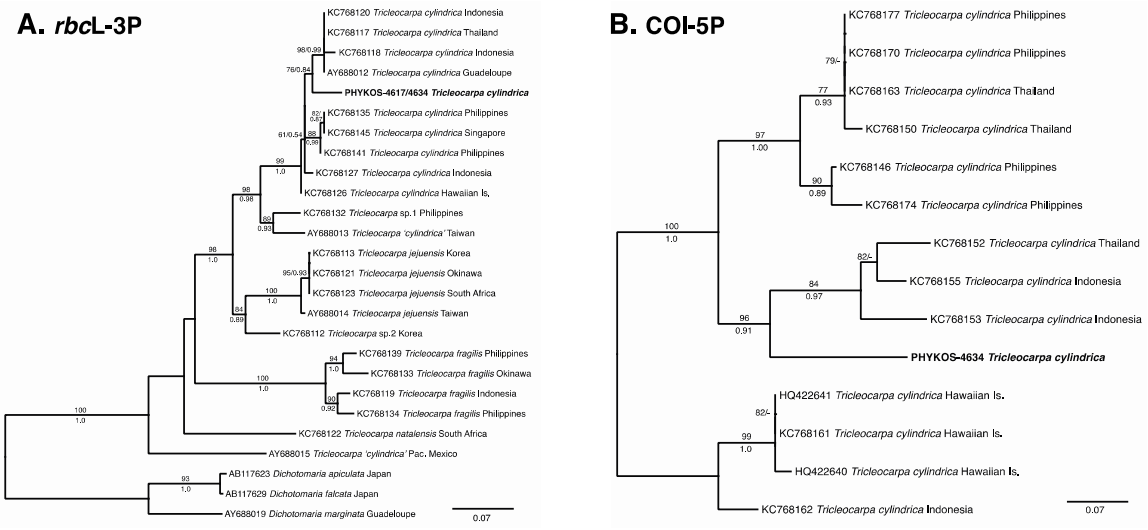

Figure 3. Maximum likelihood trees for the (**A**) *rbc*L-3P alignment of *Tricleocarpa* species and (**B**) *T. cylindrica* specimen COI-5P sequences. Bootstrap support and Bayesian posterior probability values are given for branches when >70% and >0.75, respectively.

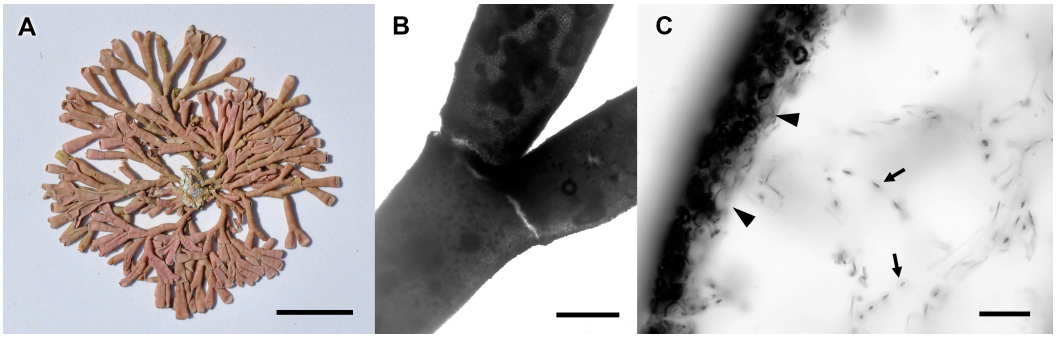

Figure 4. *Tricleocarpa cylindrica*. (**A**) Herbarium specimen, scale = 1 cm; (**B**) Surface view of whole mount showing non-calcified joints at branching point, scale = 500 μm; (**C**) Transverse section with widely spaced medullary filaments (arrows) and inflated subsurface cells (arrowheads), scale = 50 μm.

3.2.2. *Hommersandiophycus borowitzkae* (Huisman) S.-M. Lin and Huisman

BLAST searches of the *rbc*L-3P sequence for PHYKOS-4618 revealed a 99% similarity with specimens of *Hommersandiophycus borowitzkae*. The UPA sequence BLAST showed a 99% similarity with specimens of both *H. borowitzkae* and *Ganonema yoshizaki Huisman*, I.A. Abbott and A.R. Sherwood, and the COI-5P BLAST showed no close homology (Table 2). *Hommersandiophycus* species were resolved as a strongly to fully supported clade (maximum likelihood bootstrap [ML] = 99%; Bayesian posterior probabilities [PP] = 1.00) in phylogenetic analyses of *rbc*L-3P sequences for Liagoraceae species (Figure 5). PHYKOS-4618 is positioned in a clade with *H. borowitzkae* specimens from Taiwan and The Philippines (ML = 87%; PP = 1.00) that is sister to *H. samaensis* (C.K. Tseng) Showe M. Lin and Huisman. The Western Pacific *H. borowitzkae* specimens shared identical *rbc*L sequences that were only 0.2% different from that of the Panama specimen. Divergences between *H. borowitzkae* specimens and sequences available from other *Hommersandiophycus* species ranged from 3.9–9.1%.

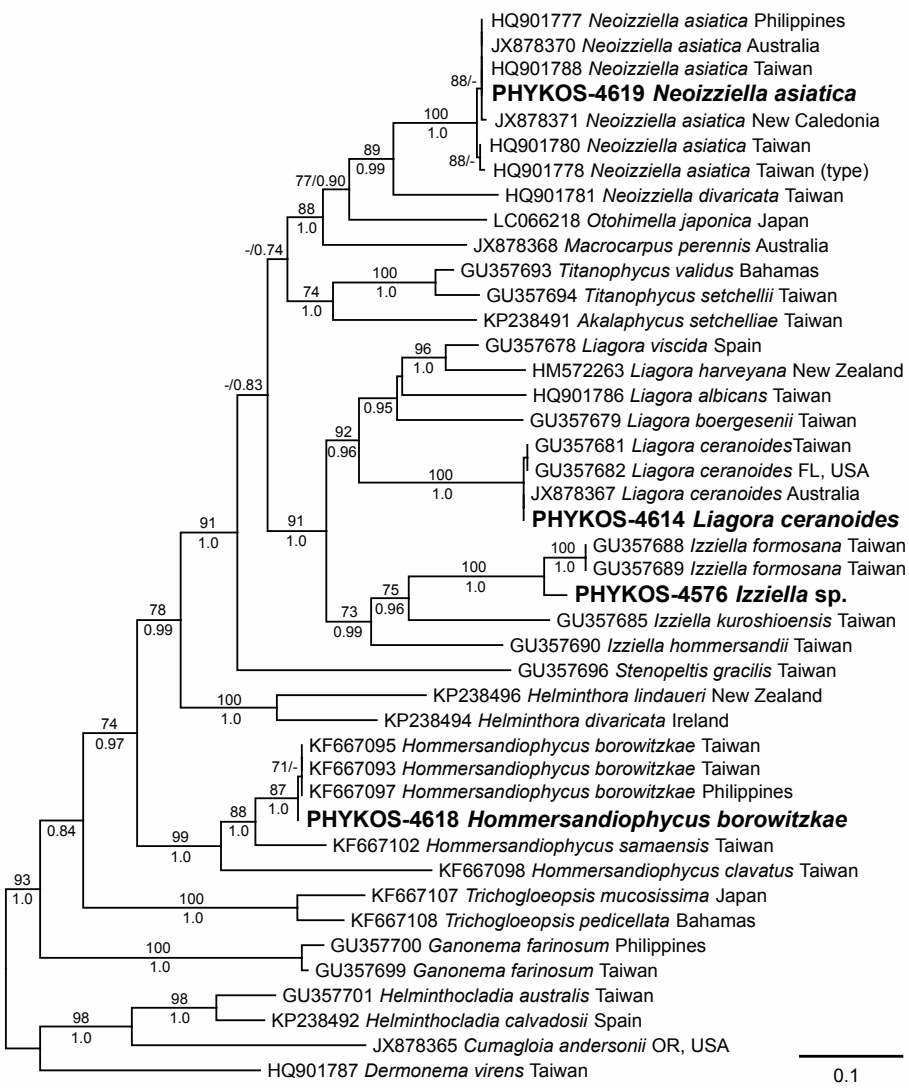

Figure 5. Maximum likelihood tree for Liagoraceae species *rbc*L-3P sequences with Punta Burica species shown in bold font. Bootstrap support and Bayesian posterior probability values are given for branches when >70% and >0.75, respectively.

PHYKOS-4618 was collected from mudstone substrate in a tide pool along the Punta Burica shoreline (Figure 1).

Remarks: Hommersandiophycus borowitzkae was originally described as *Ganonema borowitzkae* Huisman based on Western Australia specimens [40]. Lin et al. [41] established *Hommersandiophycus* for *G. borowitzkae* and two other *Ganonema* species after molecular and morphological analyses resolved these species in a distinct clade with a number of synapomorphic characters states for carpogonial branches and carposporophytes. The examined PHYKOS-4618 specimen was male and matched the description of spermatangia initials being cut off from subapical cells and developing in clusters [41] (Figure 6A,B). The species was known only from East Asia (Taiwan, The Philippines) and Western Australia, and this is the first report from the Eastern Pacific.

3.2.3. *Izziella* sp.

Sequences of PHYKOS-4576 and PHYKOS-4599 shared identical COI-5P and UPA sequences, but an *rbc*L-3P sequence was only generated from PHYKOS-4576. These sequences were found to have closest homology to sequences from *Izziella formosana* (Yamada) Showe M.Lin, S.-Y.Yang and Huisman

(*rbc*L-3P, UPA) and *I. orientalis* (J. Agardh) Huisman and Schils (COI-5P) in BLAST searches (Table 2). An *Izziella* clade received weak (ML = 73%) to strong (PP = 0.99) support in the Liagoraceae *rbc*L-3P analyses (Figure 5). PHYKOS-4576 was fully supported as the sister taxa to *I. formosana* and *rbc*L-3P sequence divergences between the sequenced specimens were only 2.4%. The only COI-5P sequence data available in GenBank is for three specimens from the Hawaiian Islands identified as *I. orientalis* that share identical sequences. The COI-5P sequence divergence between specimens from Panama and Hawai'i is 5%.

The PHYKOS-4576 and PHYKOS-4599 specimens were collected from intertidal pools along the Punta Burica shoreline (Figure 1). They were 8–9 cm in height and irregularly branched with many short branchlets (Figure 6C). Assimilatory filaments had cylindrical lower cells and ellipsoidal to obovoid upper cells (Figure 6D).

Remarks: Four *Izziella* species are currently recognized and distinguished by size, branching and specifics of carpogonial branches and involucral filaments [42,43]. Two of these species, are known only from Taiwan. *Izziella orientalis* has been recorded in both the Indian and Pacific Oceans, while *I. formosana*, originally described from Taiwan, was also recently reported from Pacific Costa Rica [39]. This is the first report of an *Izziella* species from Pacific Panama. Few Liagoraceae are reported along the Pacific coast of Central America [5], and the family is little studied in the region. The Punta Burica specimens likely represent a new *Izziella* species, but a better understanding of Eastern Pacific Liagoraceae is needed to make this determination.

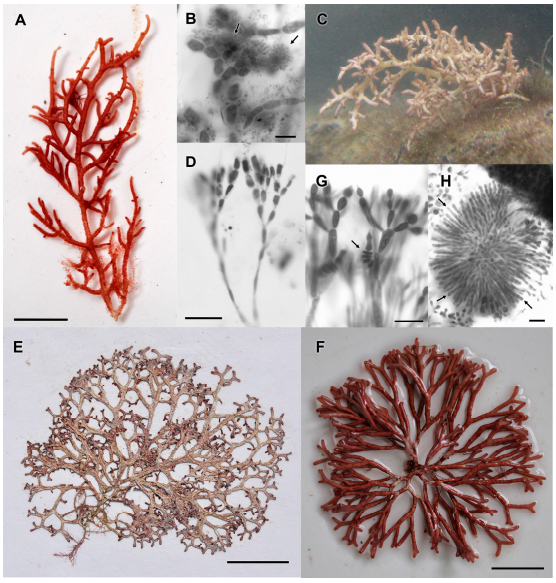

Figure 6. Liagoraceae species of Punta Burica. (**A**) Habit of *Hommersandiophycus borowitzkae*, scale = 1 cm; (**B**) Clusters of spermatangia (arrows) developing on *H. borowitzkae* assimilatory filaments, scale = 20 μm; (**C**) *Izziella* sp. in situ; (**D**) *Izziella* sp. assimilatory filaments, scale = 50 μm; (**E**) *Liagora ceranoides* habit, scale = 1 cm; (**F**) *Neoizziella asiatica* habit, scale = 1 cm; (**G**) *N. asiatica* assimilatory filament with 5-celled carpogonial branch (arrow), scale = 20 μm; (**H**) *N. asiatica* carposporophyte with terminal chains of carposporangia (arrows), scale = 20 μm.

3.2.4. *Liagora ceranoides* J.V. Lamouroux

BLAST searches with the PHYKOS-4614 sequences revealed a closest affinity with specimens identified as *Liagora ceranoides* (*rbc*L-3P), *Liagora* sp. (COI-5P), and *L. ceranoides* (UPA) (Table 2). *Liagora* species formed a well supported monophyletic clade (ML = 92%; PP = 0.96) in the Liagoraceae *rbc*L-3P analyses (Figure 5). PHYKOS-4614 was resolved within a clade of *L. ceranoides* specimens collected from multiple geographic locations. The *rbc*L-3P sequence divergences within this clade were only 0.0–0.3% despite the wide geographic separation of the analyzed specimens.

PHYKOS-4614 was collected from mudstone stubstrate in tide pool along the Punta Burica shoreline (Figures 1C and 6C). It was moderately calcified and relatively small (3 cm high), with sparse, irregular branching proximally and prolific, widely dichotomous branching distally (Figure 6E). The ultimate branches were generally short, 1–2 mm diameter tapering to 0.5 mm diameter apices.

Remarks: Morphologically PHYKOS-4614 closely matched descriptions of *L. ceranoides* [44,45]. *Liagora ceranoides* was originally described from the Virgin Islands in the Western Atlantic, and it has been reported in tropical waters around the globe. Although Lin et al. [45] demonstrated that some specimens identified as *L. ceranoides* in the Indo-Pacific represented a different genus and species, they also showed that *L. ceranoides* was distributed in Indo-Pacific as well as Western Atlantic. This species has been reported from the Pacific coast of Costa Rica [5]. Taylor [2] stated that it was common in Golfo Dulce, Costa Rica, which opens to the Pacific on the western side of the Burica penninsula, but it was not found during recent sampling.

3.2.5. *Neoizziella asiatica* Showe M. Lin, S.-Y. Yang and Huisman

The *rbc*L and UPA sequences of PHYKOS-4619 were found by BLAST searches to match sequences of *Neoizziella asiatica* specimens. BLAST searches of the PHYKOS-4619 COI-5P sequences returned a 99% sequence similarity to an Hawaiian specimen identified as *N. divaricata* (C.K. Tseng) Showe M. Lin, S.-Y. Yang and Huisman. This is the only *Neoizziella* COI-5P sequence available in GenBank and may represent a mis-identified specimen of *N. asiatica*. Sequences of *rbc*L-3P for PHYKOS-4619 and *N. asiatica* specimens from geographically diverse locations in the western Pacific were resolved in a fully supported clade that was well supported (ML = 89%; PP = 0.99) as the sister species to *N. divaricata* in the Liagoraceae *rbc*L-3P phylogeny (Figure 5). Pairwise sequence divergences among PHYKOS-4619 and the western Pacific *N. asiatica* specimens ranged from 0.0–0.6%, and these sequences were 5.9–6.2% divergent from the two available Taiwan *N. divaricata rbc*L sequences.

The specimen was collected from a tide pool along the Punta Burica shoreline (Figure 1). It was attached to the substrate by a discoid holdfast, and axes had 6–7 orders of dichotomous to subdichotomous branching and untapered ultimate branches with obtuse apicies (Figure 6F). The specimen was a female gametophyte with many carpogonial branches of 5–6 cells and mature carposporophytes (Figure 6G,H).

Remarks: PHYKOS-4619 matches the morphological descriptions of *N. asiatica* [46] except in its stature, which was less than 3 cm, and number of carpogonial branch cells, which was 5–6 versus the 4–5 reported by Lin et al. [46]. *Neoizziella* currently includes only two species, *N. asiatica* and *N. divaricata* [42,46]. A recent Costa Rica record of *Neoizziella asiatica* was the first from outside of the Indo-Pacific region [39]. This is the first report of the species from Pacific Panama.

3.2.6. *Gelidium sclerophyllum* W.R. Taylor

PHYKOS-4514, PHYKOS-4629, and PHYKOS-4645 shared identical COI-5P and UPA sequences. An *rbc*L-3P sequence was not generated for PHYKOS-4514, but PHYKOS-4629 and 4645 also shared identical sequences at this locus. BLAST searches indicated exact matches and 98% identity with COI sequences from Pacific Costa Rica specimens of *Gelidium sclerophyllum* (Table 2).

All three collections were made from subtidal hard substrates. PHYKOS-4514 was collected from the mixed sediment-mudstone bottom just beyond the surf zone off the Punta Burica shoreline. PHYKOS-4629 and PHYKOS-4645 were collected from subtidal boulders at 3–5 m depth off the southwest point of Isla Burica (Figure 1).

Remarks: The *rbc*L-3P and COI-5P sequences of the Punta Burica specimens and Costa Rica GenBank records have been tied to a partial *rbc*L [31] and full *rbc*L and COI [32] sequences from type specimens providing positive identifications. *Gelidium sclerophyllum* and *G. floridanum* W.R. Taylor have been consistently resolved together in a strongly supported clade that increased sampling is revealing to represent a closely related species complex [31,32,47]. *Gelidium sclerophyllum* has been reported from Mexico south to Ecuador, but this is the first report from Panama. A detailed description

of the species was recently provided by Grusz and Freshwater [31], and the Punta Burica specimens match this description including the characteristic wide sterile margin around tetrasporangial sori and axes frequently with emarginate apices (Figure 7A,B).

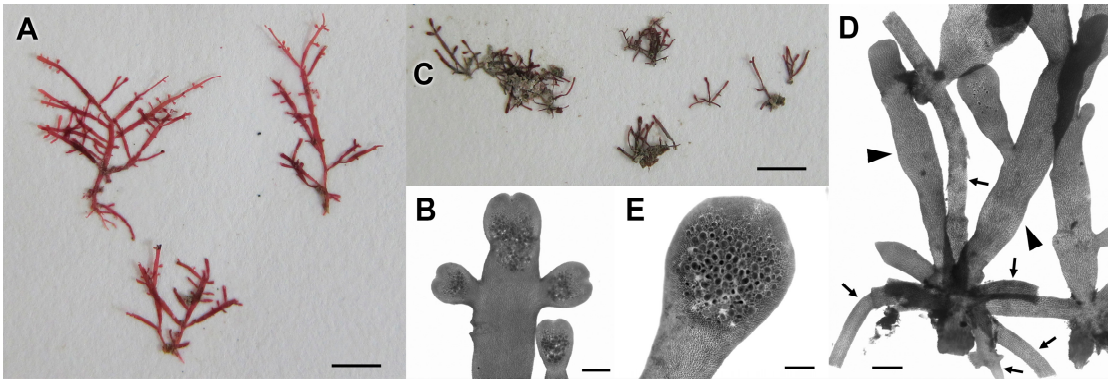

Figure 7. *Gelidium sclerophyllum* and *Gelidium* sp. (**A**) *G. sclerophyllum* habit, scale = 500 μm; (**B**) *G. sclerophyllum* tetrasporangial sori on erect axis and branchlets showing emarginate apices, and wide sterile margins surrounding sori, scale = 200 μm; (**C**) *Gelidium* sp. habit, scale = 500 μm; (**D**) *Gelidium* sp. branching point of stoloniferous branch where multiple prostrate (arrows) and erect axes originate, scale = 200 μm; (**E**) *Gelidium* sp. tetrasporangial sorus at the tip of an erect axis, scale = 100 μm.

3.2.7. *Gelidium* sp.

PHYKOS-4564, PHYKOS-4566, and PHYKOS-4597, shared identical sequences for the three tested loci except for PHYKOS-4566 where the COI sequence was one base pair different from the other two sequences. BLAST searches revealed a close affinity with *Gelidium floridanum* and *G. sclerophyllum* (Table 2), and this Punta Burica *Gelidium* species is believed to be part of a complex of closely related species.

All three collections were made from the intertidal mudstone sustrate along the Punta Burica shoreline (Figure 1) where they grew as a short turf. The thalli were composed of an extensive system of tangled stoloniferous axes that gave rise, sometimes in clusters, to generally short (<5 mm), simple to irregularly branched erect axes (Figure 7C,D), although some taller axes with more regular distichous branching were observed. The latter were similar in outline to small specimens of *G. sclerophyllum* but lacked emarginate tips and the sometimes swollen sterile margins around tetrasporangial sori (Figure 7E).

Remarks: Phylogenetic analyses of *rbc*L-3P revealed four separate groupings of specimens within this clade (Figure 8). These include a well supported *G. floridanum*, a strongly supported clade of specimens from Brazil and one from Caribbean Costa Rica that was originally identified as *G. floridanum*, a weakly supported clade of *G. sclerophyllum* specimens from Punta Burica, Pacific Costa Rica and Galapagos Islands, and the clade of *Gelidium* sp. specimens collected from Punta Burica. Thomas and Freshwater [48] suggested based on *rbc*L sequence divergences and slight morphological differences, that there was a genetic dicontinuity between specimens they identified as *G. floridanum* from Caribbean Costa Rica and *G. floridanum* in the Western Atlantic. Recent species delimination analyses with both UPA and COI-5P data distinguished *G. floridanum* from a closely related sister species that included the Caribbean Costa Rica *G. 'floridanum'* [18], and this separation was also present in the analyses here (Figure 8). *Gelidium sclerophyllum* and *G. floridanum* are considered to represent geminate sister species with a transisthmian distribution [31]. Our current analyses suggest that these three species are part of a complex of closely related taxa that also includes the second unidentified Punta Burica *Gelidium* species. Analyses of additional specimens and the inclusion of more variable COI data are required to determine the exact number and relationships of the species involved in this complex.

Gelidium pusillum (Stackhouse) Le Jolis was the only *Gelidium* species recorded from Pacific Panama by Taylor [2], and Littler and Littler [49] included both *G. pusillum* and *G. pusillum* var. *pacificum* W.R. Taylor in their web database. The Punta Burica *Gelidium* species has the general morphological characteristics that were associated with the previous concept of a widely distributed *G. pusillum*, however, the current concept of *G. pusillum* restricts its distribution to the North Atlantic [50]. A number of small *Gelidium* and *Pterocladiella* species are reported from the eastern tropical Pacific [5], but they are generally poorly known. Additional morphological and molecular analyses are required to determine the relationship of this species to others reported in the region and if it represents a new species.

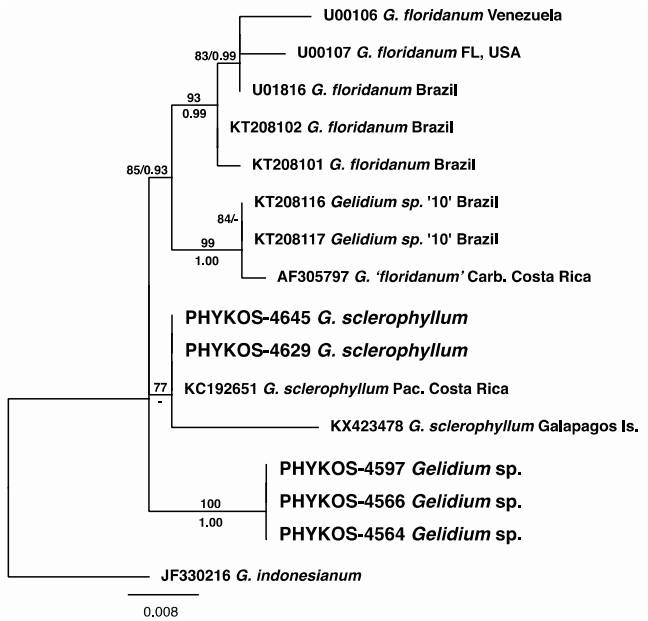

Figure 8. Maximum likelihood tree of *rbc*L-3P sequences for *Gelidium* specimens in the *G. floridanum*–*G. sclerophyllum* species complex. Bootstrap support and Bayesian posterior probability values are shown on branches when >70% and >0.75 respectively.

3.2.8. *Millerella* sp.

BLAST searches with sequences of PHYKOS-4570 returned a closest affinity with *Millerella* spp. (Table 2). The largest amount of available Gelidiellacaeae sequence data is for the *rbc*L locus, and trees generated with these data resolve PHYKOS-4570 within a strongly supported *Millerella* clade but with no close relationship to any of the other sequenced species (Figure 9).

The specimens were growing as a short turf on the intertidal mudstone substrate along the Punta Burica shoreline (Figure 1). PHYKOS-4570 matched the states of all important morphological characters described for *Millerella* species. Attachment rhizoids were in irregularly arranged clumps on stoloniferous axes and developed from outer cortical cells (Figure 10A,B). Second-order cell filaments developed from the axial cell filament in a distichous pattern during vegetative growth (Figure 10C,D). Tetrasporangial sori formed at the tips of erect axes and branches, and had acropetally developing tetrasporangia that were regularly arranged following the pattern of the second-order cell filaments, with six tetrasporangia in a row on both sides of a sorus (Figure 10E).

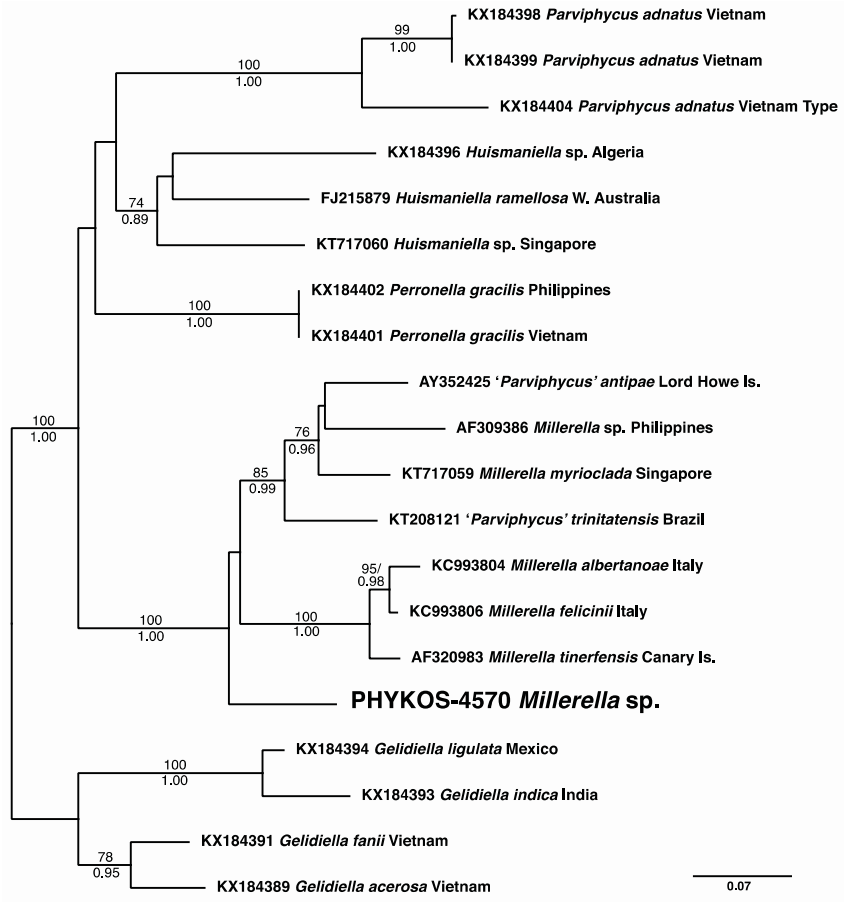

Figure 9. Maximum likelihood tree of *rbc*L-3P sequences for Gelidiellaceae species. Bootstrap support and Bayesian posterior probability values are shown on branches when >70% and >0.75 respectively.

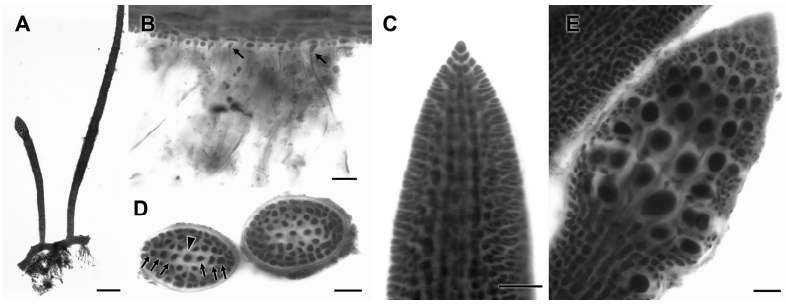

Figure 10. *Millerella* sp. morphological characteristics. (**A**) Habit of simple erect axes growing from a stoloniferous axis with irregular clumps of attachment rhizoids; left erect axis with a tetrasporangial sorus at the apical tip, scale = 200 μm; (**B**) Optical longitudinal section of stoloniferous axis with rhizoids developing from outer cortical cells (arrow), scale = 20 μm; (**C**) Tip of erect axis with a distinct apical cell and second-order cell filaments arising from the central axial cell filament in a distichous pattern, scale = 20 μm; (**D**) Transverse sections through erect axes at earlier (left) and later (right) stages of vegetative development; the central axial cell (arrowhead) and cells of the second-order cell filaments (arrows) are clearly visible, scale = 20 μm; (**E**) Tetrasporangial sorus with acropetally developing tetrasporangia in a regular arrangement, scale = 20 μm.

Remarks: Santelices [51] described *Parviphycus* to accomodate Gelidiellaceae species with a distichous arrangement of second-order cell filaments and the development of sporangia in regularly arranged rows. Further morphological and DNA sequence analyses lead Boo et al. [52] to describe

Millerella and *Perronella* for some of the species classified within *Parviphycus*. Dawson [6,53,54] described a number of small *Gelidiella* species from the tropical East Pacific, and other small Gelidiellaceae such as *Parviphycus tenuissimus* (Feldmann *et* Hamel) B. Santelices and *P. trinitatensis* (W.R. Taylor) M.J. Wynne have also been reported from Pacific Central America e.g., [5,55]. The status of the Punta Burica *Millerella* species as new or representative of one of these reported species will require a reassessment of the Gelidiellaceae in the East Pacific.

3.2.9. *Asparagopsis* sp.

BLAST searches of PHYKOS-4636 sequences returned identical matches (COI-5P, UPA) or close homology (*rbc*L-3P) to sequences of specimens identified as *Asparagopsis taxiformis* (Delile) Trevisan. Only a UPA sequence was generated for PHYKOS-4646, and it was identical to that of PHYKOS-4636. GenBank specimens with identical COI-5P and UPA sequences were from the Pacific.

Both PHYKOS-4636 and PHYKOS-4646 were collected from subtidal boulders at 4–5 m depth off the southwest point of Isla Burica (Figures 1A and 11A). The specimens were all in the *Falkenbergia*-stage and some had tetrasporangia (Figure 11B,C).

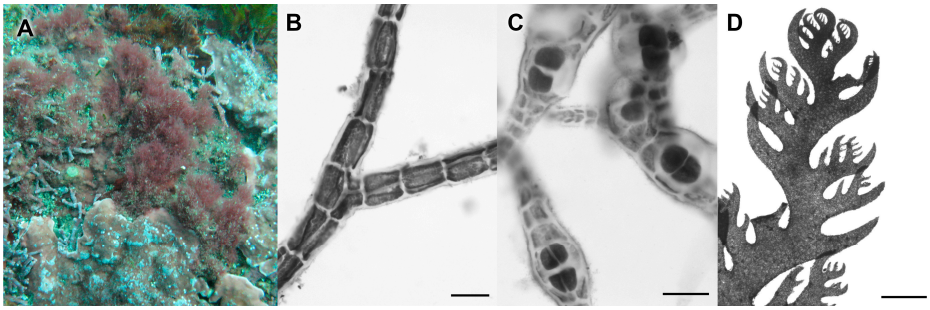

Figure 11. *Asparagopsis* sp. and *Plocamium* sp. from Punta Burica. (**A**) *Asparagopsis* sp. "Falkenbergia" stage growing on subtidal rock at 5 m depth, southwest of Isla Burica; (**B**) *Asparagopsis* sp. "Falkenbergia" stage vegetative filament with characteristic three pericentral cells, sclae = 50 μm; (**C**) *Asparagopsis* sp. "Falkenbergia" stage filaments producing tetrasporangia, scale = 50 μm; (**D**) *Plocamium* sp. branching pattern with lower most ramuli in a group that are not larger or recurved, scale = 500 μm.

Remarks: Currently there are two recognized species of *Asparagopsis*, *A. armata* Harvey, and *A. taxiformis*. The most recent DNA sequence analyses of the genus utilized nuclear-encoded LSU, chloroplast-encoded RuBisCO spacer, and mitochondria-encoded *cox2-3* spacer sequences to identify two major lineages within *A. armata* and five within *A. taxiformis* [56]. Dijoux et al. [56] designated lineages based on *cox*2-3 spacer haplotypes, which were not generated here, but RuBisCO spacer sequences for PHYKOS-4636 and PHYKOS-4646 matched the haplotypes of *A.* '*taxiformis*' specimens found in their Lineage 4. This lineage contained specimens distributed widely within the Pacific and Indian Oceans including specimens from the Gulf of Panama and Costa Rica [56]. The taxonomic status of this lineage requires further study, but its genetic distiction [56,57] and recent morphological differentiation from other lineages [58] suggests that it represents a biological species distinct from *A. taxiformis*.

Asparagopsis (as *A. taxiformis*) was first reported from Panama by Andreakis et al. [57], who included tetrasporophyte (*Falkenbergia*-stage) specimens collected from the Pacific terminus of the Panama Canal, Gulf of Panama in their biogeography study. Zanolla et al. [58] reported that *Falkenbergia*-stage specimens of *A. taxiformis* lineages could be morphologically distinguished by the length and width of axial cells, width of apical cells and thickness of cell walls. While measurements of these characters from Punta Burica specimens overlap with those cited for Lineage 4 by Zanolla et al. [58], a greater level of variation was seen.

3.2.10. *Plocamium* sp.

PHYKOS-4643 sequences were found to have closest homology to GenBank sequences from specimens identified as *Plocamium pacificum* Kylin (*rbc*L-3P 99%; COI-5P 95%), or *P. 'cartilagineum'* (Linnaeus) P.S. Dixon and *P. telfairiae* (W.J. Hooker and Harvey) Harvey ex Kützing (UPA 99%). However, phylogenetic analyses of available *rbc*L-3P sequences and cluster analyses of available COI-5P sequences did not strongly associate PHYKOS-4643 with *P. pacificum* or any other *Plocamium* species.

PHYKOS-4643 was collected from subtidal boulders at 3–5 m depth off the southwest point of Isla Burica.

Remarks: The only reports of *Plocamium* species from the Pacific coast of Central America are found in the Littler and Littler [49] web database of Pacific Panama marine algae. They list two species, *P. cartilagineum subsp. pacificum* (Kylin) P.C. Silva [=*P. pacificum* Kylin] and *P. violaceum* Farlow. The included images of the former show recurved pinnae and these are also part of the description and figure of this species in Abbott and Hollenberg [59]. Recurved branches were not present in PHYKOS-4643, which more closely matches the images of *P. violaceum* in the Littler and Littler [49] list. *Plocamium violaceum* was described by Farlow [60] based on California specimens, and he noted that ramuli were arranged in alternate groups of 3 or 4, with the lower most ramulus in a group larger than the others and slightly recurved. Lower ramuli in the Punta Burica specimen were not noticably larger or recurved (Figure 11D). COI-5P divergences between the Punta Burica and California *P. violaceum* specimens (5.6–5.9%) were greater than that between Punta Burica and specimens identified as *P. pacificum* from California, *P. oregonum* Doty from Oregon, and *P. nanum* G.W. Saunder and K.V. Lehmkuhl from France. Both the morphological and molecular discripancies indicate that the PHYKOS-4643 *Plocamium* specimen is not *P. violaceum*, and likely represents a new species.

3.2.11. *Hypnea flava* Nauer, Cassano and M.C. Oliveira

BLAST searches with PHYKOS-4574 sequences returned records from specimens identified as either *Hypnea flava* from Brazil (*rbc*L-3P 99%) or *H. 'spinella'* from Hawai'i (COI-5P 96%, UPA 99%). Phylogenetic analyses of *rbc*L-3P sequences from *Hypnea* species resolved the Punta Burica specimen within a fully-supported *H. flava* clade (Figure 12). The *rbc*L-3P divergence between the Pacific specimen from Punta Burica and the Brazilian specimens was only 0.9–1.0%.

PHYKOS-4574 specimens were collected from tide pools in the mudstone substrate along the Punta Burica shoreline (Figure 1) and included tetrasporophytes, which are described here for the first time. Tetrasporangial sori surround the median, swollen portions of generally short branchlets, 200–300 µm in diameter and may cover a majority of the branchlet surface, especially during early stages of development (Figure 13A). Sori sometimes also develop on the apical portions of longer branchlets or as patches on the surface of predominately vegetative axes. Tetrasporangia mother cells are cut off laterally from inner cortical cells and develop into zonately divided tetrasporangia (Figure 13B).

Remarks: The Punta Burica specimens closely matched the vegetative morphology of Brazilian *H. flava*, but reproductive specimens were not available when the species was orginally described by Nauer et al. [61], and tetrasporophytic thalli are described here for the first time. This is the first report of the species outside of Brazil.

3.2.12. *Hypnea pannosa* J. Agardh

PHYKOS-4631 *rbc*L-3P and COI-5P sequences were found to be identical, or nearly identical with GenBank sequences assigned to *Hypnea pannosa*. BLAST searches of the PHYKOS-4631 UPA sequence returned sequences of five species all at 99% similarity. Sequences of Eastern- and Western Pacific *H. pannosa* specimens formed a well- to fully-supported clade in the *Hypnea rbc*L-3P tree (Figure 12). *Hypnea pannosa* was originally described based on specimens from the Pacific coast of Mexico, and the Punta Burica *rbc*L-3P sequence was a match for that of a Pacific Mexico specimen.

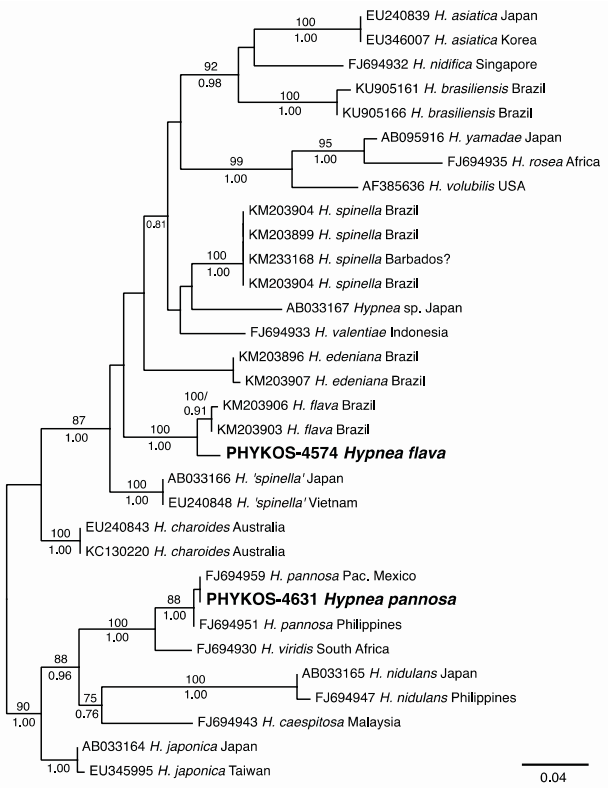

Figure 12. Maximum likelihood tree of *rbc*L-3P sequences for *Hypnea* species of the sections Pulvinatae and Spinuligerae. Bootstrap support and Bayesian posterior probability values are shown on branches when >70% and >0.75 respectively.

The PHYKOS-4643 collection was made from subtidal boulders at 4–5 m depth off the southwest point of Isla Burica (Figure 1). Specimens had compressed to subterete, entangled, irregularly branched axes with little difference between the diameter of axes and branches. Axes also anastomosed and had multiple attachments to the substrate. The Punta Burica specimens had generally acute to widely acute branch tips and tetrasporangial sori developed on both small branchlets and major axes (Figure 13C,D).

Remarks: Hypnea pannosa is a widely recorded species from tropical waters and also Pacific Central America [5]. Taylor [2] collected specimens from high tide pools at Isla Secas in the central Gulf of Chiriqui, Panama. The Punta Burica specimens matched the morphological descriptions of this species from Pacific Mexico and Hawai'i [44,62,63]. Some specimens identified as *H. pannosa* in the western Pacific are described as being mostly terete [64–66] and probably represent a different species; however, no DNA sequence data is currently available for these specimens.

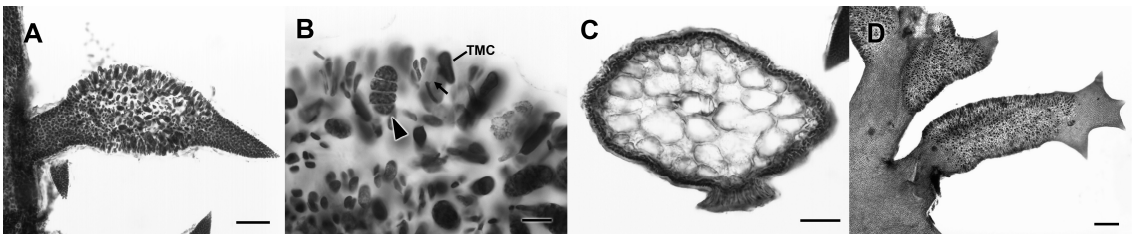

Figure 13. *Hypnea flava* and *H. pannosa*. (**A**) H. flava tetrasporangial sorus surrounding the median portion of branchlet, scale = 100 μm; (**B**) *H. flava* tetrasporangia mother cell (TMC) cut off laterally from cortical cell (pit connection shown with an arrow), and mature zonately divided tetrasporangium (arrowhead), scale = 20 μm; (**C**) *H. pannosa* transverse section through compressed prostrate branch with holdfast structure, scale = 100 μm; (**D**) *H. pannosa* branches bearing tetrasporangial sori, scale = 200 μm.

3.2.13. *Neorubra parvolacertoides* Freshwater and P.W. Gabrielson *sp. nov.*

BLAST searches with PHYKOS-4528, PHYKOS-4589 *rbc*L-3P sequences revealed a closest affinity with sequences of *Neorubra decipiens* (Montagne) M.S. Calderon, G.H. Boo and S.M. Boo (Table 2). COI-5P and UPA sequence BLAST searches provided little context for the identification of this species. Phylogenetic analyses with available *rbc*L-3P sequences from species of *Grateloupia sensu lato* resolved PHYKOS-4528 and PHYKOS-4589 within a fully supported *Neorubra* clade (Figure 14). Cluster analyses of a 137 bp section of the *rbc*L-3P locus from Eastern Pacific types and contemporary topotype specimens further indicated that the Punta Burica *Neorubra* species is unique (Figure 15).

Remarks: Gargiulo et al. [67] recently described morphological character states of the female reproductive system and post-fertilization development for a number of newly distinguished genera previously synonymized under *Grateloupia* that also were resolved as distinct clades within *rbc*L phylogenies of *Grateloupia sensu lato*. Continued molecular and morphological investigation has led to descriptions of additional genera [68–70], including *Neorubra*, which contains two species, *N. decipiens* and *N. denticulata* (Montagne) M.S. Calderon, G.H. Boo and S.M. Boo. Considering the position of PHYKOS-4528 and PHYKOS-4589 specimens within a fully supported *Neorubra* clade and their uniqueness among known eastern Pacific taxa we regard the Punta Burica specimens as a new *Neorubra* species that we describe here.

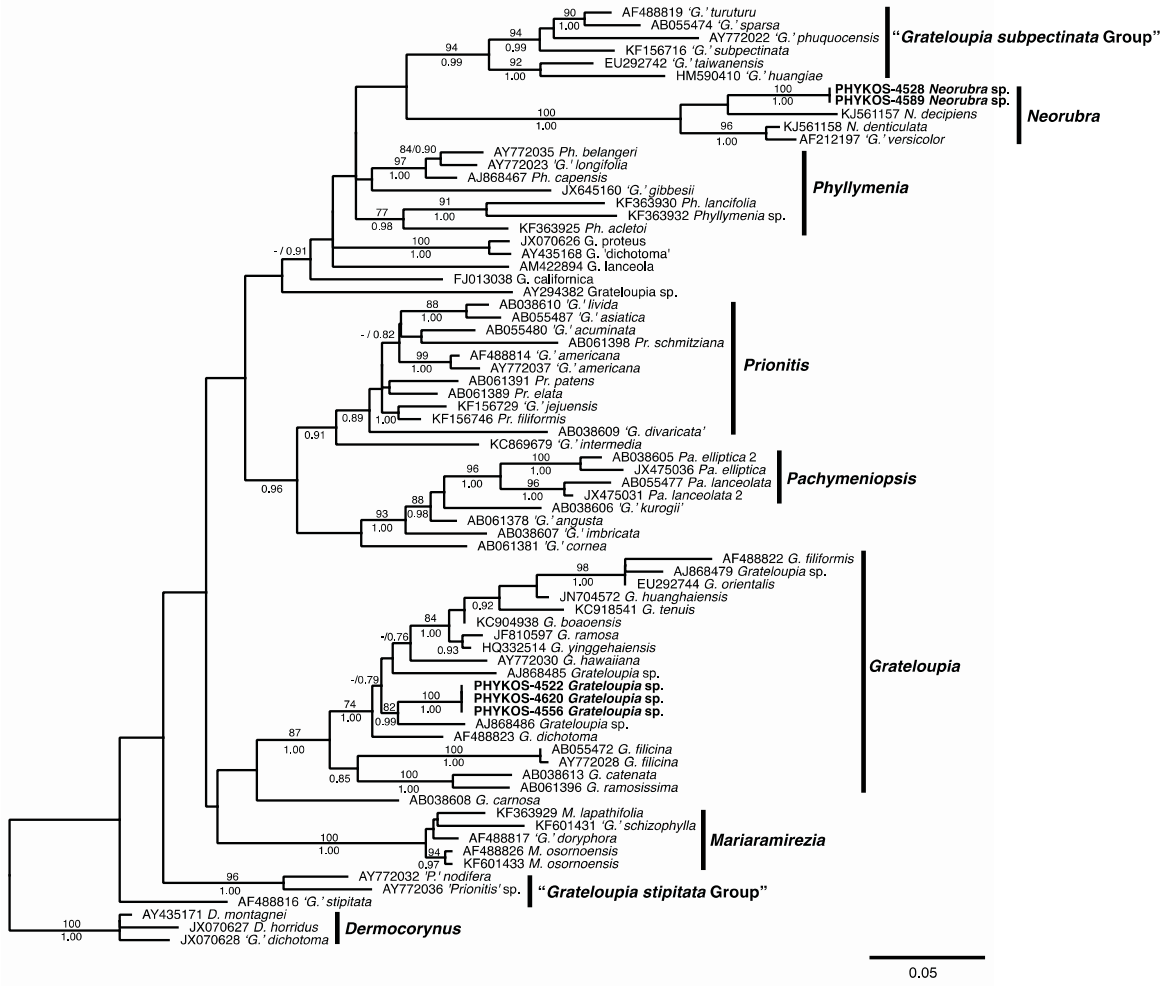

Figure 14. Maximum likelihood tree of *rbc*L-3P alignment for 74 'Grateloupia' sensu lato taxa. Bootstrap support values and Bayesian posterior probabilities are given for branches when >70% and >0.75 respectively. Punta Burica *Neorubra* and *Grateloupia* specimens are shown in bold type.

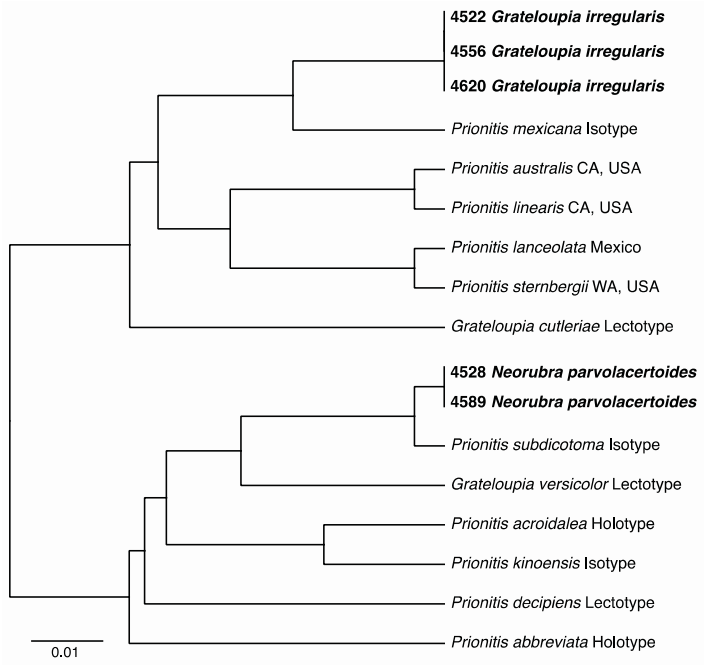

Figure 15. UPGMA cluster diagram of a 137 bp section of the *rbc*L-3P locus from eight historical type specimens, four contemporary specimens from or near species type localities and specimens of the Punta Burica *Neorubra* and *Grateloupia* species.

Neorubra parvolacertoides Freshwater and P.W. Gabrielson *sp. nov.*
Figure 16A–D.

Description: Thalli dark red with horizontal bands, sometimes wavy, of lighter color; small and erect, 9–12 mm high, laceolate to clavate with extended proximal tapering (Figure 16A,B); bases terete to subterete, becoming compressed to flattened distally, 0.5–1.5 mm wide, 150–400 μm thick; with one order of short, irregular distichous branching; axes multiaxial; tips obtuse to widely acute; medulla of longitudinal filaments of elongated cells; cortex of anticlinal filaments; medulla-cortex transition gradual; outer cortex of 5–7 layers of small, globose to elliptical, pigmented cells, 1–5 μm × 2–10 μm; inner cortex of 3–4 layers of elliptical to stellate cells, 4–7 μm × 10–18 μm (Figure 16C,D); COI-5P sequence = GenBank accession KY656538, *rbc*L-3P sequence = GenBank accession KY573975, UPA sequence = GenBank accession KY573934.

Holotype: WNC 34262, herbarium specimen and two slides (WNC 2012-s073, WNC 2012-s074) in packet on sheet, collection # PHYKOS-4528, attached to rock on mixed sediment-rock bottom just beyond breakers, 3 m depth, near Mono Feliz, Punta Burica, Panama, 8 January 2011, leg. B. Wysor and D.W. Freshwater.

Paratype: slide # WNC 2011-s072, collection PHYKOS-4589, growing with brown algal turf, shallow subtidal, <1 m depth, near Mono Feliz, Punta Burica, Panama, 9 January 2011, leg. B. Wysor and D.W. Freshwater.

Type Locality: Punta Burica, Panama, 08.03042° N; 082.87574° W

Etymology: The epithet refers to the field name given to this species by its collectors who thought that it resembled small lizards of the region.

Comment: The diminutive size, <15 mm tall, and having only 1 order of branches distinguishes *N. parvolacertoides* from all other Halymeniales in the eastern tropical Pacific. It is an order of magnitude smaller than both described species of *Neorubra*, *N. decipiens* and *N. denticulata* (Montagne) M.S. Calderon, G.H. Boo and S.M. Boo and, being tropical, does not overlap the distributions of the aforementioned species.

3.2.14. *Grateloupia irregularis* P.W. Gabrielson and Freshwater *sp. nov*

PHYKOS-4522, PHYKOS-4556, and PHYKOS-4620 all shared identical sequences for the three examined loci. BLAST searches returned a variety of *Grateloupia sensu lato* species, but none at a level of homology indicating a potential species match. Cluster analyses with a short segment of the *rbc*L-3P locus showed that this species was distinct from all the included eastern Pacific *Grateloupia sensu lato* types and contemporary topotype specimens (Figure 15). Phylogenetic analyses of the *rbc*L-3P locus resolved this specimen within a well supported *Grateloupia sensu stricto* clade (Figure 14). Similar to *Neorubra parvolacertoides*, the uniqueness among known eastern Pacific taxa and position within the well resolved *Grateloupia* clade, indicates that these specimens represent a previously undescribed *Grateloupia* species.

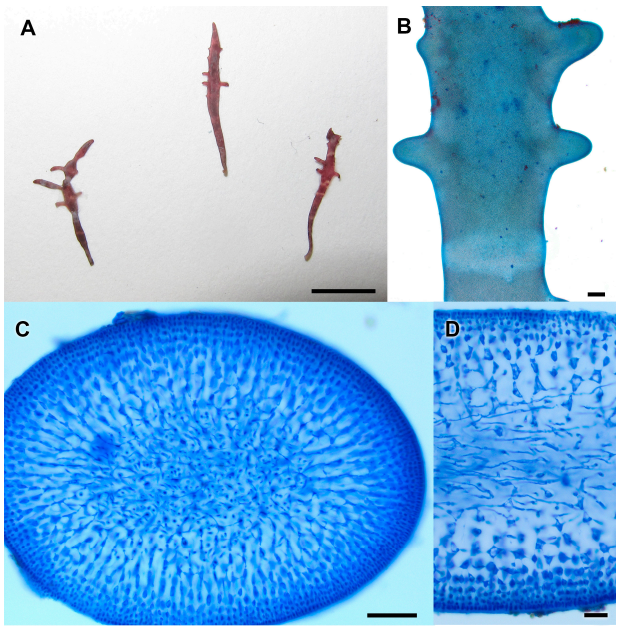

Figure 16. *Neorubra parvolaertoides*. (**A**) Holotype specimen WNC34262, scale = 5 mm; (**B**) Surface view of wholemount specimen with short distichous branches and horizontal band of lightly pigmented cortical cells, scale = 200 μm; (**C**) Transverse section of compressed axis with medulla of longitudinal cell filaments and cortex of anticlinal cell filaments, scale = 50 μm; (**D**) Tangential section of axis showing elongated medullary cells and anticlinal cortical cell filaments, scale = 20 μm.

Grateloupia irregularis P.W. Gabrielson and Freshwater *sp. nov.*
Figure 17A–E.

Description: Thalli various shades of red; small and erect, 10–20 mm high, lanceolate to linear, fractiflexus in part (Figure 17A), attached by discoid holdfast, narrow terete to subterete bases, quickly becoming compressed to flattened except at tips and young ultimate branches where terete, 0.25–1.5 mm wide; 200–350 μm thick; with up to four orders of irregular, disticus branching and sometimes sparsly scattered small denticulate proliferations; axes multiaxial; tips acute; medulla of widely spaced, longitudinal filaments of elongated cells; cortex of anticlinal filaments; medulla-cortex transition sharp; outer cortex of 5–6 layers of small, mostly elliptical cells, 2–5 μm × 3–10 μm; inner cortex of 2–3 layers of globose to elliptical or stellate cells, 5–10 μm × 10–20 μm (Figure 17B); tetrasporangia across entire thallus surface of main axes and branches (Figure 17C); tetrasporangial mother cells cut off from inner cells of the outer cortex, becoming somewhat elongated and irregular in shape during development; tetrasporangia cruciate to decussately cruciate divided(Figure 17D); spermatiangial sori covered by thick sheath, outer cortical cells develop into elongated spermatangial mother cells (Figure 17E); COI-5P sequence = GenBank accession KY656551, *rbc*L-3P sequence = GenBank accession KY573991, UPA sequence = GenBank accession KY573951.

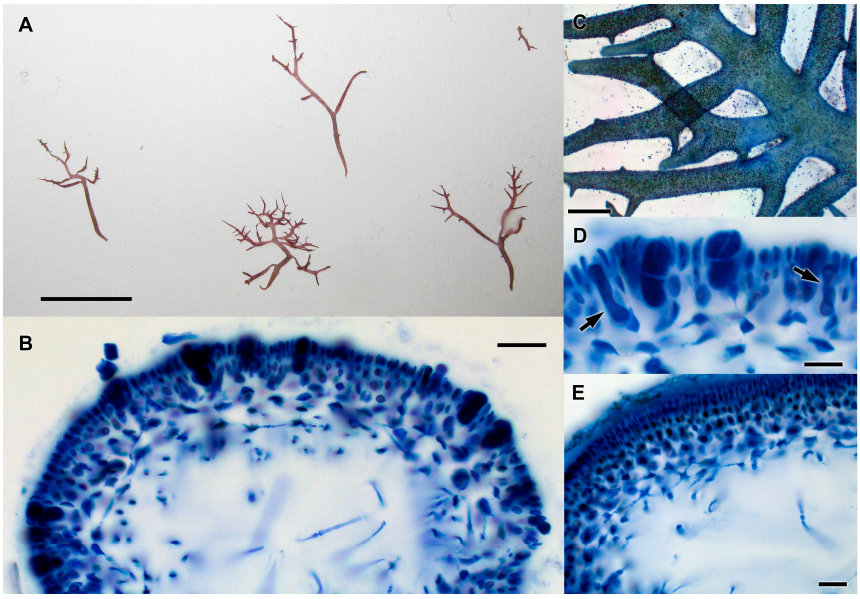

Figure 17. *Grateloupia irregularis*. (**A**) Holotype specimen WNC34263, scale = 1 cm; (**B**) Transverse section through tetrasporophytic axis with loosely arranged, widely spaced longitudinal filaments of elongated medullary cells and anticlinal cortical cell filaments, scale = 30 µm; (**C**) Whole mount of tetrasporphytic specimen, scale = 500 µm.; (**D**) Transverse section of tetrasporophytic axis showing elongated and irregularly shaped tetraspore mother cells (arrows) and decussately cruciate tetrasporangia, scale = 15 µm; (**E**) Transverse section of fertile male axis with widely spaced medullary filaments, anticlinal cortical filaments, and elongated spermatangial mother cells covered by a thick outer sheath, scale = 20 µm.

Holotype: WNC 34263, herbarium specimen and two slides (WNC 2012-s067, WNC 2012-s068) in packet on sheet, collection # PHYKOS-4556, attached to rock on mixed sediment-rock bottom just beyond breakers, 3 m depth, near Mono Feliz, Punta Burica, Panama, 8 January 2011, leg. B. Wysor and D.W. Freshwater

Paratypes: WNC 34264, herbarium specimen and two slides (WNC 2012-s071, WNC 2012-s072) in packet on sheet, collection # PHYKOS-4522, attached to rock on mixed sediment-rock bottom just beyond breakers, 3m depth, near Mono Feliz, Punta Burica, Panama, 8 January 2011, leg. B. Wysor and D.W. Freshwater; Slides WNC 2012-s069 and WNC 2012-s070, collecction # PHYKOS-4620, growing in mixed algal turf, shallow subtidal, <1 m depth, near Mono Feliz, Punta Burica, Panama, 9 January 2011, leg. B. Wysor and D.W. Freshwater

Type locality: Punta Burica, Panama, 08.03042° N; 082.87574° W

Etymology: The epithet reflects the irregular branching pattern exhibited by the species.

Comment: Similar to *N. parvolacertoides*, the diminutive size of *G. irregularis*, <15 mm tall, distinguishes it from other previously described species of eastern tropical Pacific Halymeniales. *Grateloupia irregularis* has more orders of branches (Figure 17A) and its medulla is comprised of loosely arranged filaments (Figure 17B) compared to *N. parvolacertoides* (Figure 16 A,C respectively).

3.2.15. *Ceratodictyon repens* (Kützing) R.E. Norris

BLAST searches of COI-5P and UPA sequences for PHYKOS-4627, PHYKOS-4632 and PHYKOS-4635 returned closest matches (94% and 99% respectively) with sequences of *Ceratodictyon scoparium* (Table 2). An *rbc*L-3P sequences was not generated for PHYKOS-4627, but searches with this locus for the other two samples showed them to be matches for the sequence from a South African specimen of *C. repens*. Phylogenetic analyses of *rbc*L-3P data for members of the Lomentariaceae resolve these *C. repens* specimens within a strongly supported *Cearatodictyon* clade (Figure 18).

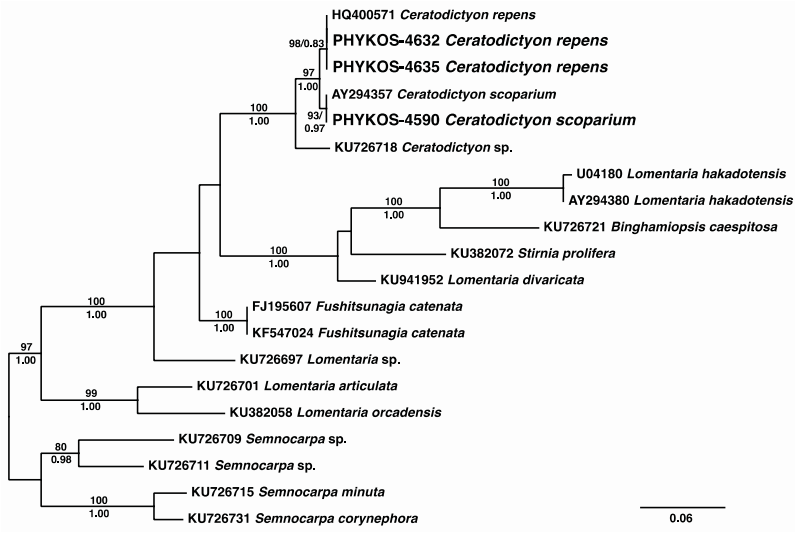

Figure 18. Maximum likelihood tree of Lomentariaceae *rbc*L-3P sequences available in GenBank. Bootstrap support values and Bayesian posterior probabilities are given for branches when >70% and >0.75 respectively. Punta Burica *Ceratodictyon* specimens are shown in larger type.

All Punta Burica specimens were growing in tufts on subtidal rocks at 4–5 m depth off the southwest point of Isla Burica (Figure 1). The thalli consisted of prostrate axes giving rise to erect axes up to 2–3 cm in height (Figure 19A). Erect axes were proximally terete and became distally compressed.

Remarks: Saunders et al. [71] established that *Gelidiopsis* was a genus within the Rhodymeniales family Lomentariaceae. *Ceratodictyon* was originally described for an algal species living symbiotically with a sponge [72]. Norris [73] proposed the merger of *Ceratodictyon* and *Gelidiopsis*, but this synonymy was generally not followed until DNA sequence analyses supported their congeneric status [74,75]. *Ceratodictyon repens* was originally described from New Caledonia and is reported from many Indo-Pacific localities e.g., [65,76]. The species is known from El Salvador in the tropical East Pacific (as *Gelidiopsis repens* (Kützing) Weber-van Bosse [55,77]), but this is the first report from Panama.

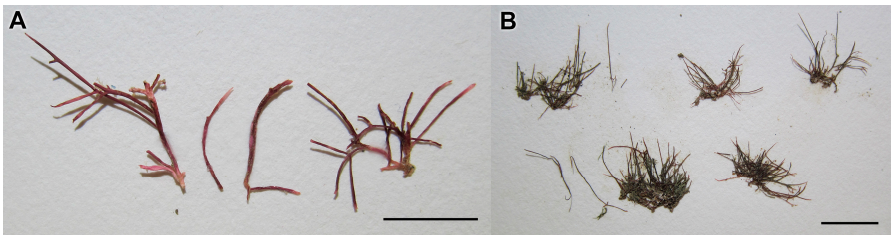

Figure 19. Habits of Punta Burica *Ceratodictyon* species. (**A**) *Ceratodictyon repens*, scale = 1 cm; (**B**) *C. scoparium*, scale = 1 cm.

3.2.16. *Ceratodictyon scoparium* (Montagne and Millardet) R.E. Norris

BLAST results with sequences of PHYKOS-4590 showed that they were identical to the *rbc*L-3P sequence of an unidentified *Ceratodictyon* sp. from Pacific Mexico, and identical (UPA) and 99% (COI-5P) similar to sequences from Hawaiian specimens of *C. scoparium*. PHYKOS-4590 was resolved sister to the Punta Burica *C. repens* specimens in the *rbc*L-3P phylogeny (Figure 18).

The PHYKOS-4590 specimens were growing as a turf on the exposed intertidal mudstone substrate and in tide pools along the Punta Burica shoreline (Figure 1).

Remarks: Ceratodictyon scoparium was originally described from Réunion Island in the western Indian Ocean and similar to *C. repens* is reported throughout much of the Indo-Pacific [35]. Morphologically the two species are also similar, with both demonstrating the condensed branching

that results in a pseudopalmate appearance for some axes [44,76]. Punta Burica *C. scoparium* specimens were smaller in stature than both the collected *C. repens* specimens (Figure 19B) and Hawaiian specimens of *C. scoparium* described by Abbott [44] although they otherwise closely match Abbott's description. This small stature may have an environmental basis as the Punta Burica *C. scoparium* was growing as a turf in the intertidal mudstone and tide pool habitat. This is the first record of *C. scoparium* from the eastern Pacific.

3.2.17. *Gracilaria* Species

Sequences for the three analyzed loci from five of the Punta Burica specimens (PHYKOS-4519, 4541, 4548, 4630, and 4640) separated out as three related species: (1) PHYKOS-4640; (2) PHYKOS-4519, 4541 and 4630; (3) PHYKOS-4548 (Figure 2). BLAST searches with these sequences returned *Gracilaria* specimen hits, but none with homology at a level to suggest species identifications (Table 2). Phylogenetic analyses of publically available *rbc*L-3P sequence data for *Gracilaria* species resolved all three of the Punta Burica species within *Gracilaria sensu stricto* (Figure 20). *Gracilaria* sp. 2 was strongly supported in a sister position to G. galetensis Gurgel, Fredericq and J.N. Norris, a species of the tropical Western Atlantic that was originally described from Caribbean Panama. Thus, these two species potentially represent a transisthmian geminate species pair. *Gracilaria* sp. 1 and *Gracilaria* sp. 3 were resolved within a strongly supported clade of *Gracilaria* species from both the Western Atlantic and Pacific (Figure 20).

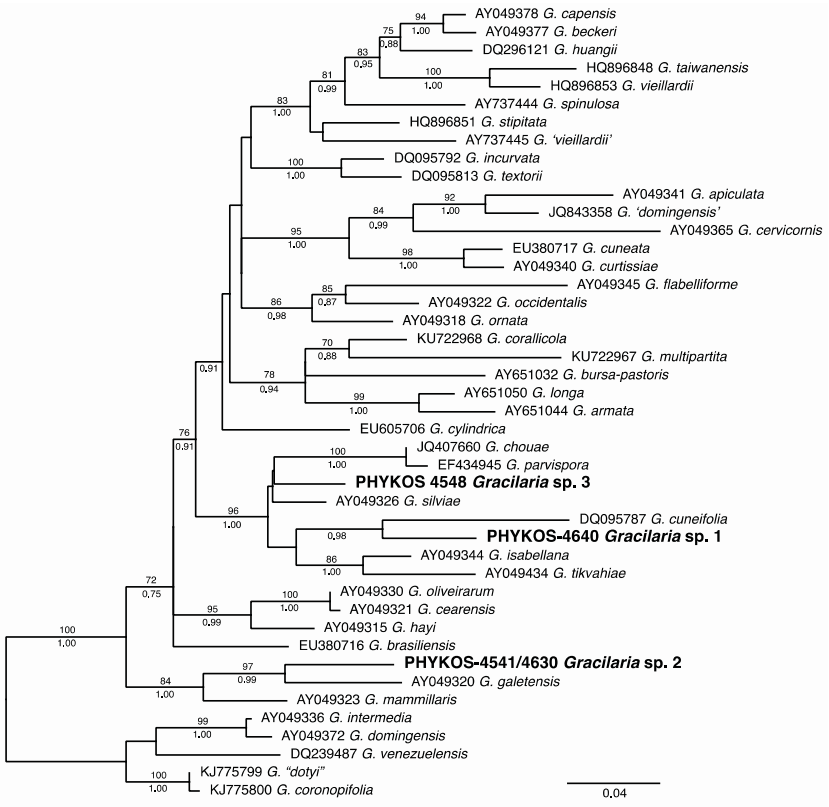

Figure 20. Maximum likelihood tree of *Gracilaria rbc*L-3P sequences from GenBank. Bootstrap support values and Bayesian posterior probabilities are given for branches when >70% and >0.75 respectively. Punta Burica *Gracilaria* specimens are shown in larger type.

Gracilaria sp. 1 was collected from subtidal rock at 4–5 m depth off the southwest point of Isla Burica (Figure 1). Specimens of *Gracilaria* sp. 2 were collected subtidally from both the mixed sediment and mudstone bottom just beyond the surf zone along Punta Burica (PHYKOS-4519; PHYKOS-4541),

and rocks at 4–5 m depth off the southwest point of Isla Burica. *Gracilaria* sp. 3 was also collected from the mixed sediment and mudstone bottom just beyond the Punta Burica surf zone. Observed specimens of the three species were small and mostly less than 2 cm high (Figure 21). *Gracilaria* sp. 3 differed from the others in having dissected, acute branch apices (Figure 21C).

Remarks: Eight species of *Gracilaria* are reported from Pacific Central America. Most of these are relatively large compared to the Punta Burica species. *Gracilaria brevis* W.R. Taylor was originally described as reaching only 5 cm in height [2], and it was included in the Littler and Littler [49] web database of Pacific Panama species. The Punta Burica *Gracilaria* sp.3 is similar to *G. crispata* Setchell and Gardner and *G. spinigera* E.Y. Dawson in having acute, dentate-like apices, but the collected Punta Burica specimens were much smaller than the reported sizes for these species [62]. A comprehensive examination of tropical eastern Pacific *Gracilaria* species will be needed to determine if the Punta Burica species are simply juvenile growth forms or undescribed species. At least two, if not all three of these species are unreported for Panama.

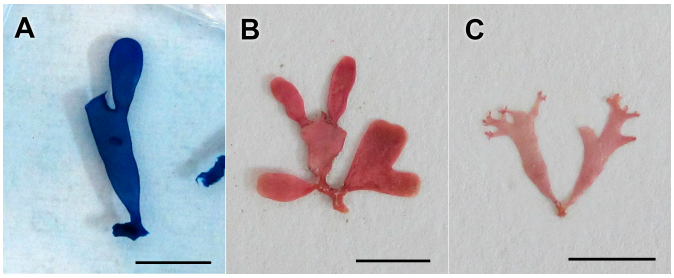

Figure 21. Habits of Punta Burica Gracilaria species. (**A**) *Gracilaria* sp. 1 (aniline blue stained, whole mount slide specimen), scale = 5 mm; (**B**) *Gracilaria* sp. 2, scale = 5 mm; (**C**) *Gracilaria* sp. 3, scale = 5 mm.

3.2.18. *Aglaothamnion* sp.

The PHYKOS-4553 *rbc*L-3P BLAST search returned sequences of *Aglaothamnion halliae* (Collins) N.E. Aponte, D.L. Ballantine and J.N. Norris and *A. hookeri* (Dillwyn) Maggs and Hommersand at 96% similarity, and a variety of *Aglaothamnion* and *Callithamnion* species without close homology in BLAST searches with COI-5P and UPA. Analyses of the *rbc*L-3P locus with other available Callithamnieae sequences resolved the Punta Burica species within a clade that also included *A. halliae*, *A. hookeri* and another unidentified *Aglaothamnion* species (Figure 22).

PHYKOS-4553 was growing attached to mudstone substrate on the mixed sediment and mudstone bottom just beyond the surf zone at Punta Burica (Figure 1).

Remarks: There currently is little available sequence data for species of *Aglaothamnion* or *Callithamnion*, and their relationships within the tribe Callithamnieae has been best characterized by concatenated data sets of multiple loci, albeit with limited taxon sampling [78,79]. *Aglaothamnion* and *Callithamnion* species are similar in general habit with both genera having distichous or radial, alternate branching from a uniseriate main axis. The genera are separated by the presence of multinucleate cells in *Callithamnion*, in contrast to Aglaothamnion having only uninucleate cells [80]. *Aglaothamnion* has been relatively well characterized in the tropical Western Atlantic [81], but both *Aglaothamnion* and *Callithamnion* are poorly known in the tropical East Pacific [5]. Taylor [2] included five species in his list, four of which were newly described by him. However, two were from Pacific Mexico and the remaining three were only collected in the Galapagos Islands. *Callithamnion marshallense* E.Y. Dawson is the only species of either genus known from Pacific Central America with reports from Costa Rica [5], and it is also included in the Littler and Littler [49] web database of Pacific Panama species. The Punta Burica species had lateral branchlets that develop from nearly all main axis cells in an alternate distichous pattern (Figure 23A,B), in contrast to *C. marshallense* that has branchlets develop in an alternate radial pattern and not from every main axis cell [82]. The Punta Burica specimen was male, and spermatangia development was similar to that described for other *Aglaothamnion* species [44,81]. Spermatangial initials were cut off from the adaxial side

of lateral branchlet cells towards their distal ends and divided to form "antheridial stands" [83] that cut off spermatia and may overlap with the adjacent branchlet cell (Figure 23B,C). PHYKOS-4553 was similar to Taylor's [2] description of *C. pacificum* W.R. Taylor, which is only known from the type collection, (epiphytic on larger algae dredged from 41–84 m depth near Isla Soccoro, Mexico) an environment that is likely quite different from the turbulent mixed sediment and mudstone bottom where the Punta Burica specimen was collected. *Callithamnion pacificum*, may be further distinguished by its more irregular branching, though clearly, additional study of both is needed to determine their relationship. Regardless, this species has not be reported previously from Pacific Panama or Pacific Central America and may represent a new species.

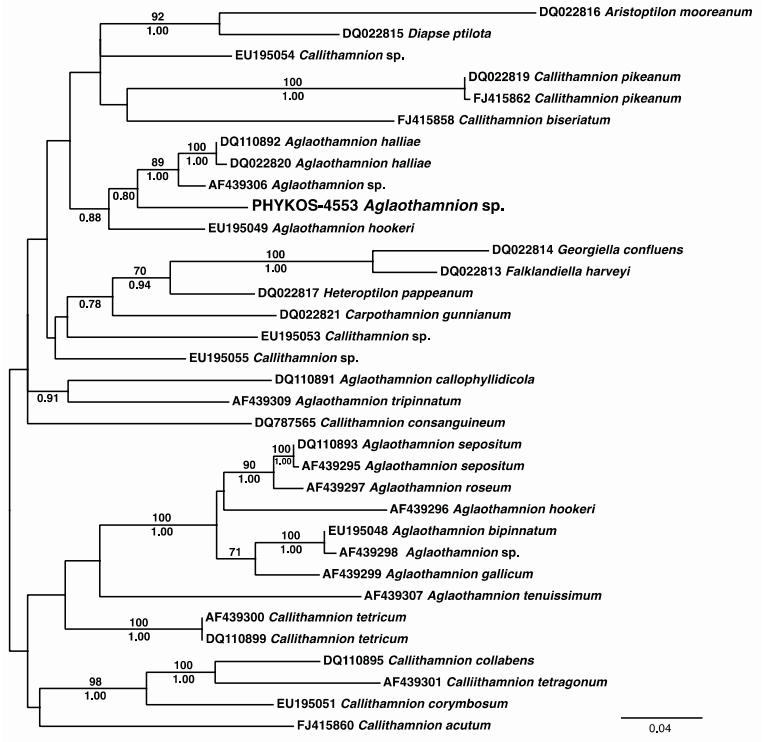

Figure 22. Maximum likelihood tree of Callithamnieae *rbc*L-3P sequences from GenBank. Bootstrap support values and Bayesian posterior probabilities are given for branches when >70% and >0.75 respectively. The Punta Burica *Aglaothamnion* species is shown in larger type.

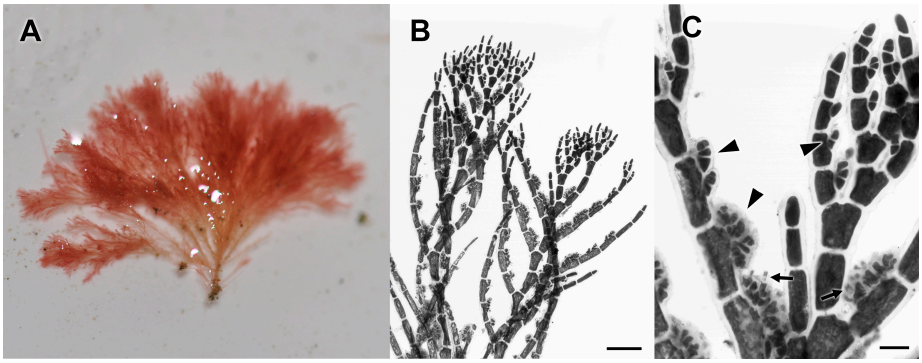

Figure 23. *Aglaothamnion* sp. (**A**) Habit of Punta Burica specimen; (**B**) Apical portions of two axes with alternate distichous pattern of branching and antheridial stands on the adaxial side of branchlets, scale = 100 μm; (**C**) Fertile male axis with antheridial stands at various stages of development (arrowheads) and spermatia being cut off (arrows), scale = 20 μm.

3.2.19. *Centroceras gasparrinii* (Meneghini) Kützing

PHYKOS-4594 and PHYKOS-4600 shared identical sequences for the three analyzed loci. BLAST searches with COI-5P and UPA returned specimens identified as *Centroceras clavulatum* (C. Agardh) Montagne (94%) and *Centroceras* sp. (99%) respectively. The Punta Burica specimens *rbc*L-3P sequences were found to be exact matches with a specimen identified as *C. gasparrinii* that had been collected from near the Pacific terminus of the Panama canal. Phylogenetic analyses of *rbc*L-3P sequence data available from GenBank resolved Pacific Panama specimens in a well supported *C. gasparrinii* clade (Figure 24).

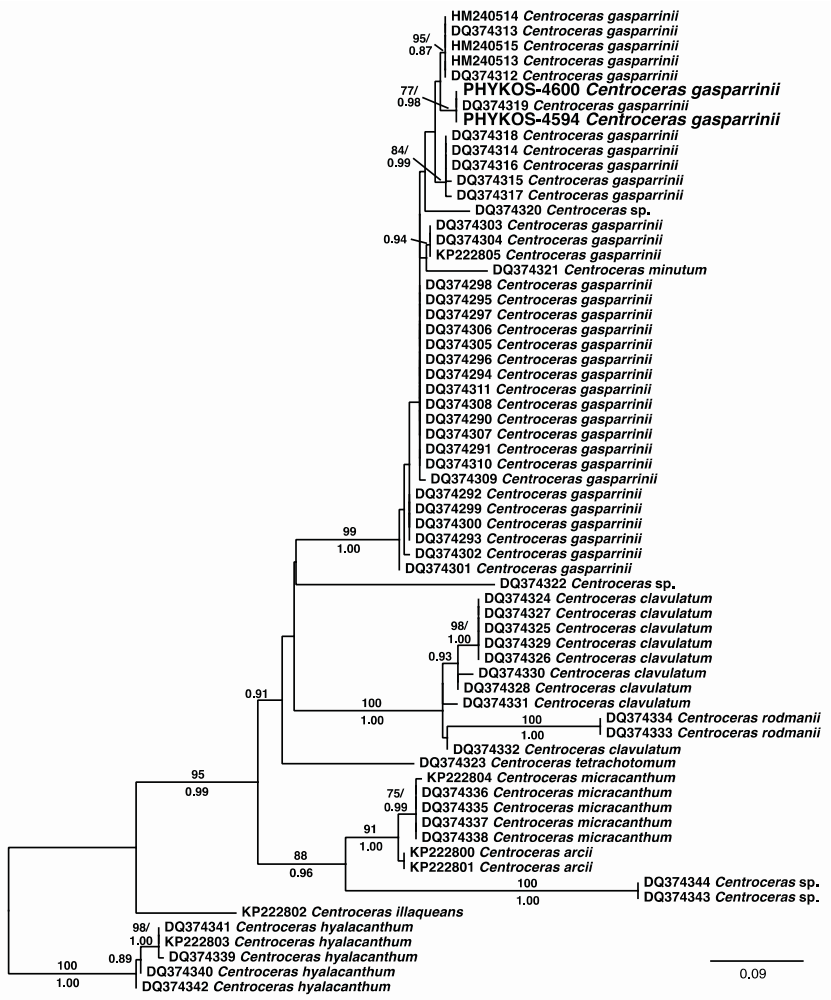

Figure 24. Maximum likelihood tree of *Centroceras* species *rbc*L-3P sequences from GenBank. Bootstrap support values and Bayesian posterior probabilities are given for branches when >70% and >0.75 respectively. The Punta Burica *C. gasparrinii* specimens are shown in larger type.

Both specimen collections were made from intertidal turfs growing on the mudstone substrate along the Punta Burica shoreline (Figure 1).

Remarks: Centroceras gasparrinii was long considered a synonym of *C. clavulatum* until the detailed morphological and molecular analyses of Won et al. [30] established that the shape of acropetal cortical cells, spines and gland cells were vegetative characters that could be used to distinguish most *Centroceras* species and these have been used in subsequent studies describing new species [84,85]. Erect axes of Punta Burica specimens had forcipate, slightly inrolled apices, di-trichotomous branching and nodal cortication characteristics as descibed for *C. gasparrini* [30] (Figure 25A,B). Tetrasporangia were produced in a whorl around the axes at upper nodes (Figure 25C).

Dawson [86] reported *C. minutum* Yamada from Pacific Panama, and this was the only record of *Centroceras* from Pacific Central America until the study of Won et al. [30] that included *C. gasparrinii*. The two species are easily distinguished by branching pattern, which is alternate in *C. minutum* and di-trichotomous in *C. gasparrinii*. *Centroceras gasparrinii* is reported from both Caribbean and Pacific Panama, and notably at both termini of the Panama Canal. Whether these populations are more closely related than their intra-oceanic conspecifics remains to be studied.

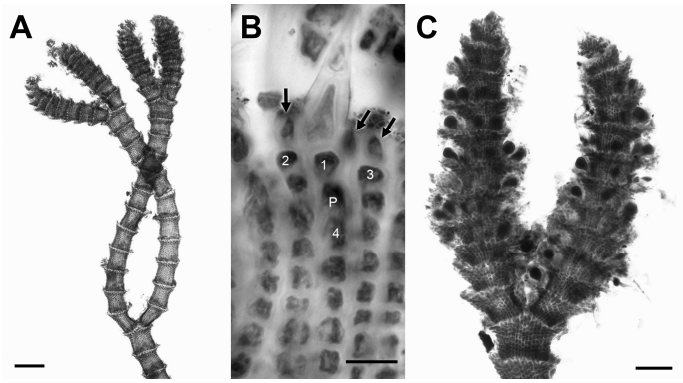

Figure 25. *Centroceras gasparrinii*. (**A**) Axis with dichotomous branching and forcipate, slightly enrolled tips, scale = 200 μm; (**B**) Cortical unit with four cortical initials (numbered cells) derived from a pericentral cell (P) with the acropetal cortical cells cut off from initials 1–3 indicated (arrows), scale = 20 μm; (**C**) Fertile tetrasporophyte axis with tetrasporangia produced in whorls at the nodes, scale = 100 μm.

3.2.20. *Spyridia* sp.

Only a COI-5P sequence was generated from PHYKOS-4584, and BLAST searches revealed a closest match (95%) with a specimen identified as *Spyridia filamentosa* (Wulfen) Harvey from Hawaii. The next closest matches (88–89%) were to other Hawaiian specimens also identified as *S. filamentosa*.

The PHYKOS-4584 collection was made from a tide pool along the Punta Burica shoreline (Figure 1). The specimen displays the general morphological characteristics of *Spyridia* including fully corticated indeterminate axes with a determinate branchlet produced by each indeterminate axis cell; inderminate axis cortication alternating bands of shorter, broader nodal cells and longer, narrower internodal cells, and determinate branchlets corticated only at nodes and with some tips terminated by acuminate spines (Figure 26).

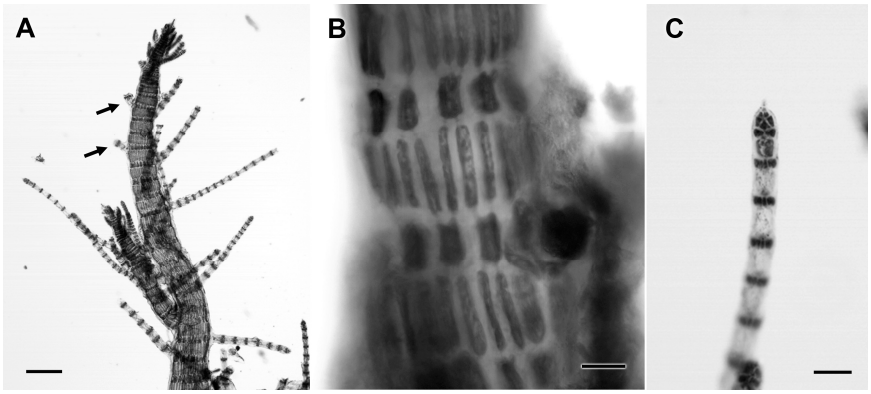

Figure 26. *Spyridia* sp. from Punta Burica. (**A**) Apical region of interminate axis with one determinate branchlet produced by each axial cell in a radial pattern. Some of the determinate branchlets have been broken off during specimen preservation (arrows), scale = 200 μm; (**B**) Inderminate axis cortication pattern of alternating bands of shorter, broader and narrower, longer cells, scale = 20 μm; (**C**) Determinate branchlet with nodal bands of corticating cells and an acuminate tip, scale = 50 μm.

Remarks: Spyridia filamentosa is widely reported around the globe [42], but previous studies have revealed that it is a complex of cryptic species [87–89]. Conklin and Sherwood [89] identified two separate *S. filamentosa* lineages within the Hawaiian Islands based on nuclear-encoded LSU sequences and determinate branchlet cell dimensions. The Hawaiian specimen with a 95% COI-5P similarity to the Punta Burica species was part of Conklin and Sherwood's LSU lineage-1, and the specimens for the next closest BLAST hits were part of their LSU lineage-2 [89]. Although the dimensions of determinate branchlet cells were found by Conklin and Sherwood [89] to be consistent character states separating the Hawai'i *S. filamentosa* LSU lineages, other morphological characters used to distinguish *Spyridia* species have been found to be variable within species and even the same thallus [90,91]. Taylor [2] reported *S. filamentosa* from Isla Taboga in the Bay of Panama and Littler and Littler [49] included it in their web database. Based on its Adriatic Sea type locality and the established diversity of cryptic species that are included under this name, the Punta Burica species and other reports of *S. filamentosa* from Pacific Central America most likely do not belong in this species and should be considered as unidentified *Spyridia* species.

3.2.21. *Melanothamnus* sp.

BLAST searches with *rbc*L-3P and COI-5P sequences for PHYKOS-4568 showed a closest match with sequences from a specimen identified as *Melanothamnus pseudovillum* (Hollenberg) Díaz-Tapia and Maggs (as *Polysiphonia pseudovillum* Hollenberg) from Caribbean Panama (Table 2). The Caribbean Panama *M. pseudovillum* and PHYKOS-4568 *Melanothamnus* sp. were also fully supported as sister species in phylogenetic analyses of *rbc*L sequences of *Polysiphonia sensu lato* species (Figure 27).

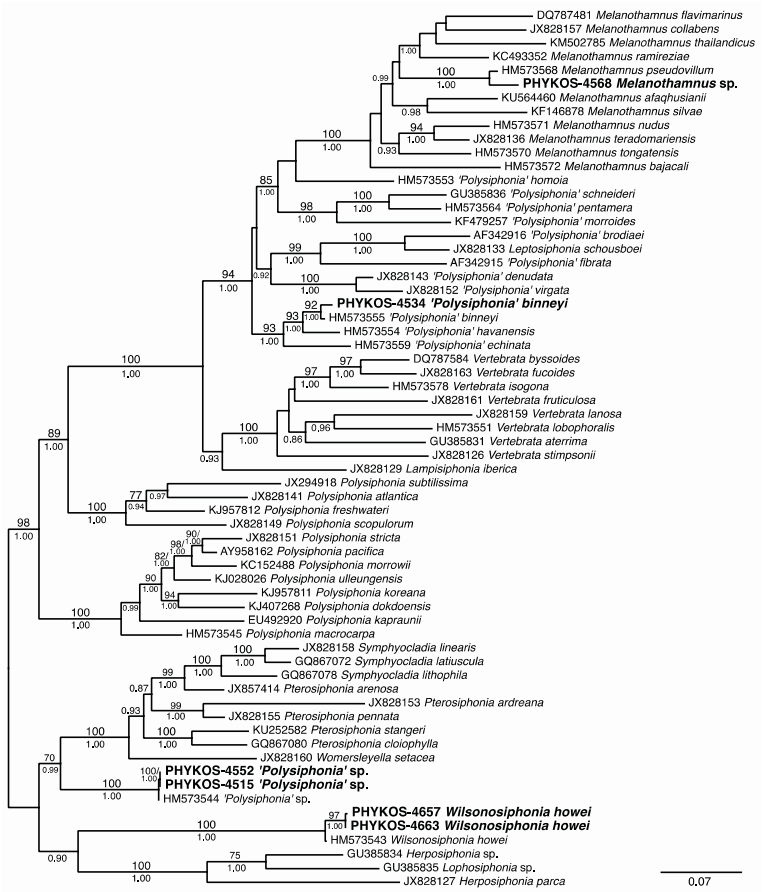

Figure 27. Maximum likelihood *rbc*L tree of *Polysiphonia sensu lato* and other species of closely related genera. Bootstrap support values and Bayesian posterior probabilities are given for branches when >70% and >0.75 respectively. The Punta Burica specimens are shown in larger type.

The Punta Burica *Melanothamnus* species was found growing as patches of near monospecific turf within tide pools (Figures 1A and 28A). Axes were ecorticate with four pericentral cells displaying plastids restricted to their radial walls, and had rhizoids that were cut off from pericentral cells (Figure 28B–D). Tetrasporangia developed in short straight series that shifted to offset positions around the axis (Figure 28E).

Remarks: Melanothamnus was a poorly known genus of two relatively robust rhodomelacean species restricted in distribution to northeast Africa and southwest Asia [92,93]. Recent DNA sequence analyses of *Polysiphonia sensu lato* species did not resolve *Melanothamnus*, *Fernandosiphonia*, and *Neosiphonia* as independent monophyletic lineages [94]. This combined with a re-evaluation of morphological characters lead to the synonymy of *Fernandosiphonia* and *Neosiphonia* under *Melanothamnus*. The restriction of plastids to radial walls and their absence from the outer walls of pericentral cells along with 3-celled carpogonial branches are synapomorphic character states for *Melanothamnus* species [94]. Rhizoids that are cut off from pericentral cells is another character state shared by *Melanothamnus* species, but this is a state that is found in many Rhodomelaceae genera.

Many of the character states observed in the Punta Burica *Melanothamnus* species match those described for Caribbean Panama *M. pseudovillum* [95], but the two species differ in the development of tetrasporangia, which is considered to be a consistent character within *Polysiphonia sensu lato* species [25]. Caribbean Panama *M. pseudovillum* had tetrasporangia that developed in a long spiral series, in contrast to the offset short straight series of tetrasporangia observed in the Punta Burica species.

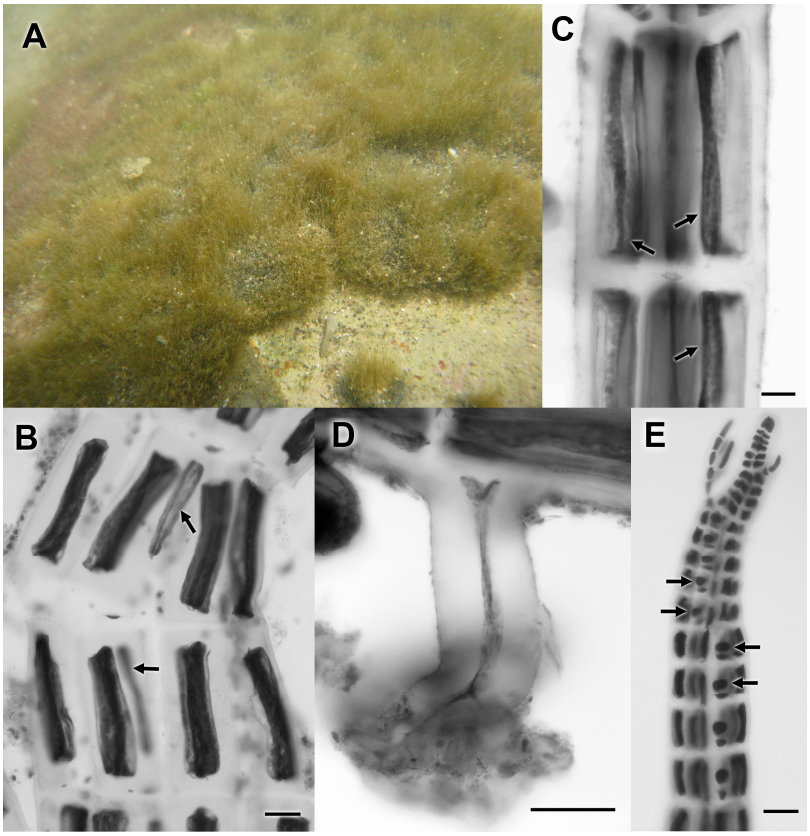

Figure 28. *Melanothamnus* sp. from Punta Burica. (**A**) In situ image of specimens growing as a turf within tide pools; (**B**) Smash preparation showing one central axial (arrows) and four pericentral cells per segment, scale = 20 μm; (**C**) Pericentral cells displaying plastids (arrows) restricted to radial walls, scale = 20 μm; (**D**) Rhizoid cut off from pericentral cell, scale = 50 μm; (**E**) Tip of fertile axis producing tetrasporangia mother cells (arrows) in offset, short straight series, scale = 20 μm.

The *rbc*L-3P divergence between the Caribbean Panama *M. pseudovillum* and Punta Burica *Melanothamnus* sp. was 2.0%, which is generally considered to represent the low end of interspecific divergence values for *Polysiphonia sensu lato* species e.g., [96–98]. COI-5P divergence values in red algae are generally greater than those for *rbc*L in complementary comparisons e.g., [99,100]. A recent study of the closely related species *Melanothamnus japonica* (Harvey) Díaz-Tapia and Maggs, *M. harveyi* (Bailey) Díaz-Tapia and Maggs, and *Polysiphonia akkeshiensis* Segi found interspecific COI-5P divergences ranging from 1.7–3.2% [101]. The COI-5P divergence of 2.8% between the Caribbean Panama *M. pseudovillum* and Punta Burica *Melanothamnus* sp. falls within this range of interspecific values for closely related species, and they represent a transisthmian geminate species pair.

3.2.22. '*Polysiphonia*' *binneyi* Harvey

PHYKOS-4534 COI-5P and UPA sequences were found by BLAST searches to be closest matches with specimens of '*Polysiphonia*' *echinata* Harvey and a '*Polysiphonia*' sp. respectively. Searches with the PHYKOS-4534 *rbc*L-3P sequence revealed a close homology (99%) with the sequence from a Caribbean Panama '*P.*' *binneyi* specimen, and they were resolved together with strong support in the *Polysiphonia sensu lato rbc*L phylogeny (Figure 27).

The PHYKOS-4534 sample was collected with a mixture of other algae from mixed sediment-mudstone bottom just beyond the Punta Burica surf zone (Figure 1), and a limited amount of material was available for study. The specimens shared character states described for Caribbean Panama '*P.*' *binneyi* including four pericentral cells; basally attenuated lateral branches; segments that were mostly shorter than broad with this being especially prominent near axes' tips; trichoblasts or scar cells at each segment, and tetrasporangia in spiral series (Figure 29).

Remarks: Mamoozadeh and Freshwater [95] generated *rbc*L sequences from four Caribbean Panama specimens identified as '*P.*' *binneyi*. Three shared identical sequences and these were 1.1% different from the fourth specimen over the *rbc*L-3P region. Direct comparisons of the Punta Burica and Caribbean '*P.*' *binneyi rbc*L-3P sequences showed that they differed by 0.8–1.6%. This is within what has been generally considered an intraspecific level of variation e.g., [95,96]. Comparisons of COI-5P sequences among these specimens are currently not possible because Mamoozadeh and Freshwater [95] were unable to amplify and sequence this locus from the Caribbean '*P.*' *binneyi* specimens. '*Polysiphonia*' *binneyi* is resolved in a strongly supported clade that is part of a series of clades only distantly related to *Polysiphonia sensu stricto* that need reclassification at the genus level see [94]. Originally described from Key West, FL, USA, this species was previously known only from the tropical Western Atlantic and Caribbean [42].

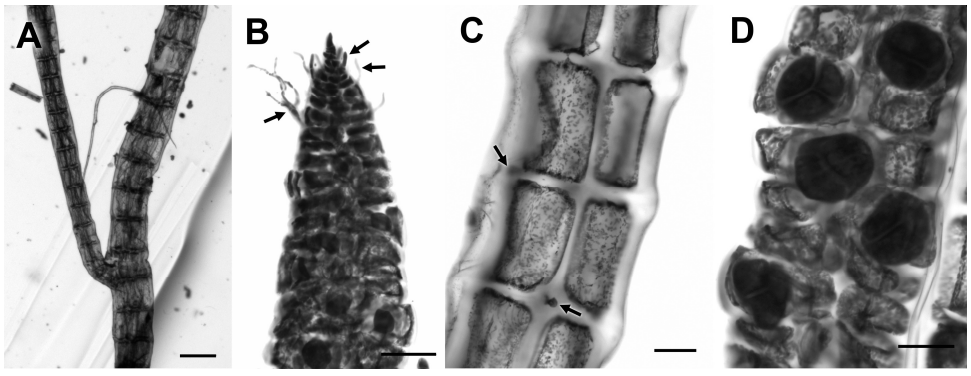

Figure 29. '*Polysiphonia*' *binneyi* from Punta Burica. (**A**) Axes with segments shorter than broad, scale = 200 μm; (**B**) Tip of axis with segments much shorter than broad and trichoblasts (arrows) produced on every segment, scale = 50 μm; (**C**) Pericentral cells with plastids present on outer walls and two visible scar cells (arrows), scale = 50 μm; (**D**) Tetrasporangia developing in a spiral series, scale = 50 μm.

3.2.23. '*Polysiphonia*' sp.

BLAST searches with sequences of PHYKOS-4515, PHYKOS-4525, PHYKOS-4526 and PHYKOS-4552 resulted in hits with sequences from Caribbean Panama specimens identified as '*Polysiphonia*' sp. for *rbc*L-3P and COI-5P, and species of *Herposiphonia* and *Symphyocladia* for UPA. Analyses of *rbc*L sequences in this study resolve the Caribbean and Pacific Panama specimens within a fully supported clade that terminates an independent lineage among representatives of *Herposiphonia*, *Lophosiphonia*, *Pterosiphonia*, *Symphyocladia*, and *Wilsonosiphonia* (Figure 27).

All four specimen collections were made from the mixed sediment-mudstone bottom just beyond the Punta Burica surf zone. Axes were ecorticate and had four pericentral cells with relatively large central axial cells; rhizoids were cut off from pericentral cells, and trichoblasts and scar cells were variable in pattern. Mid-axis segments were approximately 1× as long as wide, but this abruptly shifted to 0.5× as long as wide in upper portions of axes giving them an elongated clavate shape (Figure 30A).

Remarks: The Caribbean Panama '*Polysiphonia*' sp. *rbc*L and COI-5P sequences were generated from four specimens that shared identical sequences at both loci [102], and direct comparisons with the Punta Burica sequences revealed only 0.1% (*rbc*L) and 0.3% (COI-5P) divergence. The Punta Burica and Caribbean Panama '*Polysiphonia*' sp. specimens shared states for all observed key morphological characters. Reproductive structures were not observed in Caribbean specimens [102], but tetrasporangia in spiral series were seen developing in short lateral branches of the Punta Burica specimens (Figure 30A,B). Previous phylogenetic analyses have resolved this species as an independent lineage with no close affinity to any *Polysiphonia sensu lato* genera [95].

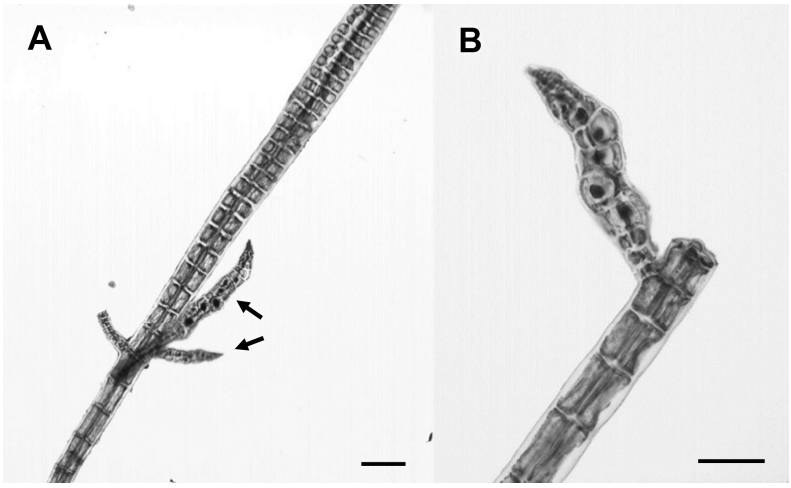

Figure 30. '*Polysiphonia*' sp. from Punta Burica. (**A**) Erect axis with a short lateral branches bearing tetrasporangia (arrows) and segments that become shorter than broad distally, scale = 200 μm; (**B**) Short lateral branch with tetrasporangia in a spiral series, scale = 100 μm.

3.2.24. *Wilsonosiphonia howei* (Hollenberg) D. Bustamante, Won and T.O. Cho

Sequences for the three analyzed loci from PHYKOS-4657 and PHYKOS-4663 returned BLAST hits for Caribbean Panama specimens of *Wilsonosiphonia howei* (Table 2). Variation between the Pacific and Caribbean specimens in nearly complete *rbc*L sequences was 1.1–1.2% and in COI-5P sequences 2.7–2.9%. Phylogenetic analyses of *rbc*L sequences for *Polysiphonia sensu lato* species and closely related genera resolve the Pacific and Caribbean *Wilsonosiphonia howei* together in a fully supported clade (Figure 27).

Both PHYKOS-4657 and PHYKOS-4663 were collected from short turf patches growing on high intertidal rocks along the eastern shoreline of the Burica Peninusla (Figure 1). These specimens displayed the key morphological characters states attributed to *Wilsonosiphonia* species including

rhizoids cut off from the distal ends of pericentral cells and "taproot shaped", multicellular rhizoid tips (Figure 31A,B). The specimens were also ecorticate; had 10–14 pericentral cells around relatively wide central axial cells (Figure 31C); displayed a pericentral cell shift to offset positons across segments; tetrasporangia that develop in a spiral series (Figure 31D), and cystocarps that were ovate and without enlarged pericarp cells around the ostiole (Figure 31E).

Remarks: Wilsonosiphonia howei was originally described as *Polysiphonia howei* Hollenberg based on specimens from the Bahamas [2]. Taylor [2] also refered Pacific Panama specimens collected from Isla Taboga in the Bay of Panama to this species, and it has been widely reported in warm-temperate and tropical waters since then e.g., [90,103–105]. Mamoozadeh and Freshwater [95] suggested that the concept of *W. howei* (as *Polysiphonia howei*) encompassed multiple species based on reported variation in spermatiagial branch development e.g., [104,106,107], pericentral cell numbers e.g., [44,90], and divergence between available nuclear-encoded 18S rRNA gene sequences. Bustamante et al. [103] included two new species in *Wilsonosiphonia* when they described the genus, *W. fujiae* D. Bustamante, Won and T.O. Cho, the generitype, and *W. indica* D. Bustamante, Won and T.O. Cho. They also considered the previous concept of *W. howei* to included multiple species, and suggested that its distribution was limited to the western Atlantic. Two recent barcoding analyses of Polysiphonieae [101] and Pterosiponieae [108] species found maximum intraspecific divergences in *rbc*L and COI-5P sequences to be 0.7% and 0.9% respectively. The *rbc*L (1.1–1.2%) and COI-5P (2.7–2.9%) divergences between the Pacific Panama and Caribbean Panama *W. howei* specimens analyzed here suggest that they are separate species and represent a transisthmian geminate pair. An examination of *W. howei* from throughout its current distribution in needed to determine how many species may be included under this name.

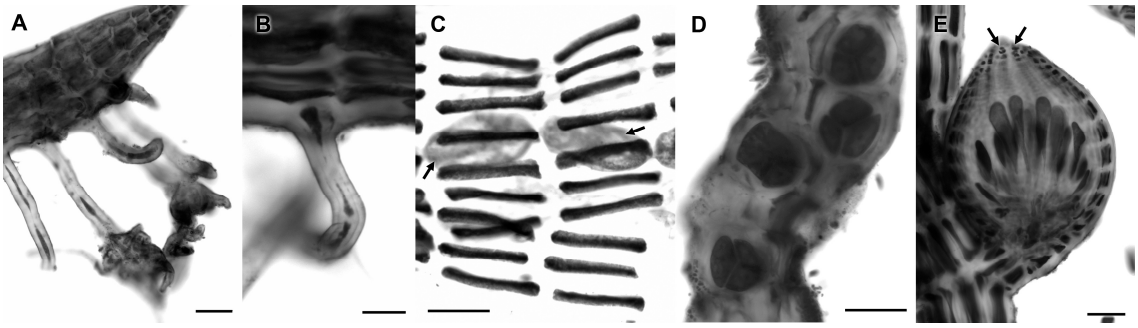

Figure 31. *Wilsonosiphonia howei* morphological characters. (**A**) Rhizoids with multicellular "taproot" tips near the apical tip of a prostrate axis, scale = 50 μm; (**B**) Base of a rhizoid showing that it is cut off from the distal end of the pericentral cell, scale = 25 μm; (**C**) Squash preparation of axis with 10 elogated pericentral cells and a relatively wide central axial (arrows) per segment, scale = 50 μm; (**D**) Tetrasporangia developing in a spiral series, scale = 50 μm; (**E**) Optical section through ovate cystocarp with developing narrowly obovate carpospores and non-enlarged pericarp cells surrounding the ostiole (arrows), scale = 50 μm.

4. Discussion

Molecular assisted identification proved to be a useful tool in this study of the previously unexplored Punta Burica marine algal flora. The three loci employed in the initial DNA barcoding approach showed different levels of variation with COI-5P having the highest, *rbc*L-3P the next highest and UPA the lowest. Despite these differences, cluster analyses with the three loci grouped the specimens into nearly identical genetic species, and analyses of the more variable COI-5P sequences resolved only one more species than analyses of complementary *rbc*L-3P and UPA sequences. Searches of publicly available databases with these sequences suggested at minimum possible genus-level classifications, and guided the assessment of morphological characters for the verification of species-level classifications. However, careful evaluation of search results is required

because many database entries have not been updated to the most recent classification and others represent misidentifications.

The recent compilation of marine algae reported in the Central American Pacific by Fernández-García et al. [5] recorded 379 species in the region. Pacific Panama had the second highest number with 174, yet there were no records of marine algae from the western Gulf of Chiriqui mainland coast, and fewer than 20 species had been reported from islands in this area [2–4,109]. As pointed out in Fernández-García et al. [5], much of the discrepancy in the number of reported species along Pacific Central America is a product of exploration effort, as the region's high heterogeneity in habitats and physical environmental factors should be conducive to generally high marine algal diversity. In contrast to the small number of marine algal records in the western Gulf of Chiriqui, the adjacent coastal area of southeastern Costa Rica to the west has a high number of reported species, but it is also one of the better-explored areas in Pacific Central America [5].

Despite our Punta Burica collecting being limited to only four short events, numerous species of red, green and brown algae were found. The 26 species treated here represent only the collections of foliose red algae and are the first reports of marine algae from the mainland coast of the western Gulf of Chiriqui. Twenty-one of these are new reports for Panama, and nine are new reports for Pacific Central America. Four records appear to be new to the Pacific entirely, including the two newly described species, *Neorubra parvolacertoides* and *Grateloupia irregularis*, as well as '*Polysiphonia*' *binneyi* and *Hypnea flava*. It is perhaps not surprising, given the paucity of historical study for this remote region of Panama that 12 of the 26 species reported here likely represent novel species, requiring further morphological and molecular examination.

The mudstone substrate in the Punta Burica intertidal zone provides an environment more conducive to the attachment and growth of marine algae than many other hard substrates along the Pacific Panama coast. Perhaps this distinctive environment has driven evolutionary adaptation resulting in a local biodiversity hotspot. Alternatively, it may simply be, as others have asserted [56], that the more we look, the more we find and that as our geographic coverage expands within Panama or throughout Central America, redundancy in species richness assessment will be revealed.

Another important aspect of the work presented here is the recognition of transisthmian geminate species pairs. Evolutionary divergence between geminate species may facilitate the calibration of important DNA barcode markers, at least among closely related species. The greater the availability of geminate species pairs for marine algae lacking a meaningful fossil record, the more accurately can molecular calibrations be estimated, while recognizing the inherent challenge e.g., [110] to isthmian-derived evolutionary rate calculations. Here, the value of DNA barcoding as a floristics tools is remarkable; DNA barcoding in our floristic study has provided a more accurate assessment of marine algal biodiversity, even when the amount of material or specimens available for study was small. Continued DNA barcoding studies of the Burica green, brown and red algal floras, as well as continuing studies of the Panamanian marine flora more generally, will undoubtedly shed new light on the novelty of the mudrock environment as a potential refuge for algal diversity and continue to illuminate new subjects for studying the evolutionary biology of marine macroalgae.

Supplementary Materials: The following are available online at www.mdpi.com/1424-2818/9/2/19/s1, Table S1: Data matrix dimensions and analysis parameters for phylogenetic analyses carried out in this study.

Acknowledgments: This study was funded by US National Science Foundation Biotic Surveys and Inventories grant 0743334, the Center for Marine Science DNA-Algal Trust, and the Red Pond Trust. The authors wish to thank Melissa Smith for help with graphics and Melissa LaCroce for technical help with putting the manuscript together.

Author Contributions: B.W. organized and planned the collecting expedition; B.W., D.W.F., C.F.-G. and N.L. collected the studied specimens, S.L.P., P.W.G. and D.W.F. generated sequence data; J.N.I., P.W.G. and D.W.F. carried out DNA sequence and morphological analyses; D.W.F., P.W.G., C.F.-G. and B.W. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Howe, M.A. Report on a botanical visit to the Isthmus of Panama. *J. N. Y. Bot. Gard.* **1910**, *11*, 30–44.
2. Taylor, W.R. *Pacific Marine Algae of the Allan Hancock Expeditions to the Galapagos Islands*; The University of Southern California Press: Los Angeles, CA, USA, 1945; p. 528.
3. Wysor, B. An annotated list of marine Chlorophyta from the Pacific coast of the republic of Panama with a comparison to Caribbean Panama species. *Nova Hedwigi.* **2004**, *78*, 209–241. [CrossRef]
4. Littler, M.M.; Littler, D.S. Coralline algal rhodoliths form extensive benthic communities in the Gulf of Chiriqui, Pacific Panama. *Coral Reefs* **2008**, *27*, 553. [CrossRef]
5. Fernández-García, C.; Riosmena-Rodríguez, R.; Wysor, B.; Tejada, O.L.; Cortés, J. Checklist of the Pacific marine macroalgae of Central America. *Bot. Mar.* **2011**, *54*, 53–73. [CrossRef]
6. Dawson, E.Y. Marine algae from the Pacific Costa Rican gulfs. *Contrib. Sci.* **1957**, *15*, 1–28.
7. Morell, K.D.; Fisher, D.M.; Gardner, T.W.; Femina, P.L.; Davidson, D.; Teletzke, A. Quaternary outer fore-arc deformation and uplift inboard of the Panama Triple Junction, Burica Peninsula. *J. Geophys. Res.* **2011**, *116*, B05402. [CrossRef]
8. Buchs, D.M.; Baumgartner, P.O.; Baumgartner-Mora, C.; Bandini, A.N.; Jackett, S.-J.; Diserens, M.-O.; Stucki, J. Late Cretaceous to Miocene seamount accretion and mélange formation in the Osa and Burica Peninsulas (Southern Costa Rica): Episodic growth of a convergent margin. In *The Origin and Evolution of the Caribbean Plate*; Lorente, J.K., Pindell, M.A., Eds.; Geological Society: London, UK, 2007; Volume 28, pp. 411–456.
9. Mamoozadeh, N.R.; Freshwater, D.W. Taxonomic notes on Caribbean *Neosiphonia* and *Polysiphonia* (Ceramiales, Flordeophyceae): Five species from Florida, USA and Mexico. *Bot. Mar.* **2011**, *54*, 269–292. [CrossRef]
10. Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; de Waard, J.R. Biological identifications through DNA barcodes. *Philos. Trans. R. Soc. B* **2003**, *270*, 313–321. [CrossRef] [PubMed]
11. Saunders, G.W. Applying DNA barcoding to red macroalgae: A preliminary appraisal holds promise for future applications. *Phil. Trans. R. Soc. B* **2005**, *360*, 1879–1888. [CrossRef] [PubMed]
12. Robba, L.; Russell, S.J.; Barker, G.L.; Brodie, J. Assessing the use of the mitochondrial *cox1* marker for use in DNA barcoding of red algae (Rhodophyta). *Am. J. Bot.* **2006**, *93*, 1101–1108. [CrossRef] [PubMed]
13. Le Gall, L.; Saunders, G.W. DNA barcoding is a powerful tool to uncover algal diversity: A case study of the Phyllophoraceae (Gigartinales, Rhodophyta) in the Canadian flora. *J. Phycol.* **2010**, *46*, 374–389. [CrossRef]
14. Milstein, D.; Saunders, G.W. DNA barcoding of Canadian Ahnfeltiales (Rhodophyta) reveals a new species—*Ahnfeltia borealis* sp. nov. *Phycologia* **2012**, *51*, 247–259. [CrossRef]
15. Lyra, G.D.M.; Gurgel, C.F.D.; Costa, E.D.S.; de Jesus, P.B.; Oliveira, M.C.; Oliveira, E.C.; Davis, C.C.; Nunes, J.M.D.C. Delimitating cryptic species in the *Gracilaria domingensis* complex (Gracilariaceae, Rhodophyta) using molecular and morphological data. *J. Phycol.* **2016**, *52*, 997–1017. [CrossRef] [PubMed]
16. Sherwood, A.R.; Presting, G.G. Universal primers amplify a 23S rDNA plastid marker in eukaryotic algae and cyanobacteria. *J. Phycol.* **2007**, *43*, 605–608. [CrossRef]
17. Sherwood, A.R.; Sauvage, T.; Kurihara, A.; Conklin, K.T.; Presting, G.G. A comparative analysis of COI, LSU and UPA marker data for the Hawaiian florideophyte Rhodophyta: Implications for DNA barcoding of red algae. *Cryptogam. Algologie* **2010**, *31*, 451–465.
18. Iha, C.; Milstein, D.; Guimarães, S.M.P.B.; Freshwater, D.W.; Oliveira, M.C. DNA barcoding reveals high diversity in the Gelidiales of the Brazilian southeast coast. *Bot. Mar.* **2015**, *58*, 295–305. [CrossRef]
19. Chase, M.W.; Hills, H.H. Silica gel: An ideal material for field preservation of leaf samples for DNA studies. *Taxon* **1991**, *40*, 215–220. [CrossRef]
20. Hughey, J.R.; Silva, P.C.; Hommersand, M.H. Solving taxonomic and nomenclatural problems in Pacific Gigartinaceae (Rhodophyta) using DNA from type material. *J. Phycol.* **2001**, *37*, 1091–1109. [CrossRef]
21. Freshwater, D.W.; Braly, S.K.; Stuercke, B.; Hamner, R.M.; York, R.A. Phylogenetic analyses of North Carolina Rhodymeniales. I. The genus *Asteromenia*. *J. N. C. Acad. Sci.* **2005**, *121*, 49–55.
22. Saunders, G.W. A DNA barcode examination of the red algal family Dumontiaceae in Canadian waters reveals substantial cryptic species diversity. 1. The foliose *Disea-Neodilsea* complex and *Weeksia*. *Botany* **2008**, *86*, 773–789. [CrossRef]
23. Saunders, G.W.; McDevit, D.C. Methods for DNA barcoding photosynthetic protists emphasizing the macroalgae and diatoms. *Methods Mol. Biol.* **2012**, *858*, 207–222. [PubMed]

24. Freshwater, D.W.; Rueness, J. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbc*L nucleotide sequence analysis. *Phycologia* **1994**, *33*, 187–194. [CrossRef]
25. Stuercke, B.; Freshwater, D.W. Consistency of morphological characters used to delimit *Polysiphonia sensu lato* species (Ceramiales, Florideophyceae): Analyses of North Carolina, USA specimens. *Phycologia* **2008**, *47*, 541–559. [CrossRef]
26. Edgar, R.C. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **2004**, *32*, 1792–1797. [CrossRef] [PubMed]
27. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [CrossRef]
28. Stamatakis, A. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **2006**, *22*, 2688–2690. [CrossRef] [PubMed]
29. Huelsenbeck, J.P.; Ronquist, F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **2001**, *17*, 754–755. [CrossRef] [PubMed]
30. Won, B.Y.; Cho, T.O.; Fredericq, S. Morphological and molecular characterization of species of the genus *Centroceras* (Ceramiaceae, Ceramiales), including two new species. *J. Phycol.* **2009**, *45*, 227–250. [CrossRef] [PubMed]
31. Grusz, A.L.; Freshwater, D.W. Studies of Costa Rican Gelidiales (Florideophyceae). II. Two Pacific taxa including *Gelidium microglossum*, n. sp. *Pac. Sci.* **2014**, *68*, 97–110. [CrossRef]
32. Boo, G.H.; Hughey, J.R.; Miller, K.A.; Boo, S.M. Mitogenomes from type specimens, a genotyping tool for morphologically simple species: Ten genomes of agar-producing red algae. *Sci. Rep.* **2016**, *6*, 35337. [CrossRef] [PubMed]
33. Wang, W.-L.; Liu, S.-L.; Lin, S.-M. Systematics of the calcified genera of the Galaxauraceae (Nemaliales, Rhodophyta) with an emphasis on Taiwan species. *J. Phycol.* **2005**, *41*, 685–703. [CrossRef]
34. Wiriyadamrikul, J.; Geraldino, P.J.L.; Huisman, J.M.; Lewmanomont, K.; Boo, S.M. Molecular diversity of the calcified red algal genus *Tricleocarpa* (Galaxauraceae, Nemaliales) with the description of *T. jejuensis* and *T. natalensis*. *Phycologia* **2013**, *52*, 338–351. [CrossRef]
35. Huisman, J.M.; Borowitzka, M.A. A revision of the Australian species of *Galaxaura* (Rhodophyta, Galaxauraceae), with a description of *Tricleocarpa* gen. nov. *Phycologia* **1990**, *29*, 150–172. [CrossRef]
36. Le Gall, L.; Saunders, G.W. Establishment of a DNA-barcode library for the Nemaliales (Rhodophyta) from Canada and France uncovers overlooked diversity in the species *Nemalion helminthoides* (Velley) Batters. *Cryptogam. Algologie* **2010**, *31*, 403–421.
37. Machín-Sánchez, M.; Rousseau, F.; Le Gall, L.; Cassano, V.; Neto, A.I.; Sentíes, A.; Fuji, M.T.; Gil-Rodríguez, M.C. Species diversity of the genus *Osmundea* (Ceramiales, Rhodophyta) in the Macaronesian region. *J. Phycol.* **2016**, *52*, 664–681. [CrossRef] [PubMed]
38. Taylor, W.R. *Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas*; The University of Michigan Press: Ann Arbor, MI, USA, 1960; p. 870.
39. Costa, J.; Lin, S.-M.; Macaya, E.; Fernández-Garcia, C.; Verbruggen, H. Chloroplast genomes as a tool to resolve red algal phylogenies: A case study in the Nemaliales. *BMC Evol. Biol.* **2016**, *16*, 205–218. [CrossRef] [PubMed]
40. Huisman, J.M. The type and Australian species of the red algal genera *Liagora* and *Ganonema* (Liagoraceae, Nemaliales). *Aust. Syst. Bot.* **2002**, *15*, 773–838. [CrossRef]
41. Lin, S.-M.; Huisman, J.M.; Ballantine, D.L. Revisiting the systematics of *Ganonema* (Liagoraceae, Rhodophyta) with emphasis on species from the northwest Pacific Ocean. *Phycologia* **2014**, *53*, 37–51. [CrossRef]
42. Guiry, M.D.; Guiry, G.M. AlgaeBase. World-Wide Electronic Publication, National University of Ireland, Galway, 2017. Available online: www.algaebase.org (accessed on 30 January 2017).
43. Lin, S.-M.; Yang, S.-Y.; Huisman, J.M. Systematic revision of the genera *Liagora* and *Izziella* (Liagoraceae, Rhodophyta) from Taiwan based on molecular analyses an carposporophyte development, with the description of two new species. *J. Phycol.* **2011**, *47*, 352–365. [CrossRef] [PubMed]
44. Abbott, I.A. *Marine Red Algae of the Hawaiian Islands*; Bishop Museum Press: Honolulu, HI, USA, 1999; p. 477.
45. Lin, S.-M.; Huisman, J.M.; Payri, C. Characterization of *Liagora ceranoides* (Liagoraceae, Rhodopyta) on the basis of *rbc*L sequence analyses and carposporophyte development, including *Yoshizakia indopacifica* gen. et sp. nov. from the Indo-Pacific region. *Phycologia* **2013**, *52*, 161–170. [CrossRef]

46. Lin, S.-M.; Yang, S.-Y.; Huisman, J.M. Systematics of *Liagora* with diffuse gonimoblasts based on *rbc*L sequences and carposporophyte development, including the description of the genera *Neoizziella* and *Macrocarpus* (Liagoraceae, Rhodophyta). *Eur. J. Phycol.* **2011**, *46*, 249–262. [CrossRef]
47. Boo, G.H.; Kim, K.M.; Nelson, W.A.; Riosmena-Rodríguez, R.; Yoon, K.J.; Boo, S.M. Taxonomy and distribution of selected species of the agarophyte genus *Gelidium* (Gelidiales, Rhodophyta). *J. Appl. Phycol.* **2014**, *26*, 1243–1251. [CrossRef]
48. Thomas, D.T.; Freshwater, D.W. Studies of Costa Rican Gelidiales (Rhodophyta): Four Caribbean taxa including *Pterocladiella beachii* sp. nov. *Phycologia* **2001**, *40*, 340–350. [CrossRef]
49. Littler, D.S.; Littler, M.M. Marine Plants of Pacific Panama. 2009. Available online: http://biogeodb.stri.si.edu/pacificalgae/list (accessed on 30 January 2017).
50. Kim, K.M.; Boo, S.M. Phylogenetic relationships and distribution of *Gelidium crinale* and *G. pusillum* (Gelidiales, Rhodophyta) using *cox*1 and *rbc*L sequences. *Algae* **2012**, *27*, 83–94. [CrossRef]
51. Santelices, B. *Parviphycus*, a new genus in the Gelidiellaceae (Gelidiales, Rhodophyta). *Cryptogam. Algologie* **2004**, *25*, 313–326.
52. Boo, G.H.; Nguyen, T.V.; Kim, J.Y.; Le Gall, L.; Rico, J.M.; Bottalico, A.; Boo, S.M. A revised classification of the Gelidiellaceae (Rhodophyta) with descriptions of three new genera: *Huismaniella*, *Millerella* and *Perronella*. *Taxon* **2016**, *65*, 965–979. [CrossRef]
53. Dawson, E.Y. The marine algae of the Gulf of California. *Allan Hancock Pac. Exped.* **1944**, *3*, 189–454.
54. Dawson, E.Y. Marine red algae of Pacific Mexico. Part I. Bangiales to Corallinaceae subf. Corallionoidea. *Allan Hancock Pac. Exped.* **1953**, *17*, 1–239.
55. Dawson, E.Y. Plantas Marinas de la zona de las mareas de El Salvador. *Pac. Nat.* **1961**, *2*, 389–461.
56. Dijoux, L.; Viard, F.; Payri, C. The more we search, the more we find: Discovery of a new lineage and new species complex in the genus *Asparagopsis*. *PLoS ONE* **2014**, *9*, e103826. [CrossRef] [PubMed]
57. Andreakis, N.; Procaccini, G.; Maggs, C.; Kooistra, W.H.C.F. Phylogeography of the invasive seaweed *Asparagopsis* (Bonnemaisoniales, Rhodophyta) reveals cryptic diversity. *Mol. Ecol.* **2007**, *16*, 2285–2299. [CrossRef] [PubMed]
58. Zanolla, M.; Carmona, R.; De La Rosa, J.; Salvador, N.; Sherwood, A.R.; Andreakis, N.; Altamirano, M. Morphological differentiation of cryptic lineages within the invasive genus *Asparagopsis* (Bonnemaisoniales, Rhodophyta). *Phycologia* **2014**, *53*, 233–242. [CrossRef]
59. Abbott, I.A.; Hollenberg, G.J. *Marine Algae of California*; Stanford University Press: Stanford, CA, USA, 1976; p. 827.
60. Farlow, W.G. On some algae new to the United States. *Proc. Am. Acad. Arts Sci.* **1877**, *4*, 235–245. [CrossRef]
61. Nauer, F.; Guimarães, N.R.; Cassano, V.; Yokoya, N.S.; Oliveira, M.C. *Hypnea* species (Gigartinales, Rhodophyta) from the southeastern coast of Brazil based on molecular studies complemented with morphological analyses, including descriptions of *Hypnea edeniana* sp. nov. and *H. flava* sp. nov. *Eur. J. Phycol.* **2014**, *49*, 550–575. [CrossRef]
62. Dawson, E.Y. Marine red algae of Pacific Mexico, part 4. Gigartinales. *Pac. Nat.* **1961**, *2*, 191–343.
63. Norris, J.N. *Marine Algae of the Northern Gulf of California II: Rhodophyta*; Smithsonian Institution Scholarly Press: Washington, DC, USA, 2014; Volume 96, pp. 1–555.
64. Payri, C.; De Ramon N'Yeurt, A.; Orempuller, J. *Algae of French Polynesia*; Au Vent Des Iles: Tahiti, France, 2000; p. 320.
65. De Ramon N'Yeurt, A. Marine algae from the Suva Lagoon and reef, Fiji. *Aust. Syst. Bot.* **2001**, *14*, 689–869.
66. Littler, D.S.; Littler, M.M. *South Pacific Reef Plants, A Diver's Guide to the Plant Life of South Pacific Coral Reefs*; Offshore Graphics, Inc.: Washington, DC, USA, 2003; p. 331.
67. Gargiulo, G.M.; Morabito, M.; Manghisi, A. A re-assessment of reproductive anatomy and postfertilization development in the systematics of *Grateloupia* (Halymeniales, Rhodophyta). *Cryptogam. Algologie* **2013**, *34*, 3–35. [CrossRef]
68. Calderon, M.S.; Boo, G.H.; Boo, S.M. *Neorubra decipiens* gen. & comb. nov. and *Phyllymenia lancifolia* comb. nov. (Halymeniales, Rhodophyta) from South America. *Phycologia* **2014**, *53*, 409–422.
69. Calderon, M.S.; Boo, G.H.; Boo, S.M. Morphology and phylogeny of *Ramirezia osornoensis* gen. & sp. nov. and *Phyllymenia acletoi* sp. nov. (Halymeniales, Rhodophyta) from South America. *Phycologia* **2014**, *53*, 23–36.

70. Calderon, M.S.; Boo, G.H.; Boo, S.M. Corrigendum of Morphology and phylogeny of *Ramirezia osornoensis* gen. & sp. nov. and *Phyllymenia acletoi* sp. nov. (Halymeniales, Rhodophyta) from South America. *Phycologia* **2016**, *55*, 610.
71. Saunders, G.W.; Strachan, I.M.; Kraft, G.T. The families of the order Rhodymeniales (Rhodophyta): A molecular-systematic investigation with a description of Faucheaceae fam. nov. *Phycologia* **1999**, *38*, 23–40. [CrossRef]
72. Zanardini, G. Phyceae papuanae novae vel minus cognitae a Cl. O. Beccari in itinere ad Novam Guineam annis 1872-75 collectae. *Nuovo Giorn. Bot. Ital.* **1878**, *10*, 34–40.
73. Norris, R.E. The systematic position of *Gelidiopsis* and *Ceratodictyon* (Gigartinales, Rhodophyceae), genera new to South Africa. *S. Afr. J. Bot.* **1987**, *53*, 239–246. [CrossRef]
74. Le Gall, L.; Dalen, J.L.; Saunders, G.W. Phylogenetic analyses of the red algal order Rhodymeniales supports recognition of the Hymenocldiaceae fam. nov., Fryeellaceae fam. nov., and *Neogastroclonium* gen. nov. *J. Phycol.* **2008**, *44*, 1556–1571. [CrossRef] [PubMed]
75. Filloramo, G.V.; Saunders, G.W. Application of multigene phylogenetics and site-stripping to resolve intraordinal relationships in the Rhodymeniales (Rhodophyta). *J. Phycol.* **2016**, *52*, 339–355. [CrossRef] [PubMed]
76. De Clerck, O.; Tronchin, E.M.; Schils, T. Red Algae, Rhodophyceae. In *Guide to the Seaweeds of Kwazulu-Natal*; De Clerk, O., Bolton, J.J., Anderson, R.J., Coppejans, E., Eds.; Scripta Botanica Belgica 33, National Botanic Garden of Belgium: Meise, Belgium, 2005; pp. 132–267.
77. Tejada, O.L. Listado de macroalgas en el litoral de El Salvador, basado en registros entre 1961 al 2001. In *Diagnóstico de la Diversidad Biológica de El Salvador*; Flores, V.O., Handal, A., Eds.; Red Mesoamericana de Recursos Bióticos: Mexico City, Mexico, 2003; pp. 1–171.
78. Hommersand, M.H.; Freshwater, D.W.; Lopez-Bautista, J.M.; Fredericq, S. Proposal of the Euptiloteae Hommersand et Fredericq *trib. nov.* and transfer of some southern hemisphere Ptiloteae to the Callithamnieae (Ceramiaceae, Rhodophyta). *J. Phycol.* **2005**, *42*, 203–225. [CrossRef]
79. Rodríguez-Prieto, C.; Freshwater, D.W.; Hommersand, M.H. Vegetative and reproductive development of Mediterranean *Gulsonia nodulosa* (Ceramiales, Rhodophyta) and its genetic affinities. *Phycologia* **2013**, *52*, 357–367. [CrossRef]
80. McIvor, L.; Maggs, C.A.; Stanhope, M.J. *Rbc*L sequences indicate a single evolutionary origin of multinucleate cells in the red algal tribe Callithamnieae. *Mol. Phylogenet. Evol.* **2002**, *23*, 433–446. [CrossRef]
81. Aponte, N.E.; Ballantine, D.L.; Norris, J.N. *Aglaothamnion halliae* comb. nov. and *A. collinsii* sp. nov. (Ceramiales, Rhodophyta): Resolution of nomenclatural and taxonomic confusion. *J. Phycol.* **1997**, *33*, 81–87. [CrossRef]
82. Dawson, E.Y. An annotated list of marine algae from Eniwetok Atoll, Marshall Islands. *Pac. Sci.* **1957**, *11*, 92–132.
83. Børgesen, F. The marine algae of the Danish West Indies. Part 3. Rhodophyceae (3). *Dansk Bot. Arkiv.* **1917**, *3*, 145–240.
84. Won, B.Y.; Fredericq, S.; Cho, T.O. Two new species of *Centroceras* (Ceramiales, Rhodophyta) from KwaZulu-Natal, South Africa. *Eur. J. Phycol.* **2010**, *45*, 240–246. [CrossRef]
85. Schneider, C.W.; Cianciola, E.N.; Popolizio, T.R.; Spagnuolo, D.S.; Lane, C.E. A molecular-assisted alpha taxonomic study of the genus *Centroceras* (Ceramiaceae, Rhodophyta) in Bermuda reveals two novel species. *Algae* **2015**, *30*, 15–33. [CrossRef]
86. Dawson, E.Y. New records of marine algae from Pacific Mexico and Central America. *Pac. Nat.* **1960**, *1*, 31–52.
87. Zuccarello, G.C.; Sandercock, B.; West, J.A. Diversity within red algal species: Variations in world-wide samples of *Spyridia filamentosa* (Ceramiaceae) and *Murrayella periclados* (Rhodomelaceae) using DNA markers and breeding studies. *Eur. J. Phycol.* **2002**, *37*, 403–417. [CrossRef]
88. Zuccarello, G.C.; Purd'homme van Reine, W.F.; Stegenga, H. Recognition of *Spyridia griffithsiana* comb. nov. (Ceramiales, Rhodophyta): A taxon previously misidentified as *Spyridia filamentosa* from Europe. *Bot. Mar.* **2004**, *47*, 481–489. [CrossRef]
89. Conklin, K.Y.; Sherwood, A.R. Molecular and morphological variation of the red alga *Spyridia filamentosa* (Ceramiales, Rhodophyta) in the Hawaiian Archipelago. *Phycologia* **2012**, *51*, 347–357. [CrossRef]

90. Kapraun, D.F. *An Illustrated Guide to the Benthic Marine Algae of Coastal North Carolina I. Rhodophyta*; The University of North Carolina Press: Chapel Hill, NC, USA, 1980; p. 206.
91. Schneider, C.W.; Searles, R.B. *Seaweeds of the Southeastern United States, Cape Hatteras to Cape Canaveral*; Duke University Press: Durham, NC, USA, 1991; p. 553.
92. Wynne, M.J.; Banaimoon, S.A. The occurrence of *Jolyna laminarioides* (Phaeophyta) in the Arabian Sea and the Indian Ocean, and a new report of *Melanothamnus somalensis* (Rhodophyta). *Bot. Mar.* **1990**, *33*, 213–218. [CrossRef]
93. Shameel, M. *Melanothamnus afaqhusainii*, a new red alga from the coast of Karachi. *Pak. J. Bot.* **1999**, *31*, 211–214.
94. Díaz-Tapia, P.; McIvor, L.; Freshwater, D.W.; Verbruggen, H.; Wynne, M.J.; Maggs, C.A. The genera *Melanothamnus* Bornet & Falkenberg and *Vertebrata* S.F. Gray constitute well-defined clades of the red algal tribe Polysiphonieae (Rhodomelaceae, Ceramiales). *Eur. J. Phycol.* **2017**, *52*, 1–30.
95. Mamoozadeh, N.R.; Freshwater, D.W. *Polysiphonia sensu lato* (Ceramiales, Florideophyceae) species of Caribbean Panama including *Polysiphonia lobophoralis* sp. nov. and *Polysiphonia nuda* sp. nov. *Bot. Mar.* **2012**, *55*, 317–347. [CrossRef]
96. McIvor, L.; Maggs, C.A.; Provan, J.; Stanhope, M.J. *rbc*L sequences reveal multiple cryptic introductions of the Japanese red alga *Polysiphonia harveyi*. *Mol. Ecol.* **2001**, *10*, 911–919. [CrossRef] [PubMed]
97. Kim, M.S.; Yang, E.C. Taxonomic note of *Polysiphonia pacifica* (Ceramiales, Rhodophyta) complex with focus on Pacific isolates. *Algae* **2005**, *20*, 15–23. [CrossRef]
98. Bustamante, D.E.; Won, B.Y.; Cho, T.O. *Polysiphonia ulleungensis* sp. nov. (Rhodomelaceae, Rhodophyta): A new diminutive species from Korea belonging to *Polysiphonia sensu stricto*. *Algae* **2014**, *29*, 111–120. [CrossRef]
99. Freshwater, D.W.; Tudor, K.; O'Shaughnessy, K.; Wysor, B. DNA barcoding in the red algal order Gelidiales: Comparison of COI with *rbc*L and verification of the "barcoding gap". *Cryptogam. Algologie* **2010**, *31*, 435–449.
100. Tan, J.; Lim, P.-E.; Phang, S.-M.; Hong, D.D.; Sunarpi, H.; Hurtado, A.Q. Assessment of four molecular markers as potential DNA barcodes for red algae *Kappaphycus* Doty and *Eucheuma* J. Agardh (Solieriaceae, Rhodophyta). *PLoS ONE* **2012**, *7*, e52905. [CrossRef] [PubMed]
101. Savoie, A.M.; Saunders, G.W. Evidence for the introduction of the Asian red alga *Neosiphonia japonica* and its introgression with *Neosiphonia harveyi* (Ceramiales, Rhodophyta) in the Northwest Atlantic. *Mol. Ecol.* **2015**, *24*, 5927–5937. [CrossRef] [PubMed]
102. Mamoozadeh, N.R. Morphological and Molecular Analyses of Polysiphonia sensu lato in Southern Central America and the Caribbean. Master's Thesis, University of North Carolina Wilmington, Wilmington, NC, USA, 2010.
103. Bustamante, D.E.; Won, B.Y.; Miller, K.A.; Cho, T.O. *Wilsonosiphonia* gen. nov. (Rhodomelaceae, Rhodophyta) based on molecular and morpho-anatomical characters. *J. Phycol.* **2017**, *53*. [CrossRef] [PubMed]
104. Kapraun, D.F.; Lemus, A.J.; Bula Meyer, G. Genus *Polysiphonia* (Rhodophyta, Ceramiales) in the tropical western Atlantic. I. Colombia and Venezuela. *Bull. Mar. Sci.* **1983**, *33*, 881–898.
105. Skelton, P.A.; South, G.R. The benthic marine algae of the Samoan Archipelago, South Pacific, with emphasis on the Apia District. *Beih. Nova Hedwig.* **2007**, *132*, 1–350.
106. Hollenberg, G.J. An account of the species of the red alga *Polysiphonia* of the central and western tropical Pacific Ocean. II. *Polysiphonia*. *Pac. Sci.* **1968**, *22*, 198–207.
107. Dawes, C.J.; Mathieson, A.C. *The seaweeds of Florida*; University Press of Florida: Gainesville, FL, USA, 2008; p. 591.
108. Savoie, A.M.; Saunders, G.W. A molecular phylogenetic and DNA barcode assessment of the tribe Pterosiphonieae (Ceramiales, Rhodophyta) emphasizing the Northeast Pacific. *Botany* **2016**, *94*, 917–939. [CrossRef]
109. Lemoine, P. Les Corallinacees de l'Archipel des Galapagos et du Golfo de Panama. *Arch. Mus. Hist. Nat.* **1929**, *6*, 47–88.
110. Knowlton, N.; Weigt, L.A. New dates and new rates for divergence across the Isthmus of Panama. *Proc. Biol. Sci.* **1998**, *265*, 2257–2263. [CrossRef]