TYPE SPECIMEN SEQUENCING, MULTILOCUS ANALYSES, AND SPECIES DELIMITATION METHODS RECOGNIZE THE COSMOPOLITAN *CORALLINA BERTEROI* AND ESTABLISH THE NORTHERN JAPANESE *C. YENDOI* SP. NOV. (CORALLINACEAE, RHODOPHYTA)[1]

Martha S. Calderon [2]

Laboratorio de Ecosistemas Marinos Antárticos y Sub-antárticos (LEMAS), Universidad de Magallanes, Punta Arenas, Chile
Instituto de Ecología y Biodiversidad (IEB), Santiago, Chile

Danilo E. Bustamante

Instituto de Investigación para el Desarrollo Sustentable de Ceja de Selva (INDES-CES), Universidad Nacional Toribio Rodríguez de Mendoza, Chachapoyas, Peru
Department of Civil and Environmental Engineering (FICIAM), Universidad Nacional Toribio Rodríguez de Mendoza, Chachapoyas, Peru

Paul W. Gabrielson

Biology Department and Herbarium, Coker Hall CB 3280, University of North Carolina, Chapel Hill, Chapel Hill, North Carolina 27599-3280, USA

Patrick T. Martone

Botany Department & Biodiversity Research Centre, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

Katharine R. Hind

Department of Biology, University of Victoria, PO Box 1700 Station CSC, Victoria, BC V8W 2Y2, Canada

Soren R. Schipper

Botany Department & Biodiversity Research Centre, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

and Andrés Mansilla

Laboratorio de Ecosistemas Marinos Antárticos y Sub-antárticos (LEMAS), Universidad de Magallanes, Punta Arenas, Chile
Instituto de Ecología y Biodiversidad (IEB), Santiago, Chile

A partial *rbc*L sequence of the lectotype specimen of *Corallina berteroi* shows that it is the earliest available name for *C. ferreyrae*. Multilocus species delimitation analyses (ABGD, SPN, GMYC, bPTP, and BPP) using independent or concatenated COI, *psb*A, and *rbc*L sequences recognized one, two, or three species in this complex, but only with weak support for each species hypothesis. Conservatively, we recognize a single worldwide species in this complex of what appears to be multiple, evolving populations. Included in this species, besides *C. ferreyrae*, are *C. caespitosa*, the morphologically distinct *C. melobesioides*, and, based on a partial *rbc*L sequence of the holotype specimen, *C. pinnatifolia*. *Corallina berteroi*, not *C. officinalis*, is the cosmopolitan temperate species found thus far in the NE Atlantic, Mediterranean Sea, warm temperate NW Atlantic and NE Pacific, cold temperate SW Atlantic (Falkland Islands), cold and warm temperate SE Pacific, NW Pacific and southern Australia. Also proposed is *C. yendoi* sp. nov. from Hokkaido, Japan, which was recognized as distinct by 10 of the 13 species discrimination analyses, including the multilocus BPP.

Species are a fundamental unit in biological research, yet species delimitation can be objectively challenging (Kuchta et al. 2016). Multilocus genetic data are increasingly being used to delimit species and to identify distinct evolutionary lineages (Jackson et al. 2017). In addition, DNA-based methods delimit species using various strategies such as genetic distance (e.g., automated barcode gap discovery algorithm, ABGD; statistical parsimony, SPN), coalescence (e.g., generalized mixed Yule coalescent, GMYC; Poisson tree processes, PTP; Bayesian

phylogenetics and phylogeography, BPP; and phylogeographic inference using approximate likelihoods, PHRAPL), and genealogical concordance (genealogical concordance phylogenetic species recognition, GCPSR; Luo et al. 2018, Bustamante et al. 2019b). These approaches have been used to delineate eukaryotic species and investigate diversification processes (Carstens et al. 2013, Liu et al. 2015); lately these are being widely used on coralline algae (Pardo et al. 2014, Torrano-Silva et al. 2018, Costa et al. 2019, Pezzolesi et al. 2019, Twist et al. 2019, Brodie et al. 2020).

To date, studies of *Corallina* systematics using DNA sequencing have focused primarily on resolving whether or not species belong in the genus and on correctly applying names by sequencing type material. For example, based on DNA sequence data – and despite morphological similarity to *Corallina* species – *C. elongata* was shown to belong in its own genus, *Ellisolandia* (Hind and Saunders 2013). In addition, the type species of three other genera thought to be distinct based on morpho-anatomical characters, namely *Yamadaia* (Segawa 1955), *Marginisporum* (Yendo 1902), and *Pachyarthron* (Manza 1937), were all shown by DNA sequencing to belong in *Corallina* (Martone et al. 2012, Hind and Saunders 2013, Hind et al. 2014). No morpho-anatomical character or suite of characters has proved diagnostic for *Corallina* or for *Ellisolandia*. Sequencing type specimens of 18th through mid-20th century historical species (e.g., *C. officinalis* [epitype], *C. cretaceum* [lectotype], and *C. ferreyrae* [isotype]) has been critical for researchers to correctly apply these names (Brodie et al. 2013, Hind et al. 2014, Bustamante et al. 2019a). Just as no morpho-anatomical characters have proved diagnostic at the generic rank for tribe Corallinoideae, likewise none have proved diagnostic for *Corallina* species.

Herein, we provide additional examples of the necessity to sequence type specimens to apply names and of the inadequacy of morpho-anatomical characters to distinguish *Corallina* species. We show that *C. berteroi* (type locality: Chile) is the oldest available name for the *Corallina* species currently known as *C. ferreyrae* (type locality: Pucusana, Peru, including *C. caespitosa* [type locality: Devon, England]) and that *C. pinnatifolia* (type locality: California, USA) is also a junior synonym, as is the morpho-anatomically divergent *C. melobesioides* (type locality: Izu, Japan) with an extensive base and unigeniculate uprights. We also show the non-congruence of different markers for resolving the phylogenetic relationships among *Corallina* species and the difficulty of applying species delimitation methods when the sequence differences between species are small and variable.

MATERIALS AND METHODS

Specimens. A total of 108 specimens of coralline algae were collected in Australia, Chile, China, England, Japan, Korea, Mexico, Peru, and USA. Specimens were dried with a towel and immediately placed in silica gel for morphological and molecular analyses. Voucher specimens were deposited in the herbarium of the Universidad de Magallanes (LEMAS), University of British Columbia (UBC), and University of North Carolina-Chapel Hill (NCU); herbarium acronyms follow Index Herbariorum online (Thiers 2021). Quantitative morpho-anatomical characters represent average values with standard deviation from approximately 30 measurements. Photographs were taken using the Leica MC170 camera attached to EZ4 Leica stereomicroscope (Leica Microsystem, Wetzlar, Germany).

Molecular procedures. DNA extraction and amplifications were performed at Universidad Nacional Toribio Rodriguez de Mendoza (UNTRM), UBC, and University of North Carolina-Chapel Hill UNC-CH. At UNTRM genomic DNA was extracted from ~ 5 mg of dried algae ground in liquid nitrogen using a NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol; at UNC-CH genomic DNA from field-collected specimens was extracted following the protocol in Gabrielson et al. (2011), but modified for type specimens by following the guidelines in Hughey and Gabrielson (2012); at UBC genomic DNA was extracted from field-collected specimens according to Saunders (2008) with modifications from Saunders and McDevit (2012). The primer pairs for amplification and sequencing at UNTRM were the newly design 133F (CGTATGGAAT-TAGCWCAACCMGG) - 947R (GCTGCWGTAAAATAAG-CACGTGT) for COI, F1- R2 (Yoon et al. 2002) for *psb*A, and F57- 897cR, F645- R1150 (Freshwater and Rueness 1994, Lin et al. 2002, Torrano-Silva et al. 2014) for *rbc*L; at UNC-CH primers for field collected material were the same for *psb*A, and for *rbc*L were F57-R1150, F753-RrbcS (Freshwater and Rueness 1994) and for *rbc*L from type material F1152Cor-R1308Cor (Gabrielson et al. 2011); at UBC primers for *psb*A were the same, for COI were GWSFn (LeGall and Saunders 2010) and GWSRx (Clarkston and Saunders 2013), and for *rbc*L were F57-R1150K (Freshwater and Rueness 1994, Lindstrom et al. 2015) and F753-rbcLrevNEW (Freshwater and Rueness 1994, Kucera and Saunders 2012).

Amplification at UNTRM used GoTaq® Green Master Mix (Promega, Madison, WI, USA), preparing a 25 µL solution. This included 3 µL of total DNA solution, 0.5 µL of each forward and reverse primer (10 pmol), 12.5 µL of master mix, and 8.5 µL MilliQ water. Reaction were cycled in a T100™ Thermal Cycler (Bio-Rad, Hercules, CA, USA) using the following parameters: 94°C for 2 min, followed by 40 cycles of 94°C for 30s, 47°C for 60 s, and 72°C for 60 s, and a final extension of 72°C for 10 min. PCR products were electrophoresed on 1% agarose gels, purified using the NucleoSpin Gel and PCR clean-up kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions, and then sequenced commercially (Macrogen, Seoul, Korea). Full-length forward and reverse strands were determined for all taxa, and the electropherograms were edited using the Chromas v1.45 software (McCarthy 1998). Amplification and cleaning of PCR products at UNC-CH followed Hughey et al. (2001); sequencing and alignment followed Gabrielson et al. (2011). Amplification of PCR products at UBC followed Hind and Saunders (2013); sequencing was done by the Genome Quebec Innovation Centre at McGill University; sequences were edited using Geneious 7.1.9 (Biomatters Ltd., Auckland, New Zealand). In total, 154 new sequences were generated and deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/; Table S1 in the Supporting Information).

Phylogenetic analyses. Sequences were initially aligned using the default settings of the MUSCLE algorithm and then manually corrected with MEGA7 (Kumar et al. 2016). Saturation of substitution tests were performed using the DAMBE7

software (Xia 2018) to evaluate COI, *psb*A, and *rbc*L data by plotting numbers of transitions and transversions against Kimura-2-parameter distance (K2P). The phylogeny was based on the concatenated data combining COI, *psb*A, and *rbc*L (78 total sequences, 2769 bp; Table S1). Also, single-locus phylogenies were constructed for COI (62 sequences; 563 bp), *psb*A (93 sequences; 851 bp), and *rbc*L (98 sequences; 1390 bp) genes (Table S1). *Crusticorallina painei* and *Ellisolandia elongata* were used as outgroups. The best-fitting nucleotide substitution model was selected using the program PartitionFinder2 (Lanfear et al. 2017) with three partitions. The best partition strategy and model of sequence evolution were selected based on the corrected Akaike Information Criterion (AICc). The general time reversible with a gamma distribution and a proportion of invariable sites substitution model (GTR + Γ + I) was selected for the Bayesian inference (BI) of multilocus data and all single-locus analyses, while the general time reversible nucleotide model (GTR) was selected only for the Maximum likelihood (ML) analysis of multilocus data. ML analyses were performed with the RAxML HPC-PTHREADS-AVX2 program (Stamatakis 2014) implemented in the raxmlGUI 2.0-beta.6 interface (Edler et al. 2019) with support assessed by 10,000 rapid bootstraps. BI was performed with MrBayes v3.2.5 software (Ronquist et al. 2012) using Metropolis coupled MCMC. We plotted likelihood versus generation using the Tracer v1.6 program (Rambaut et al. 2014) to reach a likelihood plateau and set the burn-in value. The convergence of both runs were evaluated using Tracer to observe if runs reached an effective sample size greater than 200. To evaluate posterior probabilities, we conducted two runs each with four chains (three hot and one cold) for 2,000,000 generations, sampling trees every 1,000 generations. A burn-in of 25% was used to avoid suboptimal trees in the final consensus tree (Calderon and Boo 2016). Intraspecific and interspecific pairwise divergence was estimated using the p-distance model in MEGA7.

DNA-based species delimitation. Sequences of *Corallina* species available in GenBank and generated in this study were included in the DNA-based delimitation methods. We explored five different DNA-based delimitation methods using COI, *psb*A, and *rbc*L data sets to assess species boundaries in *Corallina*: two genetic distance methods (automatic barcoding gap detection [ABGD] and statistical parsimony network analysis [SPN]) and three coalescence methods (generalized mixed Yule coalescent method [GMYC], Bayesian phylogenetics phylogeography [BPP], and Bayesian version of Poisson tree processes [bPTP]). These are single-locus based species delimitation methods, except by the multilocus BPP method.

ABGD sorts sequences into hypothetical species based on the barcode gap to infer the number of candidate species present (Puillandre et al. 2012). ABGD was performed through the web interface (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) using the model Kimura-2-parameters and 50 screening steps, variability (P) was set between 0.001 (Pmin) and 0.1 (Pmax) whereas the relative gap width (X) and the Nb bins (for distance distribution) to 1 and 20, respectively (Tineo et al. 2020).

SPN analysis was performed using TCS 1.21 (Clement et al. 2000) with gaps and missing data excluded. TCS parsimoniously associates sequences of the sample species calculating the minimum number of mutational steps at 95% statistical confidence and joins haplotypes into networks.

GMYC uses a likelihood approach on a phylogenetic tree to determine the transition threshold between speciation (Yule speciation) and the intra-species diversification (coalescent process; Lorén et al. 2018). We only performed the single-threshold GMYC because it has shown better performance than the multiple-threshold version (Luo et al. 2018). To perform the GMYC analyses, ultrametric trees were constructed by Bayesian analysis in BEAST v.2.0.2 (Drummond et al. 2012) with the GTR model for the COI, *psb*A, and *rbc*L loci. The relaxed clock log normal molecular clock model (Drummond et al. 2006) and the coalescent exponential population prior were used. Markov Chain Monte Carlo was run for 50 million generations, sampling every 1,000 generations. Output log files were visualized in Tracer v.1.6 (Rambaut et al. 2014) for assessing the stationary state of parameters based on values of estimate-effective sample size (ESS). The 25% of trees were removed as burn-in, the remaining trees were summarized in a single tree (ultrametric maximum clade credibility tree) by TreeAnnotator v.2.0.2 (Drummond et al. 2012). The single-threshold GMYC analyses were performed on the maximum clade credibility tree using the "gmyc" function of the "splits" package (Monaghan et al. 2009) in R 3.3.1 (R Development Core Team, http://www.R-project.org).

The PTP model relies on the number of substitutions (branches length) for estimating species boundaries. PTP assumes that the number of substitutions between species is significantly higher than the number of substitutions within species (Zhang et al. 2013, Rojas et al. 2018). We performed the Bayesian PTP (bPTP) on the web server (http://species.h-its.org/) using the above generated rooted ML tree as input, setting 500,000 MCMC generations, thinning value of 100, a burn-in of 10%, and removing the outgroup to improve species delimitation.

BPP is efficient in delimitating closely related species using multiple loci (Yang and Rannala 2017). Besides the sequence data, BPP uses a guide species tree with defined topology as input. An inaccurately specified guide tree can lead to a false species delimitation (Lin et al. 2017). Our multilocus BPP was run in BP&P v.2.0 (Rannala and Yang 2003, Yang and Rannala 2010, Liu et al. 2015) using the concatenated data set (COI, *psb*A, and *rbc*L) and the tree derived from the phylogenetic ML analysis as guide tree. BPP analysis were set under the A11 model (A11: species delimitation = 1, species tree = 1) and specimens were *a priori* assigned to species based on the minimum number of species resulted of the phylogenetic analysis. After several exploratory analyses, five variables (ε1˜ε5) were automatically fine-tuned following the instructions of BP&P (Bustamante et al. 2021). The prior distribution of θ and τ could have influenced the posterior probabilities for different models (Yang and Rannala 2010). Analyses were run with three different prior combinations (Leaché and Fujita 2010). Each analysis was run 10 times to confirm consistency between runs. Two independent MCMC analyses were run for 100,000 generations with the 'burn-in' = 20,000 (Bustamante et al. 2019b).

RESULTS

Phylogenetic analyses. The saturation test revealed no evidence for saturation of substitution at any codon position. The concatenated phylogenetic analysis of *Corallina* species comprised a dataset of COI + *psb*A + *rbc*L sequences from 78 individuals. The single and multilocus phylogenies obtained from ML and BI analyses supported the monophyly of *Corallina*. The tree topologies for individual markers and multilocus data were incongruent (Fig. 1, Figs. S1-S3 in the Supporting Information). The multilocus phylogeny supported 18 lineages (Fig. 1). One lineage contained sequences of type
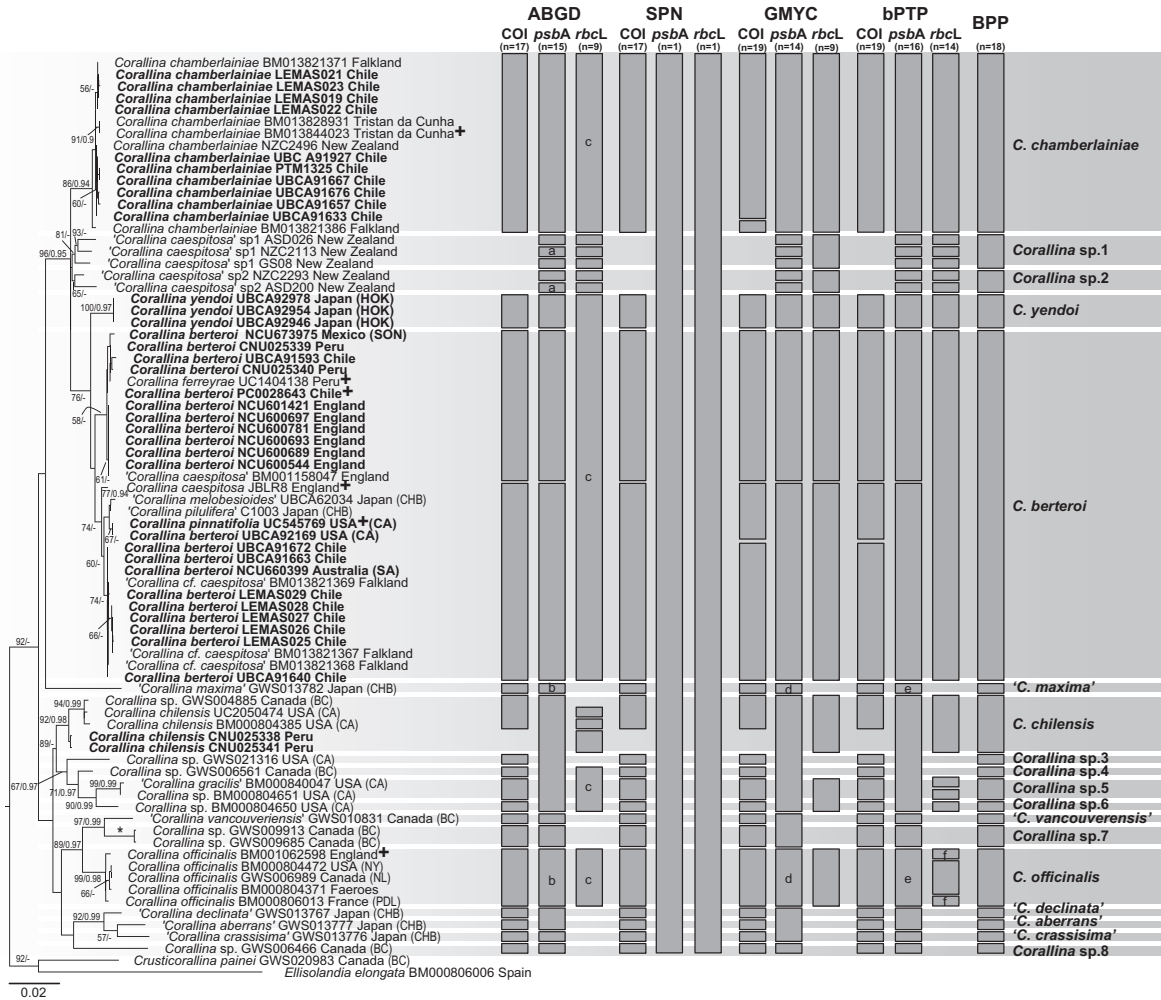
FIG. 1. Phylogenetic tree based on maximum likelihood inference of multilocus (COI, *psb*A, and *rbc*L) and species delimitation based on genetic distance (ABGD and SPN) and coalescence (GMYC, bPTP, and BPP) methods. ML bootstrap values (BS; ≥ 50%) and Bayesian posterior probabilities (BPP; ≥ 0.90) are indicated adjacent to branches. Values lower than 50% (BS) or 0.90 (BPP) are indicated by hyphens (-). Values of 100% (BS) and 1.00 (BPP) are indicated by asterisks (*). Gray bars represent species delimitation results for each method and marker, number of bars is also expressed in parentheses above each bar. Letter inside bars represents same delimited species; + represents sequence obtained from type specimens. Sequences for taxa in bold were generated in this study. Taxa in quotes indicates that type material has not been sequenced. Scale bar indicates the number of nucleotide substitution per site. BC, British Columbia; CA, California; CHB, Chiba; HOK, Hokkaido; NL, Newfoundland and Labrador; NY, New York; SA, South Australia; SON, Sonora; PDL, Pays de la Loire.

specimens of *C. berteroi, C. ferreyrae, C. caespitosa* and *C. pinnatifolia,* and a sequence of *C. melobesioides.* Other lineages corresponded to *C. aberrans, C. chamberlainiae, C. chilensis, C. crassisima, C. declinata, C. maxima, C. officinalis, C. vancouveriensis,* and other eight clades with uncertain names labeled *Corallina* sp. Of these, type material had been sequenced only for *C. chamberlainiae* and *C. officinalis.* In addition, an unnamed clade that comprised three sequences from Japan in a well-supported independent lineage (BS/BI = 100/0.97 for multilocus) was identified. The COI genetic divergence comparisons showed that this separate lineage differed from *C. berteroi* by 2.9–3.5% and from *C. chamberlainiae* by 6.6–7.1%, while *psb*A and *rbc*L divergences were 0.7–1.3%, 0.9–1.1%, 0.6–0.9%, and 1.2–1.3%, respectively

(Tables S2–S4 in the Supporting Information). The *rbc*L genetic divergences between type specimens of *C. berteroi* and *C. pinnatifolia* and those of *C. berteroi* and specimens called *C. melobesioides* were 0.2% (3 bp), whereas type sequences of *C. berteroi* and *C. ferreyrae* were identical and differed from *C. caespitosa* by 1 bp.

Species delimitation. The species-delimitation methods based on genetic distance (ABGD, SPN) and coalescence (GMYC, bPTP, BPP) showed incongruent results for the three genes (Fig. 1, Figs. S4-S6 in the Supporting Information). The delimitation performed by ABGD recognized nine species based on the analysis of *rbc*L sequences; 15 species based on *psb*A sequences, and 17 species based on COI sequences. The SPN method varied even more

widely among loci (COI = 17, *psb*A = 1, and *rbc*L = 1). The number of species delimited by the coalescent methods were somewhat more congruent. Where data were available for the same specimens at all three loci, COI recognized more species than *psb*A or *rbc*L, which were similar except for the number of new species recognized that had been incorrectly called *Corallina caespitosa*. The bPTP results were the most congruent for the three loci except for *C. berteroi*, where 1 (*rbc*L), 2 (*psb*A), or 3 (COI) taxa were recognized. The multilocus coalescent species validation (BPP) yielded the highest posterior probabilities for the 18 species that were recognized by the phylogenetic analyses (Table S5 in the Supporting Information).

We defined putative species from the groups of sequences whose boundaries agreed in at least two analyses and supported clades in our multilocus tree in order to prevent minor tip clades from being recognized as different species (Hoshino et al. 2018, Twist et al. 2019, Bustamante et al. 2021). *Corallina chamberlainiae* from New Zealand, Chile, Falkland Islands, and Tristan da Cunha was well-supported as a distinct species by BPP, bPTP, ABGD, SPN (COI), GMYC (*psb*A and *rbc*L), and the multilocus phylogeny (86/0.94). Clades named '*C. aberrans*,' '*C. crassisima*,' '*C. declinata*,' and '*C. maxima*' whose type specimens appear to be missing (Yoshida 1991), '*C. chilensis*' and '*C. vancouveriensis*,' whose type specimens have not been sequenced, and *C. officinalis*, were supported by BPP, and bPTP, GMYC, ABGD (COI and *psb*A) methods. '*Corallina caespitosa*' from New Zealand was resolved in two clades (81/- and 65/-), and also recognized by BPP and GMYC (*rbc*L). Our analysis also revealed an independent lineage, from Japan, that was strongly supported as a distinct species by the multilocus phylogeny (100/0.97) and by all of the delimitation methods with the exception of SPN (*psb*A and *rbc*L). Delimitation of *C. berteroi* was confirmed by bPTP (*rbc*L) and BPP (0.887) delimitation methods (Fig. 1, Tables S4–S6).

We limit our taxonomic conclusions to species whose type specimens have been sequenced, thereby recognizing *Corallina officinalis*, *C. chamberlainiae*, *C. berteroi*, and *C. yendoi* sp. nov. Below we present our results only for *C. berteroi*, including new proposed synonymies, and for *C. yendoi* sp. nov. (Fig. 2).

Corallina Linnaeus (1758, 805) gen. emend.

Diagnosis: Plants erect from a basal crust that may be extensive to absent; erect axes geniculate ranging from a single interaxis/geniculum pair at the dorsal surface of the crust to numerous genicula and intergenicula forming a branched frond; basal intergenicula terete, sometimes remaining so throughout the upright axes, but more frequently becoming compressed; erect axes typically branched, rarely unbranched, branching most often distichous and pinnate especially in higher order branches, but sometimes sparsely and irregularly branched throughout; intergenicula of arching tiers of medullary cells surrounded by a photosynthetic cortex and a non-photosynthetic, single layered, epithallus of dome-shaped cells; cells in contiguous filaments often fusing; secondary pit connections absent; genicula uncalcified of single tiers of long, straight unbranched cells; trichocytes present, but often not evident; conceptacles occasionally lateral, but most commonly axial and terminal; conceptacle pores central.

Synonyms: *Joculator* Manza 1937: 47; *Marginisporum* (Yendo) Ganesan 1968: 26; *Serraticardia* (Yendo) P.C. Silva 1957: 48; *Yamadaia* Segawa 1955: 241.

Lectotype species: *Corallina officinalis* Linnaeus.

Corallina berteroi Montagne ex Kützing (1849: 709; as *C.* '*berterii*').

Description: Plants erect from a basal crust that is nearly absent to extensive (>5 cm diam.); erect axes ranging from only a single intergeniculum to a sparsely to extensively branched frond with branching that is distichous and pinnate to flabellate, or radial and pinnate-plumose to flagelliform, or irregular; conceptacles axial.

Lectotype (designated herein): PC 0028643, Chile, no date, no habitat data, leg. Carlo Guiseppe Bertero.

Type Locality: Chile.

Homotypic synonym: *Corallina berteroana* Montagne 1854: 318.

Heterotypic synonyms. *Corallina caespitosa* R.H.Walker, J.Brodie and L.M.Irvine 2009: 290, figs. 3, A-D; 4 A, B, E-G.

Type Locality: Chit Rocks, Sidmouth, Devon, England.

Corallina ferreyrae E.Y.Dawson, Acleto and Folkvik 1964: 44, pl. 35, fig. B (as *C.* '*ferreyrai*').

Type Locality: Pucusana, Peru.

Corallina melobesioides (Segawa) Martone, S.C.Lindstrom, K.A.Miller and P.W.Gabrielson 2012: 864.

Basionym: *Yamadaia melobesioides* Segawa 1955: 241 (footnote), figs. 1–6.

Type Locality: Susaki, Izu Prefecture, Japan.

Corallina officinalis var. *caloclada* Harvey 1849: 104.

Lectotype (designated herein): TCD 000171, Chonos, Chiloe, Chile, 1834, no habitat data, leg. Charles Darwin 2423 (Fig. S7 in the Supporting Information).

Comment: Harvey (1849: 104) listed three syntype localities for specimens of this new variety collected by Charles Darwin on his *H.M.S. Beagle* voyage: the Falkland Islands; Chonos, Chiloe (Chile); the Cape of Good Hope. For only the first two localities was material cited, Darwin 1143 (Fig. S8 in the Supporting Information) from the Falkland Islands and Darwin 2423 (TCD 1171) from Chonos, Chiloe (Fig. S7). Wynne (2012) noted that these collections were at TCD. We have sequenced a portion of the *rbc*L gene (263 bp) from material from both of these collections, and they are two different species. The Darwin 1143 material from the Falkland Islands is *Corallina chamberlainiae*. Herein we designate as
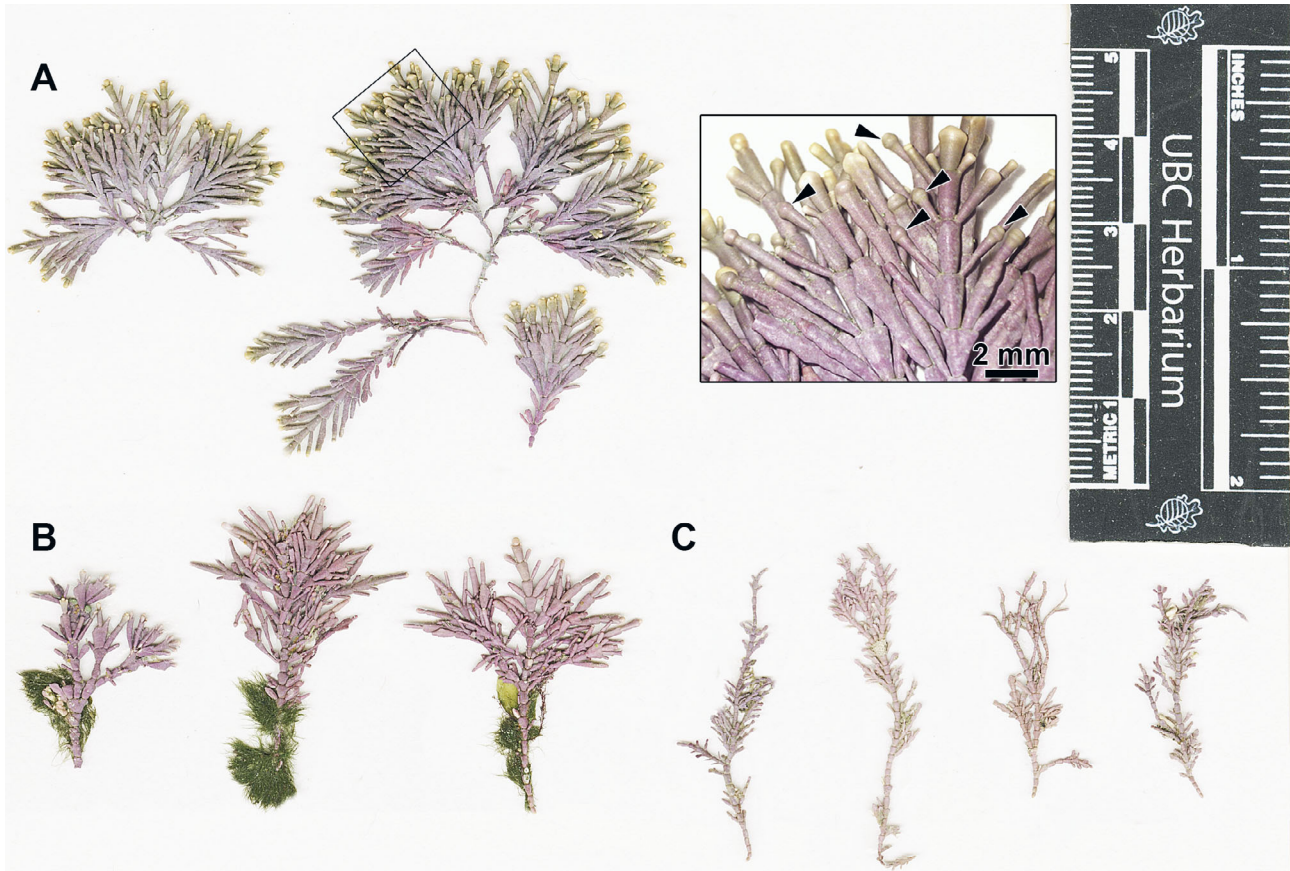
Fig. 2. Habit of *Corallina yendoi* sp. nov. All specimens collected from Hokkaido, Japan: (A) UBC A92978 (Holotype), Cape Tachimachi, Hakodate; (B) UBC A92954, Muroran; (C) UBC A92946, Oshoro Bay. Arrowheads indicate conceptacles.

lectotype of *C. officinalis* var. *caloclada* the Darwin 2423 material from Chonos, Chiloe (Chile) that is a heterotypic synonym of *C. berteroi*. Harvey (1849) also recognized that there was another different species collected and numbered 2423 by Darwin from the same locality ("HAB. Chonos, Chiloe, Mr. Darwin, No. 2423 in part") and named it, *Amphiroa darwinii* Harvey (1849: 100). Later, Johansen (1971) placed *A. darwinii* in synonymy under *Bossiella chiloensis*, but this synonymy has not been confirmed by DNA sequencing.

Corallina pinnatifolia (Manza) E.Y. Dawson 1953: 124, pl. 9, figs. 7–13.

Basionym: *Joculator pinnatifolius* Manza 1937: 47.

Holotype: UC 545769, 15.i.1934, no habitat data, *leg*. F. M. Reed.

Type locality: reef opposite Doheny State Park, Orange County, California, USA.

Comments: Manza (1937) proposed a system of classification for geniculate coralline genera based first on the position of conceptacles. He proposed *Joculator* as a new genus because it had both terminal and lateral conceptacles. Taylor (1945: 199) noted that *Corallina officinalis* in New England (USA) also had specimens with both terminal and

lateral conceptacles, and thus this character was not unique to *Joculator*. But it was Dawson (1953: 124), who transferred *J. pinnatifolia* to *Corallina*, making the combination *C. pinnatifolia* and therefore placing *Joculator* in synonymy under *Corallina*.

Corallina yendoi Martone, P.W. Gabrielson, M.S. Calderon & D.E. Bustamante **sp. nov.**

Holotype: UBC A92978, May 19, 2015, bedrock (mid-intertidal pool), *leg*. Patrick T. Martone, PTM1440 (fig. 2A).

Type locality: Cape Tachimachi, Hakodate, Japan (41.745, 140.722).

Paratypes: UBC A92954, May 18, 2015, bedrock (low intertidal), *leg*. Patrick T. Martone, PTM1416; UBC A92946, May 16, 2015, bedrock (mid-intertidal), *leg*. Patrick T. Martone PTM1408 (fig. 2, b and c).

Etymology: The epithet *yendoi* honors Dr. Kichisaburo Yendo for his pioneering work on geniculate corallines from both the Northeast (British Columbia, Canada) and Northwest (Japan) Pacific.

Description. Thalli pink-grey to medium red-purple, epilithic, densely tufted, erect fronds to 3–4 cm tall from a crustose base; axes cylindrical or subcylindrical at frond base, becoming compressed,
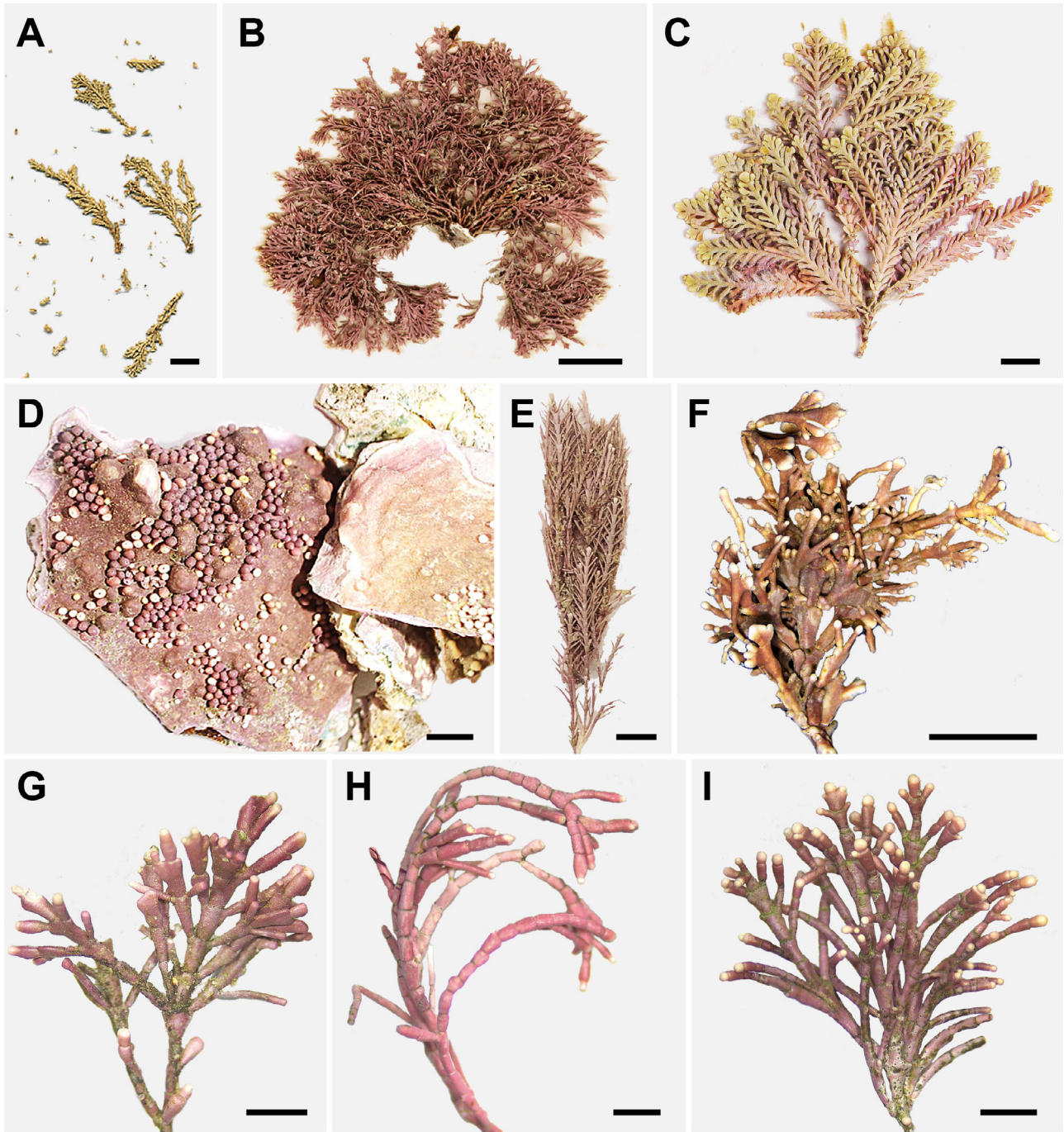
Fig. 3. Habit of *Corallina berteroi* species. (A) Lectotype of *Corallina berteroi* from Chile (PC 0028643), scale bar = 5 mm; (B) Isotype of *Corallina ferreyrae* from central Peru (UC 1404138), scale bar = 1 cm; (C) Part of the holotype of *C. pinnatifolia* from California, USA (UC 545769), scale bar = 5 mm; (D) specimen of *C. melobesioides* from Chiba, Japan (UBC A62034), scale bar = 2 mm; (E) specimen of *Corallina berteroi* from North Carolina, USA (NCU 628550), scale bar = 5 mm; (F) specimens of *Corallina berteroi* from Piura, Peru (CNU025339), scale bar = 5 mm; (G) specimens of *Corallina berteroi* from Punta Arenas, Chile (LEMAS028), scale bar = 2 mm; (H) specimens of *Corallina berteroi* from Aysén, Chile (LEMAS027), scale bar = 2 mm; (I) specimens of *Corallina berteroi* from Osorno, Chile (LEMAS026), scale bar = 2 mm.

branching dense to three to four orders, from primarily pinnate to irregularly distichous or occasionally tristichous, occasionally compound pinnate above, primary branches 1.9 ± 0.4 cm (max 2.7 cm) long, axial intergenicula 1.7 ± 0.4 mm (max 2.4 mm) long and 1.0 ± 0.3 mm (max 1.5 mm) wide, dome-shaped toward the frond apex, branch apices markedly pinnate with terminal intergenicula typically flanked by two smaller intergenicula but occasionally solitary or irregular. Conceptacles axial,
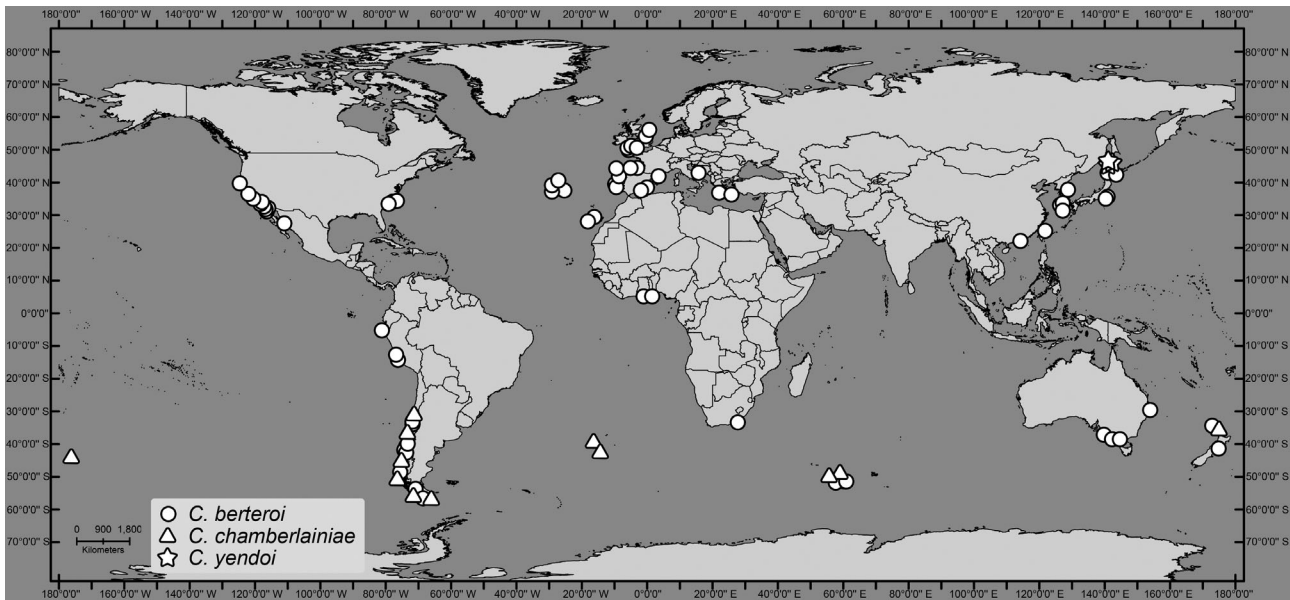
Fig. 4. Map showing updated distribution of *Corallina berteroi*, *C. chamberlainiae*, and *C. yendoi*.

each on a short determinate branch composed of one to two intergenicula branching from the main axis and appearing to replace a branch. Diagnostic DNA sequences from the holotype: GenBank MZ262566 (COI), MZ262626 (*psb*A), and MZ262637 (*rbc*L).

Habitat. Marine, growing in tufts attached to bedrock from the mid-littoral to the lower limit of the littoral zone, also in mid-pools.

Distribution. Southern and western coast of Hokkaido Island, Japan: Muroran (UBC A92954, UBC A92955), Cape Tachimachi (UBC A92978), and Otaru (UBC A92946).

DISCUSSION

DNA-sequencing defines a monophyletic *Corallina*. Recent studies, based on DNA sequences, have demonstrated the difficulty of applying morpho-anatomical characters to correctly classify geniculate corallines into *Corallina* and to delimit species. For example, at the generic rank, *Yamadaia melobesioides*, the generitype of *Yamadaia*, was found to belong in *Corallina* (Martone et al. 2012), making the former genus a synonym under the latter. The Northwest Pacific genera *Serraticardia* and *Marginisporum* suffered a similar fate and were found to be synonyms of *Corallina* (Hind and Saunders 2013), necessitating the transfer of Northeast Pacific *S. macmillanii* to a new genus, *Johansenia*. The morphologically distinct genus *Pachyarthron* was found to be not only a synonym of *Corallina*, but that *P. cretaceum* was the same species as *C. officinalis* (Hind et al. 2014). Conversely, Hind and Saunders (2013) showed that the well-studied Northeast Atlantic and Mediterranean species, *C. elongata* belonged in its own genus, *Ellisolandia*.

Sequencing type specimens. Using morpho-anatomical characters to both delimit species of *Corallina* and to apply names has been fraught with difficulties. In the Northeast Atlantic, Robba et al. (2006) sequenced the COI gene and found that two species were passing under *Corallina officinalis*, the generitype, and Walker et al. (2009) proposed the new name, *C. caespitosa* for the species not identified as *C. officinalis*. Brodie et al. (2013) were unable to amplify DNA from the lectotype specimen of *C. officinalis* (type locality: "in Oceano") and proposed an epitype, which was sequenced. Sequencing two markers, the mitochondrial encoded *cox1* and plastid encoded *rbc*L, from recently collected specimens and from historical specimens from BM, Williamson et al. (2015) found 20 clades of named and unnamed species of *Corallina*. But without sequencing type specimens the application of all named species of *Corallina* has been uncertain. This was recently demonstrated when Bustamante et al. (2019a) showed that COI, *psb*A, and *rbc*L sequences from an isotype specimen of *C. ferreyrae* were consistent to those same sequences from the holotype of *C. caespitosa*, thus placing the latter in synonymy under the former. Bustamante et al. (2019a) further noted that numerous, earlier published, valid names of *Corallina* species needed to be sequenced and might possibly replace *C. ferreyrae*. We found two older type specimens of *Corallina* species, namely *C. berteroi* (Kützing 1849) and *C. pinnatifolia* (basionym *Joculator pinnatifolius* Manza 1937, type locality: reef opposite Doheny State Park, Orange County, California, USA) and both of these are the same species as *C. ferreyrae* and *C. caespitosa*. Accordingly, *Corallina berteroi* has nomenclatural priority.

Species delimitation. We used ML and Bayesian phylogenetic analyses and five DNA-based species delimitation methods for three markers (i.e., COI, *psb*A, *rbc*L) to assess species boundaries in *Corallina* in order to prevent bias caused by any single method that would misinterpret population splits as species divergences. Species recognition based on a single locus alone may lead to misinterpreting independent lineages due to the existence of incongruent loci (Figs. S1–S6), thus necessitating the use of multilocus data as a primary source of information (Liu et al. 2016).

The use of multilocus sequence data is helpful to establish robust species boundaries (Hoshino et al. 2018, Twist et al. 2019). In our multilocus tree, most of the *Corallina* species passed the test of consistently supported monophyly, with the exception of *C. berteroi*. However, single loci, especially *psb*A, showed contradictory monophyly and paraphyly (Figs. S1–S3). According to Dettman et al. (2003), robust monophyly is observed where barriers to genetic exchange have existed for a long period relative to the population sizes of these species (e.g., *C. chamberlainiae*, *C. chilensis*, *C. yendoi*, and *C. officinalis*); whereas polyphyly and paraphyly are stages where newly diverged species pass through as a consequence of the loss of ancestral polymorphism caused by genetic drift (Avise and Ball 1990).

Our species recognition followed a conservative premise where not every independent evolutionary lineage observed was recognized as a phylogenetic species. The genetic distance methods showed conflicting results when delimiting *Corallina* (ABGD= 9-17 SPN= 1-17) compared to those from the multilocus phylogeny (18 species) mainly due to the split of *Corallina caespitosa* from New Zealand and *C. chilensis* into several species (ABGD) and the collapse of multiple *Corallina* sequences into a single species (SPN: *psb*A and *rbc*L). Previous studies have shown that ABGD over-splits species into multiple candidate species if deep divergences occur between certain populations (false positives), while SPN is prone to lumping less divergent clades in the same candidate species (false negatives; Hamilton et al. 2014, Dellicour and Flot 2015, Yu et al. 2017).

Results obtained from the coalescence methods, GMYC, bPTP, and BPP were partially congruent (14–19 species). BPP supports the conservative results obtained from the multilocus phylogeny (18 species, posterior probabilities higher than 0.88), contrary to GMYC (9–19 species; Table S6) and bPTP (14–19). These results concurred with previous empirical studies where BPP showed lower rates of over or underestimation of species when compared to GMYC and PTP methods (Carstens et al. 2013, Luo et al. 2018, García-Melo et al. 2019). In the BPP model, the misidentification of population structure as putative species is avoided by using external elements such as, morphological, ecological, biogeographic, or other classes of data types as complementary information (Zhou et al. 2012, Solís-Lemus et al. 2015, Sukumaran and Knowles 2017). We used a multilocus phylogeny as an external element to attribute our structure delimited by BPP to species boundaries rather than to populations.

Corallina yendoi. All delimitation methods and the multilocus phylogeny (100/0.97) supported recognition of *Corallina yendoi* as a distinct species except for ABGD: *rbc*L and SPN: *psb*A, *rbc*L. However, the short gap of genetic divergence observed between *C. berteroi* and *C. yendoi* (*rbc*L; Tables S2–S4) suggests that *C. yendoi* may represent an ongoing lineage diverging from the globally widespread *C. berteroi* lineage. The genetic divergence between *C. yendoi* and other species is within the range of the minimum threshold observed in species of *Corallina* (Tables S2–S4), and similar to the 0.57% *rbc*L divergence used to separate the two closely related northeast Pacific species of *Calliarthron* (Gabrielson et al. 2011).

Most unfortunately, all of Yendo's type specimens of geniculate corallines from the Northwest Pacific Ocean (Japan) are missing (Yoshida 1991, see appendix 2), making comparisons of DNA sequences from type specimens impossible. Rather than make an educated guess about which, if any, of Yendo's *Corallina* species names might apply to the sequenced field-collected material, we propose a new species, *C. yendoi*. Below we compare *C. yendoi* to the species that Yendo (1902) described from the same or nearby localities in Hokkaido Prefecture, namely *C. confusa* and *C. sessilis*, using habitat data and morpho-anatomy. In a more recent study based on collections from the same region, Baba et al. (1988) considered the former to be *C. vancouveriensis* and the latter to be *C. pilulifera*. *Corallina confusa* differs from *C. yendoi* by forming dense clusters in the high intertidal zone, having short thalli, less than 3 cm tall, and densely aggregated (confused) conceptacles near branch apices, hence the name. *Corallina sessilis*, currently considered a synonym of *C. pilulifera*, is distinguished from *C. yendoi* by the ribbed ventral surface of its main axes, shorter axial intergenicula, about 1 mm, and by having sessile, compressed conceptacles often clustered in groups of 2–3 or conceptacles borne on compressed intergenicula, called peduncles by Yendo (1902). *Corallina yendoi* coarsely resembles *C. pilulifera* identified by Baba et al. (1988) from Ôhanazaki, in Hakodate City. However, Baba et al. (1988) reported that *C. pilulifera* had evident midribs during spring and summer (absent in specimens of *C. yendoi* collected during spring) and excessively pedunculate conceptacles subtended by two to five intergenicula (only one to two subtending intergenicula in *C. yendoi*).

Corallina chamberlainiae. We expand the distribution of this species recently described by Brodie et al. (2020) from the southern Atlantic (Falkland and Tristan da Cunha Islands) and New Zealand, to

include central and southern Chile. Additionally, sequencing of the syntype material of *Corallina officinalis* var. *caloclada* collected by Darwin (no. 1143 from the Falkland Islands; Harvey 1849, Porter 1987, Wynne 2012) corresponded to *C. chamberlainiae* (Table S1, Fig. S3).

Corallina berteroi. There is no strong molecular support to recognize *Corallina berteroi* (= *C. ferreyrae*) to include *C. caespitosa*, *C. melobesioides* and *C. pinnatifolia*, nor is there any support to recognize these as separate species. The evidence indicates that specimens given these names belong to a species complex of multiple evolving populations. We have adopted the conservative position of recognizing a single, highly variable, species both molecularly and morpho-anatomically for which *C. berteroi* is the earliest available name. Biogeography also is not useful for recognizing different species or even subspecies in this complex. Moreover, the more we sequence from localities around the world, the more records we find of this species. *Corallina berteroi*, not *C. officinalis* as records based on morpho-anatomy would indicate, is the truly cosmopolitan and weedy *Corallina* species. As such, we expect that *C. berteroi* will be reported from additional localities globally.

The morphological variation exhibited by *Corallina berteroi* is striking (Fig. 3), ranging from fan-shaped, pinnately branched thalli (Walker et al. 2009 as *C. caespitosa*, Dawson 1953 as *C. pinnatifolia*, Dawson et al. 1964 as *C. ferreyrae*, Brodie et al. 2020 as *C. cf. caespitosa*) to extensive crustose thalli with diminutive upright fronds of only one intergeniculum (Martone et al. 2012 as *Corallina melobesioides*). Reviewing illustrations of thalli of *C. berteroi* in the literature from California, USA (as *C. pinnatifolia*), the Falkland Islands (as *C. cf. caespitosa*), the UK (as *C. caespitosa*), and Peru (as *C. ferreyrae*) revealed variation in the branching form of the upper lateral pinnae: pinnate-plumose in individuals from California (Dawson 1953, p. 124, pl. 9, figs. 7–20, pl. 30, fig. 1), flabellate in specimens from the Falkland Islands (Brodie et al. 2020, figs. 12–16), and flagelliform (slender and whip-like branches) in those from Peru (Dawson et al. 1964, p. 44, pl. 35, fig. B) and the UK (Walker et al. 2009, fig. 3, A–C). Dawson et al. (1953) reported a range of variation (at least 14 forms) in ultimate segments of *C. pinnatifolia* from Pacific Baja California, Mexico and the Gulf of California (as *C. pinnatifolia* and *C. pinnatifolia* var. *digitata*, respectively). Other features also have been associated with local populations such as thalli with a wider diameter of crustose base, >10 mm in populations from UK (Walker et al. 2009) versus 1–5 mm from Falkland Islands and Peru (Brodie et al. 2020), thalli with short fronds from the Falkland Islands with longer intergenicula and more numerous and compressed terminal intergenicula than populations from the UK (Brodie et al 2020), and thalli with a distinct midrib from California, USA (Dawson 1953). Our collections from Chile also showed a range of thallus variation (Fig. 3, G–I) from regular and fan-shaped to irregular forms with branches simple to compound, sometimes flagelliform and sparse, or rarely branched above with ultimate segments fused and palm-like.

Perhaps most surprising are some Japanese populations of *Corallina berteroi* that exist without branched articulated fronds, and instead grow extensive crusts with upright axes having only one intergeniculum (Fig. 3D). Originally described as *Yamadaia melobesioides*, this morphological variant was thought to be in a separate genus, later placed in synonymy under *Corallina*, as *C. melobesioides* (Martone et al. 2012) and herein synonymized with *C. berteroi*. Such an extreme range of morphological variation has never been documented in a single coralline species. Developmental and DNA sequence data have long suggested that complex articulated fronds evolved from crustose coralline ancestors at least three distinct times (Johansen 1981, Aguirre et al. 2010, Rösler et al. 2017). But recent work has demonstrated that evolutionary reversals have led to the partial or complete loss of fronds in some coralline taxa. Examples include the crustose genus *Crusticorallina*, which shares an articulated ancestor with the genus *Corallina* (Hind et al. 2016), and two species of *Bossiella*, which are the only crusts in a genus of otherwise articulated species (Hind et al. 2018). Likewise, *Chiharaea* includes one frondose species and two species with fronds of one to six intergenicula (Martone et al. 2012). Here, for the first time, we demonstrate that the near complete loss of fronds may occur not just within a single genus, but within a single species, lending additional support for the idea that gains and losses of coralline fronds over evolutionary timescales may occur rapidly and reflect simple genetic changes. Such striking morphological shifts in certain localized populations make *C. berteroi* an excellent candidate for studying mechanisms of coralline speciation.

Ecologically, nearly all collections of *Corallina berteroi* have been from rocky intertidal habitats ranging from high intertidal tidepools to exposed rock in the mid- to low intertidal. However, two collections from North Carolina are from hard bottom, subtidal habitats (13 m deep), showing the depth range of this species and tolerance to lower light levels. In general, the ability of this species to exhibit both morphological and physiological variation across a wide range of habitats is particularly noteworthy.

The multilocus tree (-/0.71), bPTP (*rbc*L), and BPP (0.887) along with pairwise sequence divergences (Fig. 1, Tables S2–S5), supports that *Corallina berteroi* is, thus far, the only widely distributed articulated coralline species confirmed by DNA sequencing. *Corallina berteroi* has been confirmed from temperate waters worldwide (Fig. 4), including the western and eastern coasts of North America (California, USA; Baja California, Mexico; North and South Carolina, USA), southwestern Pacific

Ocean (North Island, New Zealand), Pacific coast of South America (from northern Peru to Diego Ramirez Island, Chile), western and eastern Atlantic (Falkland Island, southern England and South Africa), East Asia (from Hong Kong to northern Japan), and southern Indian Ocean (southern Australia). Moreover, historical specimens collected by Claude Gay (PC28646, from San Carlos de Chiloe, Chile) and Charles Darwin (TCD1171, from Chiloé, Chile), identified by them as *C. chilensis* and *C. officinalis* (in Harvey 1849), respectively, corresponded to *C. berteroi* (Table S1, Fig. S3).

CONCLUSIONS

This study highlights not only the importance of sequencing type specimens in applying names to coralline algae, but also the need to apply various species delimitation methods and multilocus analyses to understand cryptic diversity within coralline algae (Kato et al. 2013, Hind et al. 2014, Hernandez-Kantun et al. 2016, Caragnano et al. 2018, Torrano-Silva et al. 2018, Costa et al 2019, Pezzolesi et. al 2019), particularly within the genus *Corallina*. *Corallina* species have had a long history of confusing and insufficient circumscriptions, and previous studies have emphasized the need to understand their cryptic diversity (Walker et al. 2009, Bustamante et al. 2019a). The morpho-anatomical and molecular variability of *Corallina berteroi* is an extreme example of difficulty of applying a classification system to multiple, evolving populations. The wide morphological variation of *C. berteroi* might reflect phenotypic plasticity or adaptive responses of local populations to environmental drivers (i.e., ecophysiology, hydrodynamic regimes, and biotic interactions), ultimately shaping punctuated morphotypes (Baba et al. 1988, Colombo-Pallotta et al. 2006, Gabrielson et al. 2018, Méndez et al. 2019). Additional studies are needed to understand the mechanisms underlying the variation exhibited by globally distributed populations.

Aguirre, J., Perfectti, F. & Braga, J. C. 2010. Integrating phylogeny, molecular clocks, and the fossil record in the evolution of coralline algae (Corallinales and Sporolithales, Rhodophyta). *Paleobio.* 36:519–33.

Avise, J. C. & Ball, R. M. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. *In* Futuyma, D. & Antonovics, J. [Eds.] *Oxford surveys in evolutionary biology.* Oxford Univ. Press, Oxford, UK, pp 45–67.

Baba, M., Johansen, H. W. & Masaki, T. 1988. The segregation of three species of *Corallina* (Corallinales, Rhodophyta) based on morphology and seasonality in northern Japan. *Bot. Mar.* 31:15–22.

Brodie, J., Melbourne, L., Mrowicki, R. J., Brickle, P., Russell, S. & Scott, S. 2020. *Corallina* (Corallinales, Rhodophyta) from Tristan da Cunha and the Falkland Islands: implications for South Atlantic biogeography. *Eur. J. Phycol.* 56:94–104.

Brodie, J., Walker, R. H., Williamson, C. & Irvine, L. M. 2013. Epitypification and redescription of *Corallina officinalis* L., the type of the genus, and *C. elongata* Ellis et Solander (Corallinales, Rhodophyta). *Cryptogamie Algol.* 34:49–6.

Bustamante, D. E., Calderon, M. S. & Hughey, J. R. 2019a. Conspecificity of the Peruvian *Corallina ferreyrae* with *C. caespitosa* (Corallinaceae, Rhodophyta) inferred from genomic analysis of the type specimen. *Mitochondr. DNA Part B* 4:1285–6.

Bustamante, D. E., Calderon, M. S., Leiva, S., Mendoza, J. E., Arce, M. & Oliva, M. 2021. Three new species of *Trichoderma* in the Harzianum and Longibrachiatum lineages from Peruvian cacao crop soils based on an integrative approach. *Mycologia.* https://doi.org/10.1080/00275514.2021.1917243

Bustamante, D. E., Oliva, M., Leiva, S., Mendoza, J. E., Bobadilla, L., Angulo, G. & Calderon, M. S. 2019b. Phylogeny and species delimitations in the entomopathogenic genus *Beauveria* (Hypocreales, Ascomycota), including the description of *B. peruviensis* sp. nov. *MycoKeys* 58:47–68.

Calderon, M. S. & Boo, S. M. 2016. Phylogeny of Phyllophoraceae (Rhodophyta, Gigartinales) reveals *Asterfilopsis* gen. nov. from the Southern Hemisphere. *Phycologia* 55:543–54.

Caragnano, A., Foetisch, A., Maneveldt, G., Millet, L., Liu, L. C., Lin, S. M., Rodondi, G. & Payri, C. E. 2018. Revision of Corallinaceae (Corallinales, Rhodophyta): Recognizing *Dawsoniolithon*, gen. nov., *Parvicellularium* gen. nov. and Chamberlainioideae subfam. nov. containing *Chamberlainium* gen. nov. and *Pneophyllum*. *J. Phycol.* 54:391–409.

Carstens, B. C., Pelletier, T. A., Reid, N. M. & Satler, J. D. 2013. How to fail at species delimitation. *Mol. Ecol.* 22:4369–83.

Clarkston, B. E. & Saunders, G. W. 2013. Resolving species diversity in the red algal genus *Callophyllis* (Kallymeniaceae, Gigartinales) in Canada using molecular assisted alpha taxonomy. *Eur. J. Phycol.* 48:27–46.

Clement, M., Posada, D. & Crandall, K. A. 2000. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* 9:1657–9.

Colombo-Pallotta, M. F., García-Mendoza, E. & Ladah, L. B. 2006. Photosynthetic performance, light absorption, and pigment

composition of *Macrocystis pyrifera* (Laminariales, Phaeophyceae) blades from different depths. *J. Phycol.* 42:1225–34.

Costa, I. O., Jesus, P. B. d., de Jesus, T. d. S., Souza, P. d. S., Horta, P. A. & Nunes, J. M. d. C. 2019. Reef building coralline algae from the Southwest Atlantic: filling gaps with the recognition of *Harveylithon* (Corallinaceae, Rhodophyta) on the Brazilian coast. *J. Phycol.* 55:1370–85.

Dawson, E. Y. 1953. Marine red algae of Pacific Mexico. Part 1. Bangiales to Corallinaceae subf. Corallinoidae. *Allan Hancock Pacific Expeditions* 17:1–239.

Dawson, E. Y., Acleto, O. C. & Foldvik, N. 1964. The seaweeds of Peru. *Beihefte Zur Nova Hedwigia* 13:1–111.

Dellicour, S. & Flot, J. F. 2015. Delimiting species-poor datasets using single molecular markers: A study of barcode gaps, haplowebs and GMYC. *Syst. Biol.* 64:900–8.

Dettman, J. R., Jacobson, D. J. & Taylor, J. W. 2003. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. *Evolution* 57:2703–20.

Drummond, A. J., Ho, S. Y. W., Phillips, M. J. & Rambaut, A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4:e88.

Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* 29:1969–73.

Edler, D., Klein, J., Antonelli, A. & Silvestro, D. 2019. RaxmlGUI 2.0 beta: a graphical interface and toolkit for phylogenetic analyses using RAxML. *BioRxiv* 800912.

Freshwater, D. W. & Rueness, J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species based upon *rbc*L nucleotide sequence analysis. *Phycologia* 33:187–94.

Gabrielson, P. W., Hughey, J. R. & Diaz-Pulido, G. 2018. Genomics reveals abundant speciation in the coral reef building alga *Porolithon onkodes* (Corallinales, Rhodophyta). *J. Phycol.* 54:429–34.

Gabrielson, P. W., Miller, K. A. & Martone, P. T. 2011. Morphometric and molecular analyses confirm two distinct species of *Calliarthron* (Corallinales, Rhodophyta), a genus endemic to the northeast Pacific. *Phycologia* 50:298–16.

Ganesan, E. K. 1968. Studies on the morphology and reproduction of the articulated corallines-III. *Amphiroa* Lamouroux emend. *Weber van Bosse. Phykos* 6:7–28.

García-Melo, J. E., Oliveira, C., Da Costa-Silva, G. J., Ochoa-Orrego, L. E., Garcia-Pereira, L. H. & Maldonado-Ocampo, J. A. 2019. Species delimitation of neotropical Characins (Stevardiinae): Implications for taxonomy of complex groups. *PLoS ONE* 14:e0216786.

Hamilton, C. A., Hendrixson, B. E., Brewer, M. S. & Bond, J. E. 2014. An evaluation of sampling effects on multiple DNA barcoding methods leads to an integrative approach for delimiting species: A case study of the North American tarantula genus *Aphonopelma* (Araneae, Mygalomorphae, Theraphosidae). *Mol. Phylogenet. Evol.* 71:79–93.

Harvey, W. H. 1849. *Nereis australis, or algae of the Southern Ocean: being figures and descriptions of marine plants, collected on the shores of the Cape of Good Hope, the extra-tropical Australian colonies, Tasmania, New Zealand, and the Antarctic regions; deposited in the Herbarium of the Dublin University.* Part 2. Reeve Brothers, London, 162 pp.

Hernandez-Kantun, J. J., Gabrielson, P. W., Hughey, J. R., Pezzolesi, L., Rindi, F., Robinson, N. M., Peña, V., Riosmena-Rodriguez, R., Le Gall, L. & Adey, W. H. 2016. Reassessment of branched *Lithophyllum* spp. (Corallinales, Rhodophyta) in the Caribbean Sea with global implications. *Phycologia* 55:619–39.

Hind, K. R., Gabrielson, P. W., Lindstrom, S. C. & Martone, P. T. 2014. Misleading morphologies and the importance of sequencing type specimens for resolving coralline taxonomy (Corallinales, Rhodophyta): *Pachyarthron cretaceum* is *Corallina officinalis. J. Phycol.* 50:760–4.

Hind, K. R., Gabrielson, P. W., Jensen, K. P. & Martone, P. T. 2016. *Crusticorallina* gen. nov., a nongeniculate genus in the subfamily Corallinoideae (Corallinales, Rhodophyta). *J. Phycol.* 52:929–41.

Hind, K. R., Gabrielson, P. W., Jensen, K. P. & Martone, P. T. 2018. Evolutionary reversals in *Bossiella* (Corallinales, Rhodophyta): first report of a coralline genus with both geniculate and nongeniculate species. *J. Phycol.* 54:788–98.

Hind, K. R. & Saunders, G. W. 2013. A molecular phylogenetic study of the tribe Corallineae (Corallinales, Rhodophyta) with an assessment of genus-level taxonomic features and descriptions of novel genera. *J. Phycol.* 49:103–14.

Hoshino, M., Ishikawa, S. & Kogame, K. 2018. Concordance between DNA-based species boundaries and reproductive isolating barriers in the *Scytosiphon lomentaria* species complex (Ectocarpales, Phaeophyceae). *Phycologia* 57:232–42.

Hughey, J. R. & Gabrielson, P. W. 2012. Comment on "Acquiring DNA sequence data from dried archival red algae (Florideophyceae) for the purpose of applying available names to contemporary genetic species: a critical assesssment". *Botany* 90:1191–4.

Jackson, N. D., Carstens, B. C., Morales, A. E. & O'Meara, B. C. 2017. Species delimitation with gene flow. *Syst. Biol.* 66:799–812.

Johansen, H. W. 1971. *Bossiella*, a genus of articulated corallines (Rhodophyceae, Cryptonemiales) in the eastern pacific. *Phycologia* 10:381–96.

Johansen, H. W. 1981. *Coralline Algae, A First Synthesis.* CRC Press, Boca Raton, Florida, 239 pp.

Kato, A., Baba, M. & Suda, S. 2013. Taxonomic circumscription of heterogeneous species *Neogoniolithon brassica-florida* (Corallinales, Rhodophyta) in Japan. *Phycol. Res.* 61:15–26.

Kucera, H. & Saunders, G. W. 2012. A survey of Bangiales (Rhodophyta) based on multiple molecular markers reveals cryptic diversity. *J. Phycol.* 48:869–82.

Kuchta, S. R., Brown, A. D., Converse, P. E. & Highton, R. 2016. Multilocus phylogeography and species delimitation in the Cumberland Plateau salamander, *Plethodon kentucki*: Incongruence among data sets and methods. *PLoS ONE* 11: e0150022.

Kumar, S., Stecher, G. & Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33:1870–4.

Kützing, F. T. 1849. *Species algarum.* Leipzig, F.A, Brockhaus, 922 pp.

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T. & Calcott, B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol. Biol. Evol.* 34:772–3.

Le Gall, L. & Saunders, G. W. 2010. DNA barcoding is a powerful tool to uncover algal diversity: a case study of the Phyllophoraceae (Gigartinales, Rhodophyta), in the Canadian flora. *J. Phycol.* 46:374–89.

Leaché, A. D. & Fujita, M. K. 2010. Bayesian species delimitation in west African forest geckos (*Hemidactylus fasciatus*). *Proc. R. Soc. B.* 277:3071–7.

Lin, S. M., Fredericq, S. & Hommersand, M. H. 2002. Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on large subunit rDNA and *rbc*L sequences, including the Phycodryoideae, subfam. nov. *J. Phycol.* 37:881–99.

Lin, X. L., Stur, E. & Ekrem, T. 2017. Exploring species boundaries with multiple genetic loci using empirical data from non-biting midges. *Zool. Scr.* 47:325–41.

Lindstrom, S. C., Gabrielson, P. W., Hughey, J. R., Macaya, E. C. & Nelson, W. A. 2015. Sequencing of historic and modern specimens reveals cryptic diversity in *Nothogenia* (Scinaiaceae, Rhodophyta). *Phycologia* 54:97–108.

Linnaeus, C. 1758. *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis.* Tomus I. Editio decima, reformata. Editio decima revisa. Holmiae, Stockholm, 823 pp.

Liu, F., Wang, M., Damm, U., Crous, P. W. & Cai, L. 2016. Species boundaries in plant pathogenic fungi: A *Colletotrichum* case study. *BMC Evol. Biol.* 16:81–94.

Liu, S. L., Lin, S. M. & Chen, P. C. 2015. Phylogeny, species diversity and biogeographic patterns of the genus *Tricleocarpa* (Galaxauraceae, Rhodophyta) from the Indo-Pacific region, including *T. confertus* sp. nov. from Taiwan. *Eur. J. Phycol.* 50:439–56.

Lorén, J. G., Farfán, M. & Fusté, M. C. 2018. Species delimitation, phylogenetic relationships, and temporal divergence model in the genus *Aeromonas. Front. Microbiol.* 9:770–82.

Luo, A., Ling, C., Ho, S. Y. W. & Zhu, C. D. 2018. Comparison of methods for molecular species delimitation across a range of speciation scenarios. *Syst. Biol.* 67:830–46.

Manza, A. V. 1937. The genera of the articulated corallines. *Proc. Natl. Acad. Sci. USA* 23:44–8.

Martone, P. T., Lindstrom, S. C., Miller, K. A. & Gabrielson, P. W. 2012. *Chiharaea* and *Yamadaia* (Corallinales, Rhodophyta) represent reduced and recently derived articulated Coralline morphologies. *J. Phycol.* 48:859–68.

McCarthy, C. 1998. Griffith University, Southport, Australia. http://www.technelysium.com.au/chromas.html

Méndez, F., Marambio, J., Ojeda, J., Rosenfeld, S., Rodríguez, J. P., Tala, F. & Mansilla, A. 2019. Variation of the photosynthetic activity and pigment composition in two morphotypes of *Durvillaea antarctica* (Phaeophyceae) in the sub-Antarctic ecoregion of Magallanes. *Chile. J. Appl. Phycol.* 31:905–13.

Monaghan, M. T., Wild, R., Elliot, M., Fujisawa, T., Balke, M., Inward, D. J. G., Lees, D. C., Ranaivosolo, R., Eggleton, P., Barraclough, T. G. & Vogler, A. P. 2009. Accelerated species inventory on Madagascar using coalescent-based models of species delineation. *Syst. Biol.* 58:298–311.

Montagne, C. 1854. Botánica. Tomo octavo. Flora Chileana. Plantas cellulares. Tomo segundo. Algas. *In* Gay, C. [Ed.] *Historia física y política de Chile según documentos adquiridos en esta república durante doce años de residencia en ella y publicata bajo los auspicios del supremo gobierno.* Vol. 8. Santiago de Chile, Chile, pp. 257–389.

Pardo, C., López, L., Peña, V., Hernández-Kantún, J., Le Gall, L., Bárbara, I. & Barreiro, R. 2014. A multilocus species delimitation reveals a striking number of species of coralline algae forming maerl in the OSPAR Maritime Area. *PLoS ONE* 9:e104073.

Pezzolesi, L., Peña, V., Le Gall, L., Gabrielson, P. W., Kaleb, S., Hughey, J. R., Rodondi, G. et al. 2019. Mediterranean *Lithophyllum stictiforme* (Corallinales, Rhodophyta) is a genetically diverse species complex: implications for species circumscription, biogeography and conservation of coralligenous habitats. *J. Phycol.* 55:473–92.

Porter, D. M. 1987. Darwin notes on Beagle plants. *Bull. Br. Mus. Nat. Hist. (Hist. Ser.)* 14:145–233.

Puillandre, N., Lambert, A., Brouillet, S. & Achaz, G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol. Ecol.* 21:1864–77.

Rambaut, A., Suchard, M. A., Xie, D. & Drummond, A. J. 2014. Tracer v1.6, Available at: http://beast.bio.ed.ac.uk/Tracer

Rannala, B. & Yang, Z. 2003. Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics* 164:1645–56.

Robba, L., Russell, S. J., Barker, G. L. & Brodie, J. 2006. Assessing the use of the mitochondrial *cox*1 marker for use in DNA barcoding of red algae (Rhodophyta). *Am. J. Bot.* 93:1101–8.

Rojas, R. R., Fouquet, A., Ron, S. R., Hernández-Ruz, E. J., Melo-Sampaio, P. R., Chaparro, J. C., Vogt, R. C. et al. 2018. A Pan-Amazonian species delimitation: high species diversity within the genus *Amazophrynella* (Anura: Bufonidae). *Peer J* 6:e4941.

Ronquist, F., Teslenko, M., Van der Mark, P., Ayres, D., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61:539–42.

Rösler, A., Perfectti, F., Peña, V., Aguirre, J. & Braga, J. C. 2017. Timing of the evolutionary history of Corallinaceae (Corallinales, Rhodophyta). *J. Phycol.* 53:567–76.

Saunders, G. W. 2008. A DNA barcode examination of the red algal family Dumontiaceae in Canadian waters reveals substantial cryptic species diversity. 1. The foliose *Dilsea - Neodilsea* complex and *Weeksia. Botany* 86:773–89.

Saunders, G. W. & McDevit, D. C. 2012. Methods for DNA barcoding photosynthetic protists emphasizing the macroalgae and diatoms. *In* Kress, W. J. & Erickson, D. L. [Eds.] *DNA Barcodes: Methods and Protocols. Methods in Molecular Biology.* Humana Press, Totowa, New Jersey, pp 207–22.

Segawa, S. 1955. Systematic anatomy of the articulated corallines (Supplementary Report). The structure and reproduction of *Yamadaia melobesioides* Segawa. *Bot. Mag. Tokyo* 68:241–7.

Silva, P. C. 1957. Notes on Pacific marine algae. *Madroño* 14:41–51.

Solís-Lemus, C., Knowles, L. L. & Ané, C. 2015. Bayesian species delimitation combining multiple genes and traits in a unified framework. *Evolution* 69:492–507.

Stamatakis, A. 2014. RAxML Version 8: A tool for phylogenetic analysis and postanalysis of large phylogenies. *Bioinformatics* 30:1312–3.

Sukumaran, J. & Knowles, L. L. 2017. Multispecies coalescent delimits structure, not species. *Proc. Natl. Acad. Sci. USA* 114:1607–12.

Taylor, W. R. 1945. Pacific marine algae of the Allan Hancock Expeditions to the Galapagos Islands. *Allan Hancock Pacific Expeditions* 12:i–iv, 1–528.

Thiers, B. 2021. (continuously updated) *Index Herbariorum: A global directory of public herbaria and associated staff.* New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/

Tineo, D., Bustamante, D. E., Calderon, M. S., Mendoza, J. E., Huaman, E. & Oliva, M. 2020. An integrative approach reveals five new species of highland papayas (Caricaceae, *Vasconcellea*) from northern Peru. *PLoS ONE* 15:e0242469.

Torrano-Silva, B. N., Riosmena-Rodriguez, R. & Oliveira, M. C. 2014. Systematic position of *Paulsilvella* in the Lithophylloideae (Corallinaceae, Rhodophyta) confirmed by molecular data. *Phytotaxa* 190:94–111.

Torrano-Silva, B. N., Vieira, B. R., Riosmena-Rodríguez, R. & Oliveira, M. C. 2018. Guidelines for DNA barcoding of coralline algae, focusing on Lithophylloideae (Corallinales) from Brazil. *Bot. Mar.* 61:127–40.

Twist, B. A., Neill, K. F., Bilewitch, J., Jeong, S. Y., Sutherland, J. E. & Nelson, W. A. 2019. High diversity of coralline algae in New Zealand revealed: Knowledge gaps and implications for future research. *PLoS ONE* 14:1–21.

Walker, R. H., Brodie, J., Russell, S., Irvine, L. M. & Orfanidis, S. 2009. Biodiversity of coralline algae in the northeastern Atlantic including *Corallina caespitosa* sp. nov. (Corallinoideae, Rhodophyta). *J. Phycol.* 45:287–97.

Williamson, C. J., Walker, R. H., Robba, L., Yesson, C., Russell, S., Irvine, L. M. & Brodie, J. 2015. Toward resolution of species diversity and distribution in the calcified red algal genera *Corallina* and *Ellisolandia* (Corallinales, Rhodophyta). *Phycologia* 54:2–11.

Wynne, M. J. 2012. Current taxonomic assignments of benthic marine macroalgal collections made by Charles Darwin while on the H.M.S. Beagle, with the addition of "*Laurencia papillosa*". *Nova Hedwigia* 95:243–58.

Xia, X. 2018. DAMBE7: New and improved tools for data analysis in Molecular Biology and Evolution. *Mol. Biol. Evol.* 35:1550–2.

Yang, Z. & Rannala, B. 2010. Bayesian species delimitation using multilocus sequence data. *Proc. Natl. Acad. Sci. USA* 107:9264–9.

Yang, Z. & Rannala, B. 2017. Bayesian species identification under the multispecies coalescent provides significant improvements to DNA barcoding analyses. *Mol. Ecol.* 26:3028–36.

Yendo, K. 1902. Corallinae verae japonicae. *J. Coll. Sci., Tokyo Imp. Univ.* 161–36.

Yoon, H. S., Hacket, J. D. & Bhattacharya, D. 2002. A single origin of the peredinin- and fucoxanthin-containing plastids in donoflagellates through tertiary endosymbiosis. *Proc. Natl. Acad. Sci. USA* 99:11724–9.

Yoshida, T. 1991. Catalogue of the type specimens of algae preserved in the herbarium, Department of Botany, the University Museum, the University of Tokyo. *Material Rep. Univ. Mus. Univ. Tokyo* 24:1–51.

Yu, G., Rao, D., Matsui, M. & Yang, J. 2017. Coalescent-based delimitation outperforms distance-based methods for

delineating less divergent species: the case of *Kurixalus odontotarsus* species group. *Sci. Rep.* 7:16124.

Zhang, J., Kapli, P., Pavlidis, P. & Stamatakis, A. 2013. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 29:2869–76.

Zhou, W. W., Wen, Y., Fu, J., Xu, Y. B., Jin, J. Q., Ding, L., Min, M. S., Che, J. & Zhang, Y. P. 2012. Speciation in the *Rana chensinensis* species complex and its relationship to the uplift of the Qinghai-Tibetan Plateau. *Mol. Ecol.* 21:960–73.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Fig. S1. Phylogenetic tree based on maximum likelihood inference of COI data. ML bootstrap values (BS; ≥ 50%) and Bayesian posterior probabilities (BPP; ≥ 0.90) are indicated adjacent to the branches. Values lower than 50% (BS) or 0.90 (BPP) are indicated by hyphens (-). Values of 100% (BS) and 1.00 (BPP) are indicated by asterisks (*). + represents sequence obtained from the type specimen. Sequences for taxa in bold were generated in this study. Scale bar indicates the number of nucleotide substitution per site. BC, British Columbia; CA, California; CHB, Chiba; HOK, Hokkaido; NL, Newfoundland and Labrador; NY, New York; PDL, Pays de la Loire.

Fig. S2. Phylogenetic tree based on maximum likelihood inference of *psb*A data. ML bootstrap values (BS; ≥ 50%) and Bayesian posterior probabilities (BPP; ≥ 0.90) are indicated adjacent to the branches. Values lower than 50% (BS) or 0.90 (BPP) are indicated by hyphens (-). Values of 100% (BS) and 1.00 (BPP) are indicated by asterisks (*). + represents sequence obtained from the type specimen. Sequences for taxa in bold were generated in this study. Scale bar indicates the number of nucleotide substitution per site. BC, British Columbia; CA, California; CHB, Chiba; HOK, Hokkaido; NC, North Carolina; NL, Newfoundland and Labrador.

Fig. S3. Phylogenetic tree based on maximum likelihood inference of *rbc*L data. ML bootstrap values (BS; ≥ 50%) and Bayesian posterior probabilities (BPP; ≥ 0.90) are indicated adjacent to the branches. Values lower than 50% (BS) or 0.90 (BPP) are indicated by hyphens (-). Values of 100% (BS) and 1.00 (BPP) are indicated by asterisks (*). + represents sequence obtained from the type specimen. Sequences for taxa in bold were generated in this study. Scale bar indicates the number of nucleotide substitution per site. BC, British Columbia; BCA: Baja California; CA, California; CHB, Chiba; HOK, Hokkaido; NC, North Carolina; NY, New York; PDL, Pays de la Loire;

SA, South Australia; SC, South Carolina; SON, Sonora; VIC, Victoria.

Fig. S4. Bayesian inference ultrametric gene tree obtained using a coalescent tree prior in BEAST with the statistical species delimitation results from GMYC based on COI.

Fig. S5. Bayesian inference ultrametric gene tree obtained using a coalescent tree prior in BEAST with the statistical species delimitation results from GMYC based on *psb*A.

Fig. S6. Bayesian inference ultrametric gene tree obtained using a coalescent tree prior in BEAST with the statistical species delimitation results from GMYC based on *rbc*L.

Fig. S7. Specimen (Darwin 2423, TCD 1171) collected by Charles Darwin on his *H.M.S. Beagle* voyage from Chonos, Chiloe.

Fig. S8. Specimen (Darwin 1143) collected by Charles Darwin on his *H.M.S. Beagle* voyage from the Falkland Islands.

Table S1. List of taxa used in molecular analyses along with Herbarium acronym followed by accession number, collection locality, date, habitat data, collector and, if known, collector specimen number. GenBank accession numbers under each marker; if marker not sequenced indicated by "–". Sequences generated in present study are in bold. Taxa in quotes indicates that type material has not been sequenced.

Table S2. Genetic distance (p-distances) in percentage for species of *Corallina* for COI marker based on the multilocus results. Intraspecific divergences are in grey color.

Table S3. Genetic distance (p-distances) in percentage for species of *Corallina* for *psb*A marker based on the multilocus results. Intraspecific divergences are in grey color.

Table S4. Genetic distance (p-distances) in percentage for species of *Corallina* for *rbc*L marker based on the multilocus results. Intraspecific divergences are in grey color.

Table S5. Highest posterior probabilities of the three-gene Bayesian species delimitation analysis (BPP) by jointing species delimitation and species tree inference (A11: species delimitation = 1, species tree = 1).

Table S6. Results of the Generalized Mixed Yule-Coalescent (GMYC) analyses under the single threshold model.