What is *Corallina officinalis var. chilensis*?

An examination of nomenclature, biogeography, phylogeny, and morphology

by

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B.M., University of Maryland, College Park, 2006

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

The Faculty of Graduate and Postdoctoral Studies

(Botany)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

September 2020

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What is *Corallina officinalis var. chilensis*?
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submitted by Soren Huber in partial fulfillment of the requirements for

the degree of Master of Science

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Abstract

Geniculate coralline algae are notoriously challenging to identify in the field due to confusing morphological variation. Consequently, former species delimitations based exclusively on morphology are often unsupported by sequence-based phylogenies. The purpose of my research was to determine whether *Corallina chilensis* Decaisne, basonym of *C. officinalis var. chilensis*, was a distinct species or should be considered a variety of *C. officinalis;* and consequently whether *C. chilensis* was distributed in two hemispheres.

In order to answer these questions, I sequenced *psb*A, CO1, and *rbc*L genes from 76 voucher specimens representing *Corallina* collections from ~2000 to 2019. I applied names by comparing these sequences with published sequences and type specimen sequences, including an *rbc*L sequence from the specimen collected by Darwin (#2151 from Valparaiso, Chile), the holotype specimen for *C. chilensis* designated by Harvey. I used phylogeny with additional support from morphometric, Automatic Barcode Gap Discovery, and distance matrix analyses for species delimitation.

DNA from the Chilean *C. chilensis* holotype matched an unnamed coralline species commonly found in the Northeast Pacific, and *C. chilensis* specimens formed a separate clade from *C. officinalis* specimens in my phylogenetic analyses. *Corallina chilensis* is a distinct species, not a variety of *C. officinalis*, and it is present in both hemispheres. Going forward, the name *C. officinalis var. chilensis* should be discontinued, and the older name *C. chilensis* should be used in its place.

Lay Summary

Algae come in many different colors, shapes, and sizes, and there are hundreds of species present in oceans worldwide. Sometimes it is hard to tell two species apart because they look so similar, while other times one species can have many different appearances. Thus, we must use DNA sequence data to confirm species identity and to ensure each species is given its proper binomial name.

This research involved extracting and sequencing DNA from a specimen collected by Charles Darwin, during a stop by the HMS *Beagle* at Valparaiso, Chile, and comparing it to our recent collections. We discovered that Darwin's original specimen corresponded with a species growing in the Pacific Northwest, and that this species was not a variety of *Corallina officinalis*. Given that it is a distinct species, it should not be called "*C. officinalis var. chilensis*," but rather *C. chilensis*, the name originally applied to Darwin's Chilean specimen.

Preface

I wrote this thesis based on work conducted in the Martone Laboratory (Botany Department, University of British Columbia), with collaborators Jeffery Hughey and Paul Gabrielson. I was responsible for molecular work in the laboratory, for the phylogenetic analyses, and for the overseeing and implementing of morphometric measurements.

Katherine Hind, Patrick Martone, and Paul Gabrielson were the primary collectors of specimens used in this research. Erasmo Macaya sent two specimens from Chile. Jeffery Hughey and Paul Gabrielson extracted and amplified DNA from the three 1800's herbarium specimens. Paul Gabrielson also contributed a sequence of *Corallina chilensis* from Chile. Jasmine Lai sequenced PTM specimens 1984 and 1985. Some DNA sequences, particularly the *psb*AF1 DNA sequences used for identification, were retrieved from the laboratory archive that had been sequenced by Katherine Hind or former lab volunteers and work-study students.

Brenton Twist and Mary Berbee provided advice on statistical analyses for phylogenetic trees and aided in interpreting the results. Jade Shivak took all morphometric measurements (in Table 4 and Appendix III); created the map in Figure S12, Appendix II; took the photographs that appear in Figure 25; and prepared R graphs in Figure 26. Patrick Martone took the photograph in Figure 27, and Bill Woelkerling took the photographs in Figures 4 and 5. Modifications to the figures and photographs mentioned above and all other figures and photographs are my own. Patrick Martone, Mary Berbee, Paul Gabrielson, and Amy Angert contributed manuscript edits.

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List of Symbols and Abbreviations

ABGD – Automatic Barcode Gap Discovery

aLRT – approximate Likelihood Ratio Test

bp – base pair

bs – bootstrap

CO1 – Cytochrome c oxidase subunit I

ML – Maximum Likelihood

PC – Paris herbarium

PCR – Polymerase chain reaction

PTM – Patrick T. Martone (prefix designation for specimens collected by Martone Laboratory)

*psb*A – Photosystem II protein D1 precursor

*rbc*L – Ribulose bisphosphate carboxylase large subunit

TCD – Trinity College Herbarium

UBC – University of British Columbia

Acknowledgements

I would first like to acknowledge my thesis advisor, Patrick Martone, for accepting the risk and allowing a music major into his lab. I've always appreciated Patrick's enthusiasm, love of seaweeds, and fountain of ideas. Most of all though, I have admired the tone he established and the culture of care, respect, and inclusivity that he cultivated in his lab. The Martone lab has been a second family to me the past 3 years. My lab mates taught me the ropes, including how to actually "do research" in practice, aided me with field work and experiments, critiqued my drafts and presentations, and were there for me through the best and worst of times. I'll never forget the fun times we had together "cooking" in the lab, on collecting trips, attending conferences and writing retreats, at curling championships, and playing "salad bowl."

During this degree program, I have been very fortunate to have had an exceptional Supervisory Committee. Thanks to Paul Gabrielson for entrusting me with this special project and for having patiently answered dozens of questions and helping me get all the wording just right. I am appreciative to Amy Angert for her perspective, direction, and advice. An especially huge thank you goes to Mary Berbee, (AKA "Mary Godmother"), for helping me troubleshoot PCR when all else failed, interpreting unexpected results, suggesting cool additional analyses and walking me through how to execute them, and contributing hours of edits so this research might be adequately communicated to the rest of the world. I thank Mary for her ever-optimistic perspective, commitment to good science despite the extra time investment, help in framing this story, and encouragement to produce the best possible product.

I would like to recognize Jade Shivak for her strong work ethic, upbeat attitude, and complete trustworthiness while measuring hundreds of coralline intergenicula, among other things, under the microscope and graphing those data for this thesis.

Many thanks to my students from Biol 209, 230, and 320. They were the reason I jumped out of bed for an early start during long rainy winters, and they taught me how to teach. Working with them was a highlight and one of the most rewarding aspects of grad school.

It has certainly taken a village to raise this botanist, and I would like to acknowledge the following people for their support during this journey. Lisa Brooks has been my long-term unofficial biology mentor and compass who pointed me in the right direction from the beginning. Sandy Wyllie-Echeverria gave me a critical foothold when I needed prerequisite research experience. Jeff Hughey was instrumental in helping me access the best possible place to study exactly what I wanted to study.

Although he is no longer with us, I would like to take this opportunity to acknowledge Len Dyck, to whom this thesis is dedicated. Both artist and scientist, Len loved his research and teaching, was humble despite his brilliance, and exceedingly kind to his students even bringing them cookies to stave off "the shakes" during lab exams. Len, thank you for all that you taught me about algae, for the perspective you offered me on my thesis research, but mostly for the example you set as an instructor and human being. I am so lucky to have had the opportunity to work with you. You will always be remembered.

Finally, I would like to acknowledge my entire family for their unconditional love and support. Special thanks to my father, Carl Huber, and my grandmother, Jeanne Huber, for sharing their love of plants with me for as long as I can remember; to my grandfather, Al Huber, for lessons in the garden and the fields; and to my mother, Leah Huber, for encouraging me to collect, press, and study plants and fungi from an early age. I am grateful to Ross Schipper for his overall support and infinite optimism, including through a pandemic; but most of all for celebrating all the failures and successes with me.

Dedicated in memory of
Len Dyck

(Who understood that there is more to “species” than “just a few base pairs.”)

Introduction

Marine macroalgae, or "seaweeds," are members of a morphologically diverse group of photosynthetic eukaryotes that inhabit all the planet's oceans. Ranging in size from hardly visible filamentous strands to 50-meter-long foliose kelps (Graham et al. 2009), seaweeds display a vast pallet of colors, textures, sizes, and shapes for adapting to hydrodynamic, temperature, and, for some, desiccation stress characteristic of coastal habitats (Armstrong 1989, Blanchette et al. 2002, Boller & Carrington 2006, Collado-Vides 2002, Monro & Poore 2005, Koehl et al. 2008). Macroalgae can be red, green, brown, pink, purple, yellow, or black, depending on the combination of specialized pigments they contain for photoprotection, and for absorbing light underwater (Graham et al. 2009).

Seaweed size and shape is highly variable yielding many different morphologies, i.e. ways that seaweeds can look. Having a highly variable morphology can be confusing if one is trying to identify seaweeds to species and has been the subject of phycological study for a long time. Many seaweeds can look similar due to heredity, but in other cases, morphology depends upon the conditions in which they are growing (Ramus 1972, Denny et al. 1985, Armstrong 1988, Armstrong 1989, Gaylord et al. 1994, Blanchette 1997, Blanchette et al. 2002, Collado-Vides 2002, Monro & Poore 2005, Boller & Carrington 2006). For instance, in high flow environments some kelp blades tend to be narrow and flat, while in slower flow environments they tend to be wider and ruffled (Gerard & Mann 1979, Armstrong 1988, Armstrong 1989). Higher in the intertidal zone where the shore is exposed to air for hours at a time, other algae have adapted to desiccation by reducing their surface to volume ratio or growing as turfs to maximize water retention while the tide is out (Padilla 1984, Hunt & Denny 2008, Holzinger & Karsten 2013, Guenther & Martone 2014). Thus, turf forming algae tend to be small (millimeters

to only a few centimeters), highly branched, and frilly (Gaylord et al. 1994). We do not understand the morphological variation exhibited by most seaweed species, and this has been (and continues to be) problematic for taxonomists and other researchers over the years.

I. Corallines and their significance

Coralline red algae (Phylum Rhodophyta, Subphylum Eurhodophytina) are situated within the class Florideophyceae. Members of Florideophyceae are typically characterized by a triphasic life history (Graham et al. 2009). The common name "coralline" specifically refers to three orders, Corallinales, Hapalidiales and Sporolithales, within the subclass Corallinophycidae (Hind et al. 2018). This group is characterized by their ability to incorporate calcium carbonate into their cell walls, mostly in the form of high-magnesium calcite (Hippler et al. 2009, Smith et al. 2012, Nash et al. 2017). Calcium-carbonate impregnated cell walls give corallines a hard, rock-like quality, and chalky, pink-purple appearance (Fig. 1).

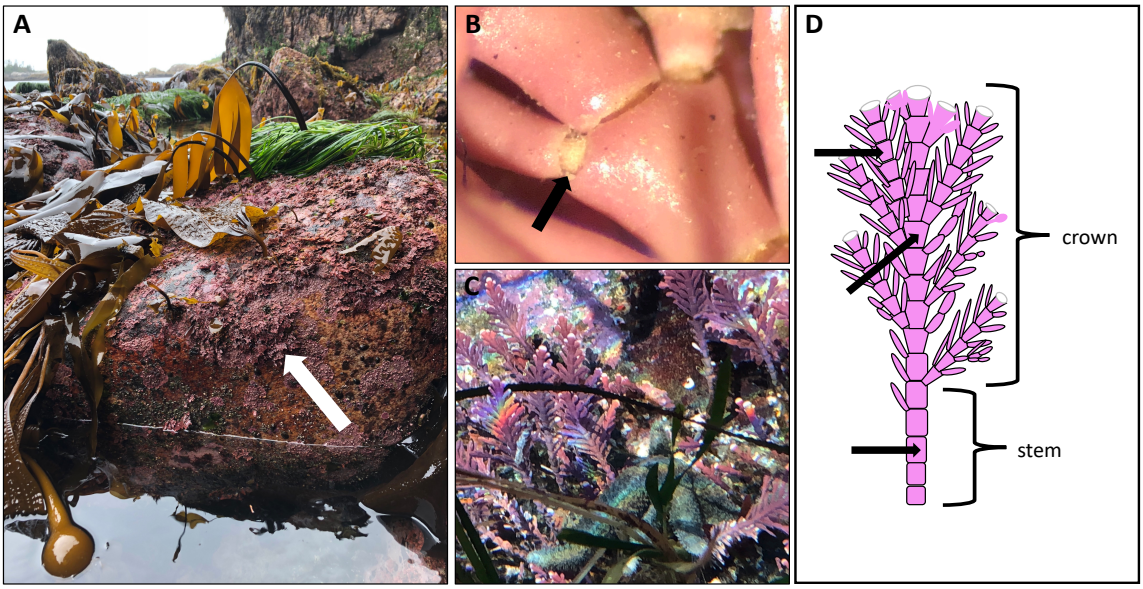


Figure 1. (A) Corallines growing on rock under kelp. (B) Articulated coralline joint "geniculum." (C) Coralline fronds growing in tidepool. (D) Diagram of generic coralline frond, arrows pointing to intergenicula.

Corallines have a range of forms. Some occur as crusts completely adhering to the substratum, others occur as free-living rhodoliths unattached to any substrata and ranging in size from pebbles to small boulders. Still other corallines grow as upright, segmented "articulated" fronds (Fig. 1A-D) several centimeters high (Johansen 1981, Gabrielson & Lindstrom 2018). While there are exceptions to these generalizations, for the purposes of clarity, in this thesis I will refer to encrusting and rhodolith-like morphologies as "non-geniculate corallines," and upright articulated forms as "geniculate corallines."

All coralline individuals begin from a single spore that divides to form a basal crust. Geniculate corallines grow upright from their basal crusts and tend to have a lower portion of unbranched axes that divide to form clusters of branches, which I call "crowns" (Fig. 1D). Fronds may grow individually (Fig. 1C) or in clumps (Fig. 1B), and can exhibit a variety of branching patterns including pinnate, irregular, dichotomous, planar, or whorled. Geniculate corallines are composed of many hard, calcified, longer segments separated by soft, very short, uncalcified regions that act as joints (Fig. 1B-D). The joints between segments are referred to as "genicula," (Fig. 1B), and the calcified segments between genicula are called "intergenicula" (Fig. 1D). Joints lend fronds the ability to flex and bend. This enables geniculate corallines to live in high-energy wave swept environments and thrive where few other organisms can survive (Johansen 1981, Martone 2006, 2007, Martone & Denny 2008A, 2008B, Denny et al. 2013, Janot & Martone 2016). Genicula have evolved at least three different times throughout evolutionary history, which is reflected in their distribution in three different subfamilies Metagoniolithoideae, Lithophylloideae, and Corallinoideae (Janot & Martone 2018). Size and shape of intergenicula may sometimes be used to help differentiate among species or genera in the field (Abbott & Hollenberg 1976, Johansen 1981, Baba et al. 1988), but intergenicular

morphology is notoriously problematic and may not consistently be used as a diagnostic character (see Hind et al. 2014A, 2014B).

II. Identification and species delimitations

While non-geniculate coralline algae have a long history of being challenging to identify (Sissini et al. 2014, van der Merwe et al. 2015, Maneveldt et al. 2017, Twist et al. 2019), it turns out that articulated corallines may be just as challenging to identify. Therefore field identifications of geniculate corallines must also be confirmed or rejected by comparing DNA sequences of unknown specimens with DNA sequences from specimens whose identities have been established.

Corallines are challenging to identify because a single species can be so morphologically variable that specimens of the same species can appear to be multiple species. For example, individuals of *Corallina vancouveriensis* growing only a few meters apart can appear morphologically different from one another (Fig. 2). *Corallina vancouveriensis* specimens may grow as brush-shaped fronds (Fig. 2A) with irregular branches and pinnules (i.e. small secondary terminal branchlets), may grow as flat, symmetrical fronds (Fig. 2B), or both flat and brush-shaped fronds from the same basal crust (Fig. 2C). In other instances, geniculate coralline field identifications may be confounded because multiple coralline species appear morphologically similar and may be mistaken for the same species (Fig 3). For example, some species of *Corallina* and *Bossiella* can look remarkably similar in the field (Fig. 3).

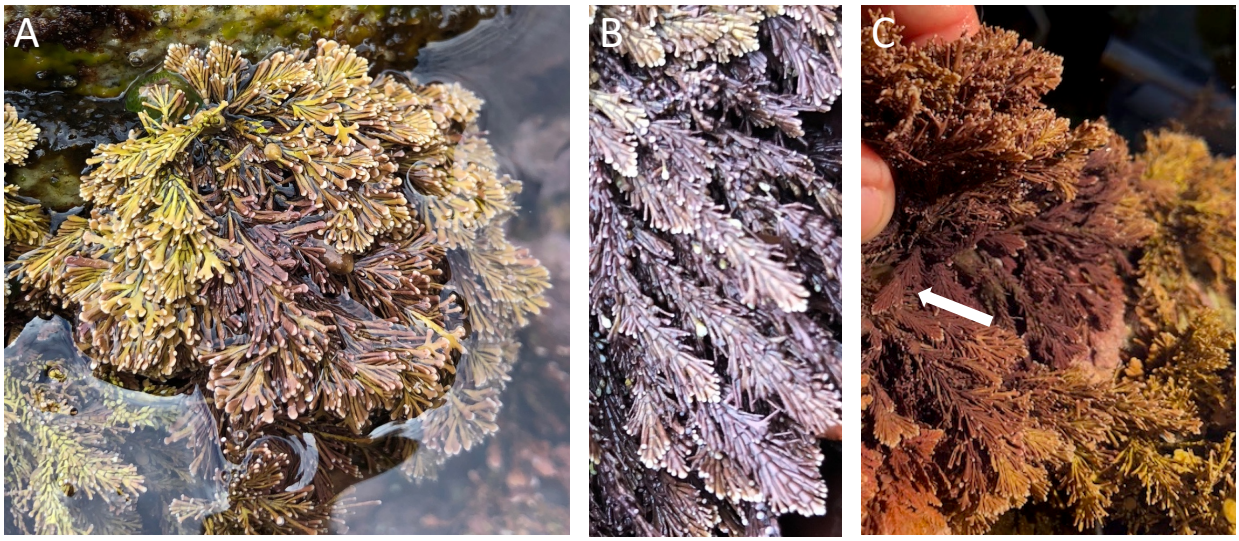


Figure 2. *Corallina vancouveriensis* growing on North Beach, Calvert Island, BC, Canada. (A) Exposed brush like form. (B) Shaded flat form. (C) Both forms in one clump. Arrow is pointing to flat fronds towards the middle of the clump where they are shaded by the outer brush-shaped fronds.

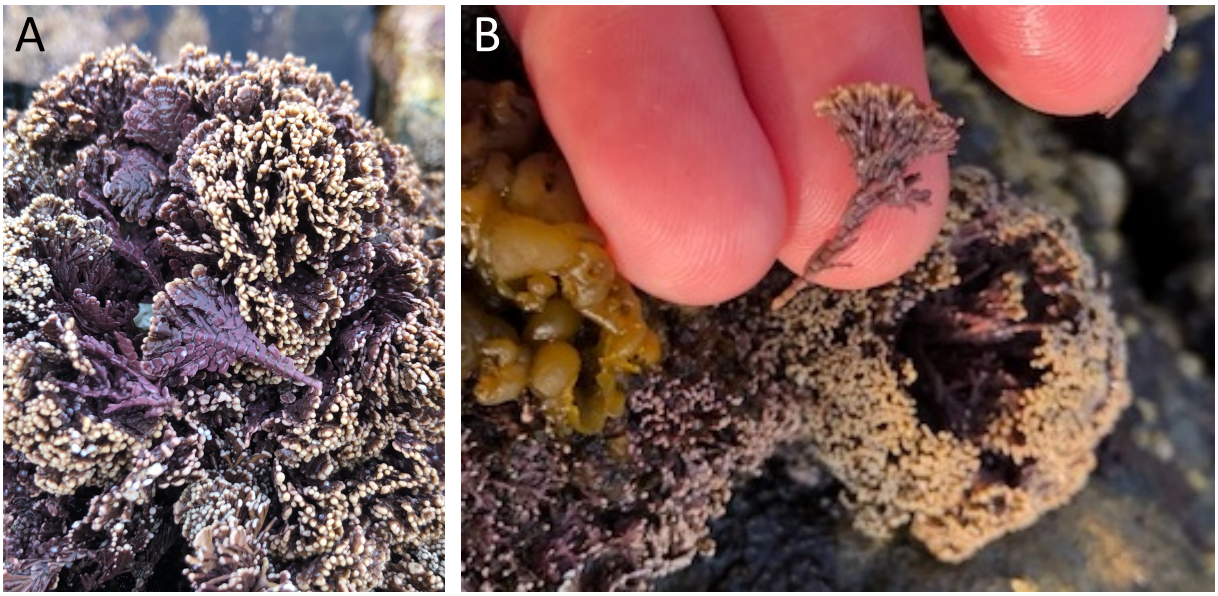


Figure 3. Corallines growing at Botanical Beach, British Columbia, Canada. (A) *Bossiella sp.* (B) *Corallina sp.*

Several different phenomena have led to taxonomic confusion in the corallines. These include convergent traits between distant relatives, that is, similar traits that have evolved independently multiple times (Janot & Martone 2018); nearly identical morphology between closely related species (i.e. cryptic speciation) (Gabrielson et al. 2011, Brodie et al. 2013, Sissini

et al. 2014, Hind et al. 2015); and morphological variation between close relatives, or "intraspecific variation," sometimes based on habitat or geographic location (Hind et al. 2015 Hind et al. 2014B, Hind et al. 2016, Hind et al. 2018, Jeong et al. 2019). Also, some corallines appear to change their morphology based on environmental influences, i.e. they exhibit phenotypic plasticity (Tyrell & Johansen 1995, DeWitt & Scheiner 2004, Maneveldt & Keats 2008). These phenomena in isolation or combination have led to instances where one name has been applied to multiple species (Gabrielson et al. 2011, Hind & Saunders 2013A, Hind et al. 2014B, Sissini et al. 2014, Hind et al. 2015) and other cases where multiple names were applied to the same species or genus (Hind et al. 2014A, van der Merwe et al. 2015, Hind et al. 2016, Hind et al. 2018, Jeong et al. 2019).

Historically, coralline taxonomy was based exclusively on morpho-anatomy and, consequently, due to the above-mentioned phenomena, names were frequently misapplied. Current studies implement DNA sequence data to designate species boundaries (Leliart et al. 2014, van der Merwe et al. 2015, Nelson et al. 2015, Hind et al. 2016, Spalding et al. 2016, Richards et al. 2017, Hind et al. 2018, Twist et al. 2019), and subsequently to determine distinguishing morphological characteristics, if any exist, based on those genetic boundaries.

The process of reconciling old and new approaches of identification and species delimitation has led to vast taxonomic fluctuation (Gabrielson et al. 2011, Brodie et al. 2013, Hind & Saunders 2013A, Hind & Saunders 2013B, Hind et al. 2014A, Hind et al. 2015, Hind et al. 2016, Rösler et al. 2016, Bustamante et al. 2019). As a result, there is an abundance of putative coralline species with provisional names in the literature that require confirmation and description (Saunders & Hommersand 2004, Le Gall et al. 2010, Martone et al. 2012, Hind & Saunders 2013A, Hind et al. 2016, Yang et al. 2016). Reconciling old and new approaches and

confirming and describing putative coralline species is necessary to obtain accurate biodiversity estimates (Kucera & Saunders 2012, Brodie et al. 2013, Williamson et al. 2015).

A species definition common across multiple species concepts is helpful when reconciling morpho-anatomical based approaches and DNA sequence-based approaches to species delimitation. Species may thus be defined as "separately evolving metapopulations" (De Queiroz 2007). Evidence that metapopulations are evolving separately may include reproductive or geographical isolation, as well as morphological, molecular, or phylogenetic distinction (De Queiroz 2007). Congruence across multiple lines of evidence is advisable for delimiting species (Carstens et al. 2013). Some molecular-based techniques implemented in species delimitation of algae include use of phylogenetic trees, Automatic Barcode Gap Discovery (ABGD) analyses, and the comparison of DNA sequences in distance matrices (Le Gall & Saunders 2010, Hind & Saunders 2013A, Nelson et al. 2015, van der Merwe et al. 2015, Jeong et al. 2019, Twist et al. 2019). In my research, I used a combination of aLRT (approximate Likelihood Ratio Test), Bayesian, and bootstrap support for monophyly in both individual and concatenated gene sequences to delimit species, with the consistent separation of species in ABGD analyses as further confirmation of speciation. I also looked for morphological differences between my study species and a congeneric species that is commonly found growing in the same vicinity in Northeast Pacific populations.

III. Nomenclature and the importance of sequencing type specimens

In botanical nomenclature, that includes vascular and non-vascular plants, algae, and fungi, each published name is permanently attached to an original "type" specimen, to which all other specimens can be compared. To clearly understand the species to which a name is

referring, researchers must link that name to the original type collection (Turland et al. 2018, *see article 7.2*). With respect to red algae, this was accomplished until 2001 using morpho-anatomy, but is ideally done by extracting DNA from type specimens for comparison with the DNA sequences from specimens in question. Many type specimens were collected in the 1700's and 1800's, and specific primers and protocols are required to extract remaining intact fragments of partially degraded DNA (Hughey et al. 2001, 2002, Gabrielson et al. 2011).

Hughey et al. (2001, 2002) were the first researchers to successfully extract and amplify DNA from red algal type specimens in the family Gigartinaceae for the purposes of molecular comparison. Gabrielson et al. (2011) adapted the technique to geniculate coralline algae where it has been used to correctly apply names in the geniculate genera *Calliarthron*, *Corallina* (Hind et al. 2014A), and *Bossiella* (Hind et al. 2014B, 2015).

This approach of using DNA sequences from type materials for comparison with recent collections enabled researchers to determine that species formerly thought to have been *Calliarthron* belonged to a different genus, that three species were synonymous, and that there were only two *Calliarthron* species (Gabrielson et al. 2011). Sequencing type material in another study demonstrated that what was formerly referred to as *Pachyarthron cretaceum* based on morphology, was molecularly identical to and should be called *Corallina officinalis* (Hind et al. 2014A). In the case of *Bossiella*, which was originally thought to consist of fewer species because of overlapping morphological characters across multiple species, DNA sequences from type specimens were correlated with genetic groups to recognize and describe over a dozen species within the genus (Hind et al. 2015, Hind et al. 2018).

While DNA sequencing of old type material has been successfully incorporated into many red algal taxonomic studies over the past two decades, the practice has not been

implemented consistently across the field (Farr et al. 2009, Walker et al. 2009, Nelson et al. 2015, Melbourne et al. 2017). Any given collection of DNA sequences may be compared and divided into molecular species groups or compared to DNA sequences published in databases or other publications, yet not comparing such groups with types and thus anchoring them to original names, can create confusion (Walker 2009, Bustamante 2019).

In some cases, it is not possible to utilize type DNA because the type specimen could not be located (e.g. Yendo's *Corallina* collections have not been found), or DNA could not be successfully extracted and amplified from old type material (Brodie et al. 2013). Resolving the nomenclature may still be possible. For instance, Brodie et al. (2013) selected an epitype for *Corallina officinalis* when they could not successfully extract and amplify DNA from the designated lectotype (BM 001062598).

In other cases, morpho-anatomical features are still used to compare specimens with types. Nelson et al. (2015) compared their specimens to Harvey et al. (2005) specimens which had been identified based on morph-anatomical examination of type specimens.

IV. *Corallina officinalis var. chilensis*

The subject of this thesis, *Corallina officinalis var. chilensis* (Decaisne) Kützing (1858), is a perfect example of the challenges facing the identification, delimitation and naming of coralline algal species. *Corallina officinalis var. chilensis* is a geniculate coralline belonging to the order Corallinales (Silva & Johansen 1986), and member of the family Corallinaceae (Lamouroux, 1812), characterized by grouped, zonate-divided, tetra- and bi-sporangia that have no plugs and are housed in uniporate, calcified conceptacles (Harvey et al. 2003). Within Corallinaceae, there are currently seven recognized subfamilies; Lithophylloideae (Setchell

1943), Corallinoideae (Areschoug) Foslie 1908, Chamberlainoideae (Caragnano, Foetisch, Maneveldt & Payri 2018), Neogoniolithoideae (Kato & Baba 2011), Mastophoroideae (Setchell 1943), Metagoniolithoideae (Johansen 1969), and Hydrolithoideae (Kato & Baba 2011). *Corallina* is one of 13 recognized genera in the subfamily Corallinoideae (Hind & Saunders 2013A, Hind et al. 2016, Hind et al. 2018, Guiry & Guiry 2020). *Corallina* contains nearly as many provisionally named species as species that have been formally described and are supported by a morpho-anatomical comparison or DNA sequence match to their type specimen (Hind and Saunders 2013A). Supported species to date include *C. aberrans* (Yendo) K.R.Hind & G.W.Saunders*, C. declinata* (Yendo) K.R.Hind & G.W.Saunders*, C. crassissima* (Yendo) K.R.Hind & G.W.Saunders 2013*, C. officinalis* Linnaeus*, C. maxima* (Yendo) K.R.Hind & G.W.Saunders*, C. vancouveriensis* Yendo, *C. ferreyrae* E.Y.Dawson, O.C. Acleto, & N. Foldvik, *C. pinnatifolia* (Manza) E.Y.Dawson, and *C. melobesioides* (Segawa) P.T.Martone, S.C.Lindstrom, K.A.Miller, & P.W.Gabrielson 2012. Putative species in need of confirmation in addition to *C. officinalis var. chilensis* include C. sp. 2 frondescens, C. sp. 3 frondescens, C. sp. 4 frondescens, C. sp. 5 frondescens, C. sp. 1 gws, C. sp. 2 vancouveriensis, C. sp. 1 california, and C. sp. 5 korea (Hind & Saunders 2013A). Typical of many corallines, *Corallina* species are difficult to tell apart in the field due to cryptic speciation and/or variable morphology. Of the *Corallina* species in the Northeast Pacific, *C. vancouveriensis* appears to be the most common inhabitant of rocky intertidal zones, although it may be sometimes challenging to identify in the field. Other *Corallina* species are found less frequently and are also difficult to differentiate based on morphology. While the name "*C. officinalis var. chilensis*" has been haphazardly applied for decades without type consultation, this is the first time that DNA has been extracted and sequenced from its holotype specimen to determine the accurate application of its name.

In this thesis, I first investigate which specimens from our recent collections are indeed *C. officinalis var. chilensis* by comparing DNA sequences from recent (e.g., collected after ~ year 2000) collections with a DNA sequence from the holotype specimen. Then I describe the species within the context of its genus, update its distribution based on sequenced specimens, and characterize Northeast Pacific populations based on morpho-anatomy.

Chapter I

What is *Corallina officinalis* var. *chilensis*?

1.1 Introduction

1.1.1 Historical context

Linnaeus (1758), when he proposed *Corallina*, listed the binomial names and descriptions of 10 species, including *Corallina officinalis*, but he did not designate a generitype species (Appendix I, Fig. S1). In the original description of *C. officinalis*, Linnaeus referenced an illustration by Ellis (1755, Appendix I, Fig. S2), which by definition was considered the holotype (Turland et al. 2018). The type locality for *C. officinalis* was "Habitat in Oceane Europaeo, Americano" (Linnaeus 1758). Schmitz (1889) placed *Corallina* within the family Corallinaceae situated within the "Florideen" [subclass Florideophycidae], and designated *C. officinalis* as the generitype (Appendix I, Fig. S3). Irvine in Jarvis (1993: 37) designated a specimen in the Linnaean Herbarium LINN 1293.9 as the lectotype. Recently, Brodie et al. (2013) were unable to obtain any viable DNA sequences from the lectotype specimen (LINN 1293.9 from Linnaeus' collection) and designated a neotype specimen from which DNA was successfully extracted and amplified (Spencer et al. 2009, Brodie et al. 2013).

Nearly a century after Linnaeus described *Corallina officinalis* from the Northern Hemisphere, Irish botanist and phycologist William Henry Harvey (1849) published in *Nereis Australis* descriptions of coralline algal taxa in the southern oceans including *C. chilensis* Decaisne in Harvey (Appendix I, Fig. S4). The holotype of *C. chilensis* that is cited in the

description is "Valparaiso C. Darwin 2151," a collection made by Charles Darwin that is currently housed in Trinity College Herbarium (TCD) (Fig. 4).

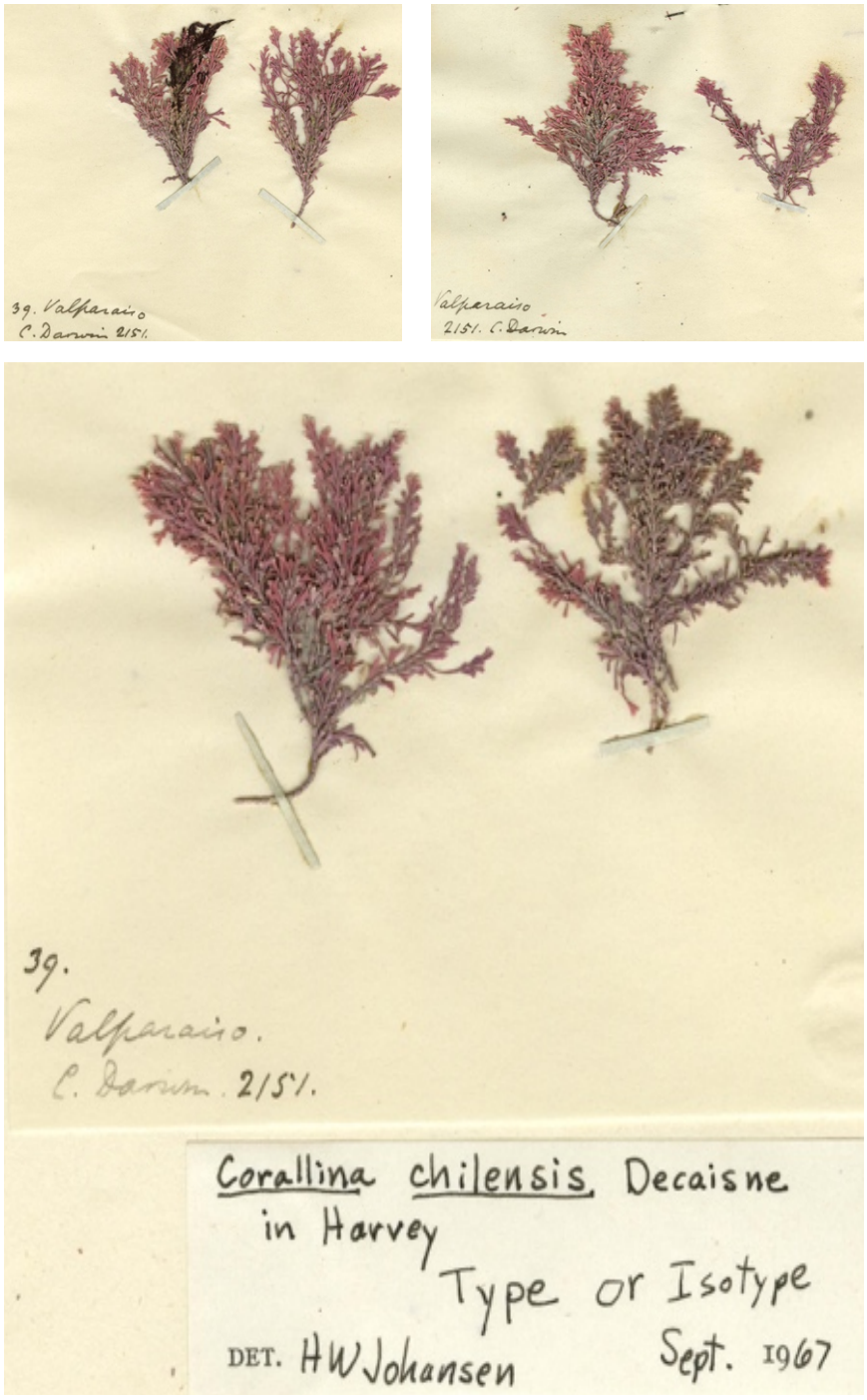


Figure 4. Darwin's *C. chilensis* specimen from Valparaiso, Chile. Housed at the Trinity College Herbarium, Dublin, Ireland (Appendix II). This specimen was designated the type specimen in Harvey 1849.

Harvey (1849) reported that *C. chilensis* was also collected from Port Famine (C. Darwin #1840) and from Norfolk Island [Australia]. There was no collection number provided in the description for the Norfolk Island specimen, but Harvey reported that the collection resides in "Herb. Hooker" (see Appendix I, Fig. S4). The description was as follows:

> "1-2 inches high, bi-tri-pinnate above, the pinnae long, erecto-patent, the upper ones gradually shorter. Articulations of the stem and branches once and half as long as broad, cuneate, simple, the upper ones longer and more expanded towards the apex, very irregular in shape, often laciniate or crenate; the apical ones, especially, frequently palmate" (Harvey, 1849).

Note that Harvey credited the French Belgian botanist Joseph Decaisne in "Herb. Paris" for the description (Harvey, 1849). Decaisne, who was at the Muséum National d'Histoire Naturelle, may have seen other *C. chilensis* specimens in the Paris herbarium (PC), but may never have seen the Darwin type material. Although describing a species without seeing the type is inconsistent with current practices, it helps to remember that the perceived importance of type specimens has increased over the years and type specimens were not required for new species descriptions until 1935 (Turland et al. 2018, See Article 10.7), a result of the 1930 Cambridge Congress (Merrill 1930). Decaisne's description of *C. chilensis* could have been based upon the collections of Claudio Gay and Alcide d'Orbigny, two French naturalist contemporaries of Darwin who explored Chile and brought back their collections to PC (Fig. 5).

While Harvey (1849) made it clear from where specifically and generally *C. chilensis* specimens were collected, details regarding the material from Norfolk Island were vague. Montagne (1852) stated that he had personally not found *C. chilensis*, but clarified that it was Darwin who had found it on the coast of Chile, specifically in Valparaiso and in Puerto del

Hambre (Port Famine) in the Strait of Magellan, and off Norfolk Island, Australia (Montagne 1852, Appendix I, Fig. S5). Aside from Montagne's report, *C. chilensis* was not reported on extensively until after Kützing's (1858) publication nearly a decade after Harvey's publication.

Figure 5. *Corallina sp.* collected by Gay from Ancud, Chile in 1836 (A-C). See Appendix II, Table S1. The designation "paratype" is in error. Small pieces from this collection (D) were sent from the Paris museum herbarium for extraction by Jeffery Hughey.

Nine years after *C. chilensis* Decaisne was published (Harvey, 1849), German phycologist Friedrich Traugott Kützing (1858 : 32) reduced *C. chilensis* to a variety, "*Corallina officinalis chilensis,*" (now *Corallina officinalis var. chilensis* (Decaisne) Kützing). Kützing's publication *Tabulae Phycologicae; oder Abbildungen der Tange* is a work of 8 volumes describing collections loaned to him by "foreign friends," and its 8th volume (1858) emphasizes corallines. In this 8th volume, Kützing recognized eight varieties of *C. officinalis* in addition to *C. officinalis var. chilensis* from Chile, acknowledging the abundant intraspecific variation characteristic of corallines. The varieties were based on specimens from the North Sea, the Adriatic, and the Atlantic Ocean, and he attributed some of the variation to geographical location. Kützing considered *C. officinalis chilensis* (Fig. 6) to have been merely a Southern Hemisphere variety of *C. officinalis*. While some sources agreed with Kützing's reduction in rank from species to variety, not all sources accepted the updated name. Thus both names, *C. chilensis* and *C. officinalis var. chilensis*, co-occur in the literature from 1858 onwards (Yendo 1902A, 1902B, Setchell & Gardener 1903, Skottsberg 1923, Dawson 1953, Papenfuss 1964, Ramírez & Santelices 1991, Hind & Saunders 2013A, Williamson et al. 2015).

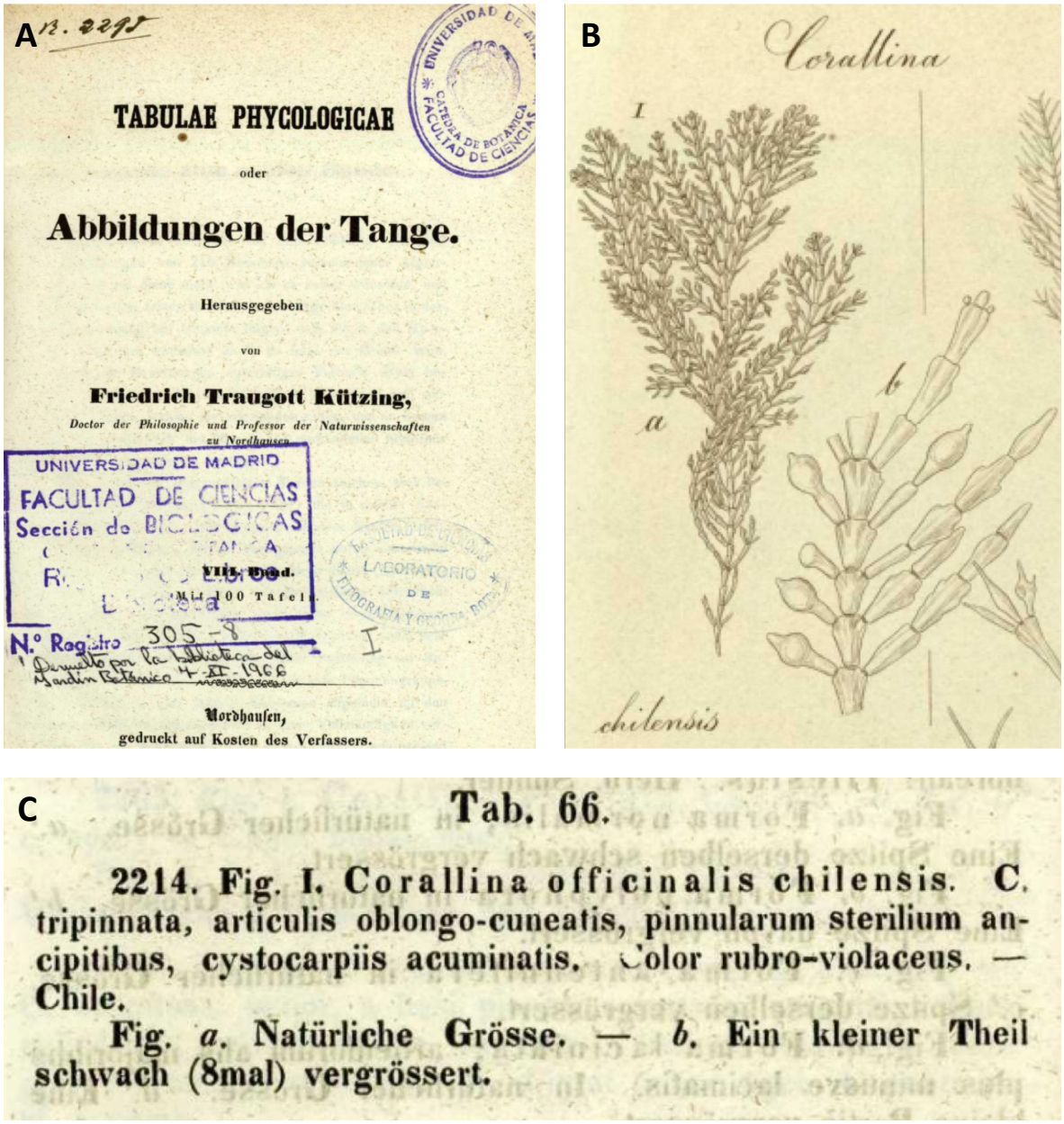


Figure 6. Screenshot of Kützing's 1858 description of *C. officinalis* [*var.*] *chilensis*, retrieved from AlgaeBase February 12, 2020. (A) Title page of publication "Illustrations of Seaweed." (B) Sketch accompanying description. (a) Normal size (b) A small piece enlarged ~ 8x. (C) Latin description accompanying illustration.

There have been numerous reports of *C. chilensis*/*C. officinalis var. chilensis* since the late 1800's, at first only in Chile. It was reported that *C. officinalis var. chilensis* was found in Magellanes province and Tierra del Fuego, Chile (Ardissone 1888), as well as in Bahia Orange,

Chile (Hariot 1889, Appendix I, Fig. S6). Then, in 1901, Kichisaburo Yendo, a newly graduated Japanese phycologist, traveled to Canada and observed and then subsequently described the corallines growing near the Minnesota Seaside Station near Port Renfrew, BC, Canada (Yendo 1902A, Zasshi 1921). He reported that *C. officinalis var. chilensis* was present, although rare, and observed that it tended to "assume very diverse forms when found at the margins of the pools, or between tidal marks" (Yendo 1902A, Appendix I, Fig. S7). He remarked that the specimens fit better with Kützing's illustration of *C. officinalis var. chilensis* than with Linnaeus' *C. officinalis* and that it was also similar to specimens from Hakodate, Japan (Yendo 1902A). He thus believed that this southern hemisphere variety was present as far north as Vancouver Island, British Columbia, Canada and Japan. Unfortunately, Yendo fell ill and died at age 46 (Zasshi 1921). Many of the articulated coralline species that he described and illustrated (Yendo 1902A, 1902B) have not been found, making it impossible to verify his identifications. [Interestingly, Yendo (1902A) refers to the specimens from Canada as *C. officinalis var. chilensis*, and Yendo (1902B) refers to the specimens from Japan as *C. chilensis*.]

Since Yendo's 1902 reports, *C. chilensis*/*C. officinalis var. chilensis* has been extensively reported across both hemispheres, using morpho-anatomy to identify specimens. Setchell and Gardner (1903) reported that *C. officinalis var. chilensis* was rare further north in the East Pacific, but that it was commonly found on the coast of California (Appendix I, Fig. S8). Foslie (1907) reported finding young *C. chilensis* specimens in the Beagle channel "infested with *Herposiphonia sullivana*," and in the Falklands, specifically Berkeley Sound, Port Louis (Appendix I, Fig. S9). Skottsberg (1923) likewise reported finding *C. chilensis* in tidepools in the Falklands, expressing that *C. chilensis* "quite possibly is only a form of *C. officinalis*, but further studies are necessary," and he also reported "feather-like" branching (Appendix I, Fig. S10).

Skottsberg (1923) also included Japan and Peru in addition to Northwest America, Chile, and the Falklands with respect to *C. chilensis*' distribution. Smith (1944) in his *Marine Algae of the Monterey Peninsula* reported that *Corallina chilensis* was "common everywhere" ranging from San Diego, California, north to Vancouver Island. He noted that it grew on rocks in the lower intertidal but was also found in tide pools higher in the intertidal, and that branches were in one plane and robust (Appendix I, Fig. S11).

Dawson (1953) provided a more detailed description of *C. officinalis var. chilensis* than any of the previous reports. He compared "common plants" from the Pacific Coast [assuming northern] with South American specimens and with *C. officinalis* specimens from all over the world noting that they all looked so similar, he did not think that Pacific American varieties were different species from the "classic" *C. officinalis* of the Northern Atlantic. The only difference he observed between North Atlantic *Corallina officinalis* and Pacific *C. officinalis var. chilensis* was the tendency of *C. officinalis var. chilensis* to be compound pinnate whereas North Atlantic *C. officinalis* was simple pinnate. Dawson (1953) also noted that *C. officinalis var. chilensis* grew "more abundantly" and "luxuriantly" in cooler waters over warmer waters, and that it commonly occurred all along the coast of Mexico, ranging from Isla Magdalena, Baja Mexico Sur, Mexico, north to British Columbia, Canada.

Papenfuss (1964) noted that *C. chilensis* was distributed in the Falklands in his catalogue of Antarctic and sub-Antarctic benthic marine algae. Interestingly, while using the name "*Corallina chilensis*" instead of "*Corallina officinalis var. chilensis*," Papenfuss also noted that Levring (1960) suspected that *C. chilensis* was not distinct from *C. officinalis*.

There are many reports of *C. chilensis* or *C. officinalis var. chilensis* having been collected from between southern Chile north through Lima, Peru between ~1900 and 1980

(Ramírez & Santelices 1991), as well as extensive reports from Mexico through British Columbia (Yendo 1902A, Setchell & Gardner 1903, Foslie 1907, Dawson 1953, Ramírez & Santelices 1991, Hind & Saunders 2013A).

Corallina officinalis var. chilensis has also been reported in South Africa (Silva et al. 1996). The reports of *C. chilensis* in the "arctic," seem to refer to specimens collected in the Falklands and Southern Chile (Foslie 1907, Skottsberg 1923, Papenfuss 1964, Ramírez & Santelices 1991).

While there exist a great number of reports indicating that the species commonly occurred within the range of southern Chile through Peru, and from Baja California through British Columbia, Canada, it is important to note that all these historical reports were made exclusively based on morpho-anatomical comparisons, not DNA sequences.

In summary, (1) *Corallina chilensis* was originally published as a species that was later reduced to a variety, *C. officinalis var. chilensis*. (2) Kützing considered *C. officinalis var. chilensis* to be a southern variety of Northern Hemisphere *C. officinalis* based on morphology. (3) Yendo and others reported that this southern variety was also in the Northern Hemisphere (including British Columbia).

1.1.2 Study objectives

The following study objectives were motivated from the historical context surrounding the name *C. officinalis var. chilensis*. (1) Is *C. chilensis* a distinct species or should it be considered a variety, *C. officinalis var. chilensis*? (2) Is *C. chilensis* distributed in both hemispheres in the East Pacific?

1.2 Materials and methods

1.2.1 Sampling

I used a diversity of samples identified as *Corallina*. For the molecular-based portion of my study, I examined 77 specimens that were collected between 2007 and 2019 from Western Canada, the United States, Chile, Japan, Taiwan, and China, representing putative *C. chilensis* and other species. Details including location for the specimens may be found in Appendix II. Care was taken to include as many different species across various geographic locations as possible (Bergsten et al. 2012). Two of the samples were collected from the Biobio region of central Chile in 2019 and were field identified as *C. officinalis var. chilensis*, and an additional specimen collected in Chile (NCU 656905, Playa Cocholgue, Appendix II) was contributed by Paul Gabrielson. In addition to the 77 specimens from our contemporary collections, I included three specimens in this study that were collected in the 1830's – 40's. These were the Darwin specimen designated by Harvey as the type specimen of *C. chilensis* (2151, Valparaiso, Appendix II, Table S1), as well as the field identified *C. chilensis* collections of Darwin's contemporaries Gay (Ancud, Chile, Appendix II, Table S1) and d'Orbigny (exact locality unknown, but thought to be Patagonia, see Appendix II, Table S1) which Decaisne may have used for his description of *C. chilensis* in the original name publication.

For the morphological-based portion of my study, I used 41 specimens collected between northern Oregon and northern British Columbia between 2007 and 2017 (Appendix II, Figure S10, Appendix III). Collections used for the morphological-based analysis overlapped with but were not identical to collections used for the molecular-based portion of this study.

1.2.2 DNA extraction, amplification, and sequence assembly

DNA was extracted following the red algal extraction protocol described in Hind et al. (2013A). Each ground sample was mixed with DNA extraction buffer, 10% Tween 20, and Proteinase K, and then treated using the Wizard® DNA extraction kit and eluted with fifty microliters of water or AE buffer.

Amplification targets were two chloroplast genes, *psb*A and *rbc*L and the mitochondrial COI (Cox1). These genes are commonly used for phylogenetic studies of coralline algae, and published DNA sequences are available for comparison (Hind et al. 2013A, Richards et al. 2017, Jeong et al. 2019, Twist et al. 2019). Including three genes can help to detect the issues that can complicate phylogenetic interpretation such as the retention of ancestral polymorphisms, hybridization, and incomplete lineage sorting that result in single gene trees inaccurately reflecting the speciation process (Leliaert et al. 2014). Amplification and one directional sequencing using either *psb*AF1 or *psb*AR2 was attempted for each DNA template to determine the success of extraction and for the purposes of confirming field identifications (Table 1). Templates of interest that were successfully sequenced in one direction using the *psb*A marker and were relatively free of contamination were then sequenced in forward and reverse directions for *psb*A, CO1, and *rbc*L genes (Table 1).

Table 1. Table of primer names, sequences, and sources for primers used in this analysis.

Gene	Primer	Direction	Primer sequence	Source
psbA	psbAF1	Forward	5' ATG ACT GCT ACT TTA GAA AGA CG 3'	Yoon et al. 2002
	psbAR2	Reverse	5' TCA TGC ATW ACT TCC ATA CCT A 3'	Yoon et al. 2002
CO1	GWSFn	Forward	5' TCA ACA AAY CAY AAA GAT ATY GG 3'	Le Gall & Saunders 2010
	GWSRx	Reverse	5' ACT TCT GGR TGI CCR AAR AAY CA 3'	Clarkston & Saunders 2012
rbcL	F57	Forward	5' GTA ATT CCA TAT GCT AAA ATG GG 3'	Freshwater & Rueness 1994
	R1150K	Reverse	5' GCA TTT GAC CAC AAT GGA TAC 3'	Lindstrom et al. 2015
	F753	Forward	5' GGA AGA TAT GTA TGA AAG AGC 3'	Freshwater & Rueness 1994
	rbcLrevNEW	Reverse	5' ACA TTT GCT GTT GGA GTY TC 3'	Kucera & Saunders 2012

In preparation for PCR, DNA extract concentration was measured using a NanoDrop 8000 (Thermo Fisher Scientific, Wilmington, DE, United States), and diluted to a concentration of 40 to 80 ng/ml.

A master mix was prepared fresh as needed for each DNA template to be amplified plus enough excess for one positive control and one negative control. Each reaction contained 13.16 µl sterile water, 2.0 µl 10X Buffer (included with Taq), 1.6 µl 25 mM $MgCl_2$, 1.6 µl 2.5 mM dNTPs, 0.28 µl 10 µM forward primer, 0.28 µl 10 µM reverse primer, and 0.09 µl 5U/µl Taq DNA polymerase (Invitrogen). For templates that failed to amplify, troubleshooting was attempted using puReTaq™ Ready-To-Go™ PCR beads following the manufacturer's protocol.

PCR product was verified via gel electrophoresis on 1.0% agarose gels stained with 3.5 µl SYBR-Safe dye. (See Appendix IV, Table S6 for amplification thermal cycler conditions.) Successful amplification materials were stored in the -20°C freezer. Non-purified PCR product was sequenced by the McGill University and Genome Quebec Innovation Centre.

Chromatograms were imported into Geneious 7.1.9 (Biomatters Ltd., Auckland, New Zealand). Raw ends were trimmed to about 950 base pairs (bp) for *psb*A forward and reverse, 650-700 bp for CO1 forward and reverse, and 600-900 bp or 800-950 bp for *rbc*L. Forward and

reverse sequences for each specimen were aligned, edited by eye and assembled into contigs, which were corrected manually to close gaps that had been inserted by single erroneous bases which tended to occur towards the ends of sequences, and to resolve ambiguous bases in highly conserved regions of the alignments. *psb*A sequences were 877 bp long, CO1 sequences were 680 bp long, and the majority of the *rbc*L sequences were 1334 bp long.

Jeffery Hughey (Department of Biology, Hartnell College) extracted and amplified DNA from the three 1800's herbarium specimens (Appendix II, Table S1) following Hughey et al. 2001, as modified by Gabrielson et al. (2011), following recommendations by Hughey and Gabrielson 2012 and Saunders and McDevit 2012. A small portion of the *rbc*L gene was targeted using the F1152cor (Gabrielson et al. 2011) and R-*rbc*S (Freshwater and Rueness 1994) primers to produce 263 bp sequences. The *Corallina chilensis* type specimen (Darwin #2151), as well as specimens collected by Gay (Ancud specimen) and d'Orbigny, were sequenced in this way (Appendix II, Table S1).

Supplemental published sequences (See Appendix II, Table S1) were selected from the literature and retrieved from GenBank. These specific DNA sequences were chosen for the purpose of supplying outgroups (Appendix II, Table S2), confirming species identities, and maintaining consistency of species representation. Additional published sequences that were not included in the molecular analyses were used for confirming the contemporary range of *Corallina chilensis* (Appendix II, Table S3).

1.2.3 Sequence alignment & phylogenetic analysis

Three individual gene trees, a separate *rbc*L gene tree containing short DNA sequences from the 1800's herbarium materials including the Darwin *C. chilensis* type, a majority rule tree, and a concatenated gene tree were generated during the phylogenetic analyses.

Individual gene trees & rbcL type tree

Edited *Corallina* sequences were aligned with published sequences including outgroups (Appendix II, Table S1). Sequences were placed in single-locus alignments using Geneious Prime® 2019.2.3, build 2019-09-24 (Biomatters Ltd., Auckland, New Zealand). The *psb*A alignment was composed of 91 sequences including outgroups, and was 851 aligned sites long. The CO1 alignment consisted of 63 sequences including outgroups, and was 660 aligned sites long. The *rbc*L alignment consisted of 47 sequences including outgroups, and was 1334 aligned sites long. The *rbc*L type alignment consisted of the same 47 sequences in the *rbc*L that were 1334 aligned sites long, along with 3 short 1800's herbarium sequences of 263 aligned sites long.

Maximum likelihood trees were created in IQ-tree 1.6.12 for MacOSx (Nguyen et al. 2014) for each gene. Sequences were partitioned by codon position. Models of sequence evolution for each locus were estimated under Bayesian Information Criterion (BIC) utilizing ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ tree (see table of evolution models implemented, Appendix IV, Table S7). Internal node robustness was assessed in IQ tree by 1,000 maximum likelihood bootstrap replicates and by approximate Likelihood Ratio Tests (aLRT) based Shimodaira-Hasegawa-like procedures (Anisimova 2006). MrBayes (Ronquist et al. 2011) was used to run Bayesian analyses on the three individual gene alignments. Since MrBayes has fewer sequence evolution models available than IQ-tree, ModelFinder in IQ-tree

was re-run on each partitioned dataset to determine the optimal sequence evolution models within the MrBayes available subset. (See Appendix IV, Table S7 for evolution models.) Two independent analyses were run on each partitioned dataset with four independent chains. Analyses ran for 4 million generations, sampled every 1,000 generations. The first 10% of the trees were discarded as burn-in, and trees from subsequent generations were saved because the log-likelihoods had plateaued after that point and estimated sample sizes of parameter values exceeded 200 when viewed in Tracer v1.7.1 (Rambaut et al. 2018). Trees were visualized using FigTree v1.4.4 (Rambaut 2018), and maximum likelihood bootstrap values and aLRT values were superimposed on the Bayesian tree topology.

Majority rule tree & concatenated trees

Individual gene trees revealed congruent species-level clades but unresolved, confusing, and incongruent relationships among species. I performed two additional phylogenetic analyses to evaluate congruence and incongruence among single gene trees (Maddison 1997, Mossel & Vigoda 2005, Liu & Pearl 2007). The first involved comparing clades appearing in majority rule bootstrap consensus trees from each locus. For each locus, 1000 bootstrap trees were generated in RAxMLGUI 1.5 beta (Silvestro & Michalak, 2012), using all PTM and published sequences listed in Appendix II, Tables S1 & S2, except for PTM 826, the 1800's herbarium materials, or as otherwise noted in Table S1. For each locus, a 50% majority rule consensus tree was created from the 1000 bootstrap trees using PAUP Version 4.0a, build 167 (Sunderland, Massachusetts, USA; Swofford 2002). A final majority rule consensus tree was then created in PAUP from the three majority rule individual gene consensus trees.

A second phylogenetic analysis explored the incongruence among sequences from different loci from *Corallina officinalis* within a tree from a concatenated alignment. The alignment, created in Geneious, included the concatenated gene sequences available from each voucher listed in Appendix II, Table S2. For *Corallina officinalis*, each *psb*A, *rbc*L and CO1 sequence was added as a separate operational taxonomic unit, without concatenating different genes from the same voucher. To confirm that the manual alignment using Geneious was not responsible for incongruences among loci, all concatenated sequences were realigned in MAFFT version 7 (Katoh 2013). The alignment was partitioned by codon and GTR gamma + I substitution model was used in creating the concatenated tree.

The concatenated gene tree was the most likely from 200 replicated searches in RAxML-HPC2 on XSEDE through the CIPRES Science Gateway V 3.3. (Miller et al. 2010). The alignment was analyzed in RAxMLGUI to produce 1000 bootstrap trees and bootstrap percentages which were then overlaid on the most likely tree.

1.2.4 Other analyses supporting species delimitation

Automatic Barcode Gap Discovery (ABGD) for delimitation of candidate species (Puillandre et al. 2011) was applied to the *psb*A, CO1, and *rbc*L alignments. The ABGD barcoding analysis may only be performed on single gene alignments, so there was no analysis of the concatenated alignment. ABGD was run with P-min set to 0.001 and P-max set to 0.1, steps set to 10, Nb bins set to 20, X relative gap width equal to 1.5, and the Jukes-Candor (J669) option selected. Output partitions were chosen based on how well they fit to currently recognized *Corallina* species delimitations, prioritizing partitions that grouped *C. vancouveriensis* as a distinct species from *C. officinalis* (Hind & Saunders 2013A).

Uncorrected pairwise percent differences between sequences were generated in Geneious Prime® 2019.2.3. For the purpose of maintaining consistency between these results and other studies, genetic distances throughout the methods, discussion, and future directions portion of this thesis were compared with distances in Hind et al. 2018 and references therein (Broom et al. 2008, Hind and Saunders 2013A, Nelson et al. 2015, Hind et al. 2016, Hind et al. 2018). These distances were 0.7-1.3 % difference between species in *psb*A, 4.5-5.8% difference between species in CO1, and 1.6-1.9% difference between species in *rbc*L (Hind et al. 2018). These percent differences differed from earlier papers with lower thresholds that will also appear later in the discussion.

Three DNA sequences corresponding with specimens that were interesting because of their geographical origin and that were thought to have been *Corallina chilensis* were contributed too late to be included in my phylogenetic analysis. Two of the specimens were collected in 2019 (Biobio, Chile) and identified in the field as *C. officinalis var. chilensis*, and one was a recent collection from Chile contributed by Paul Gabrielson. I tested their identity based on previous sequencing results and by comparison to sequences in GenBank (Appendix II, Table S3). I considered that specimens might be conspecific if their sequences shared at least 75% coverage and were at least 98% similar for *psb*A, 98.5% similar for CO1, and 99% similar for *rbc*L.

1.2.5 Morphometric analysis

Morphology of Corallina sp. 1 frondescens collections from British Columbia was analyzed because BLAST searches with preliminary DNA sequence data suggested that they might be conspecific with *C. chilensis* (Appendix III). A morphological analysis of Northeast Pacific *C. vancouveriensis* also appears in this chapter because *C. vancouveriensis* grows

abundantly along-side C. sp. 1 frondescens across its entire Northeast Pacific range. Appendix III lists the specimens used for this analysis.

The majority of C. sp. 1 frondescens specimens had multiple fronds per specimen, so one individual frond was selected arbitrarily from each specimen for measurement. Measurements included in the morphometric analysis are summarized in Fig. 7. Height and maximum width of each frond were measured, as was length of the crown and length of the stem (Fig. 7A-C). The crown was defined as the branching upper portion of the main axis with branches consisting of more than one intergeniculum per branch. (Basal branchlets, only one intergeniculum long were discounted.) The stem was defined as the region of the main axis starting from the most basal unbranched intergeniculum to the branch inflection point (Fig. 7C). On the same frond, the length of a secondary pinnate branch off the main axis was arbitrarily selected for measurement (Fig. 7D). Intergenicular dimensions were measured on haphazardly chosen mid-intergenicula from randomly selected main axes and secondary branches for each collection (Fig. 7D-F). Arbitrarily selected basal intergenicula from the main axes were measured (Fig. 7G) as well as conceptacle branches (including the subtending intergenicula) when present in the sample (Fig. 7H). Because it was often unclear if smaller fronds were branches from larger fronds within the same sample or if they were independent individuals, the frond length and width at the widest point was also measured from the tallest frond of each specimen to ensure that maximum height and width of frond data would not be deflated.

Photographs were taken of six representative C. sp. 1 frondescens (Appendix II, Tables S1 & S3) specimens from British Columbia. Two were collected from Botany Beach, Vancouver Island, and the other four are herbarium specimens collected from the Hakai conservancy on Calvert Island.

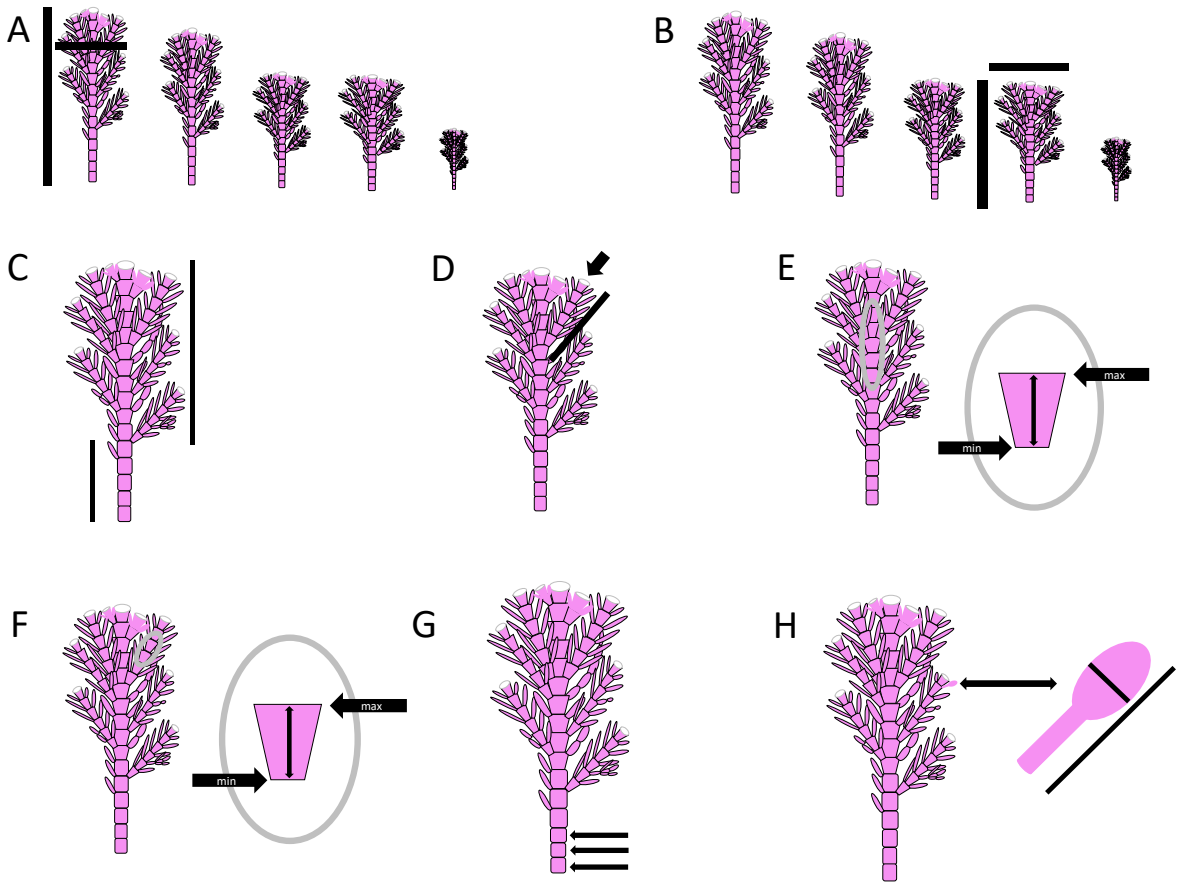


Figure 7. Summary of measurements taken for morphometric analysis. Generic *Corallina* illustrations modeled after C. sp. 1 frondescens not drawn to scale. (A) Tallest frond length and width. (B) Random frond length and width. (C) Crown length and stem length. (D) Secondary branch length. (E) Main axis mid intergenicular dimensions. (F) Secondary branch mid intergenicular dimensions. (G) Basal intergenicular dimensions on main axis. (H) Conceptacle branch dimensions. Length includes subtending intergeniculum and bulbous head.

1.3 Results

Table 2 shows the preliminary Automatic Barcode Gap Discovery (ABGD) species delimitation and the following figures (Figs. 8-22) include all phylogenetic trees described in the methods (see Methods 1.2.3). Figures 8-9 show an *rbc*L gene tree that includes sequences from mid-1800's herbarium specimens. The next three phylogenies (Figs. 10-15) are individual *psb*A (Figs. 10-11), CO1 (Figs. 12-13), and *rbc*L (Figs. 14-15) trees that do not contain sequences from the 1800's herbarium material. The individual gene trees are followed by the majority rule tree (Figs. 16-19) illustrating the disagreement across all three individual gene trees. The final tree is the concatenated tree (Figs. 20-22). The majority rule tree (and the concatenated tree likewise do not contain any sequences from 1800's herbarium material.

Table 2. Side by side results of three ABGD barcoding analyses performed on *psb*A, CO1, and *rbc*L single gene alignments. Boxes indicate species as determined by each analysis. The *psb*A analysis identified 13 species, the CO1 analysis 23 species, and the *rbc*L analysis 16 species.

psbA analysis	CO1 analysis	rbcL analysis
C. officinalis	*C. officinalis*	*C. officinalis*
C. sp. 1 california	C. sp. 1 california	C. sp. 1 california
C. chilensis	*C. chilensis*	*C. chilensis*
C. sp. 2 frondescens	C. sp. 2 frondescens	C. sp. 5 frondescens
C. sp. 5 frondscens	C. sp. 5 fondescens	C. sp. 2 frondescens
C. sp. 3 frondescens	C. sp. 3 frondescens	C. sp. 3 frondescens
C. sp. 3 frondescens-like	C. sp. 3 frondescens, PTM1400	C. sp. 3 frondescens-like
C. sp. 2 vancouveriensis	C. sp. 3 frondescens-like	C. sp. 1 gws
C. maxima	C. sp. 2 vancouveriensis	C. sp. 1 gws-like
C. sp. 1 gws	*C. maxima*	C. ferreyrae-like
C. sp. 1 gws-like	C. sp. 1 gws	*C. vancouveriensis*
C. ferreyrae-like	C. sp. 1 gws-like	C. sp. 1 chile
C. declinata	C. ferreyrae-like	C. sp. 4 frondescens
C. aberrans	*C. declinata*	*C. ferreyrae* (Bustamante)
C. vancouveriensis	*C. aberrans*	*C. ferreyrae* (PTM826 only)
C. sp. 1 chile	*C. vancouveriensis*	C. sp. 2 chile
C. sp. 4 frondescens	C. sp. 1 chile	C. sp. 2 chile
C. ferreyrae (Bustamante)	C. sp. 4 frondescens	*C. pinnatifolia*
C. ferreyrae (PTM)	C. sp. 4 frondescens	*C. crassissima*
C. sp. 2 chile	*C. ferreyrae* (PTM 826 only)	*C. aberrans*
C. crassissima	*C. ferreyrae* (Bustamante)	*C. melobesioides*
	C. ferreyrae (PTM 819 only)	
	C. sp. 2 chile	
	C. caespitosa holotype	
	C. crassissima	
	C. sp. 5 korea	

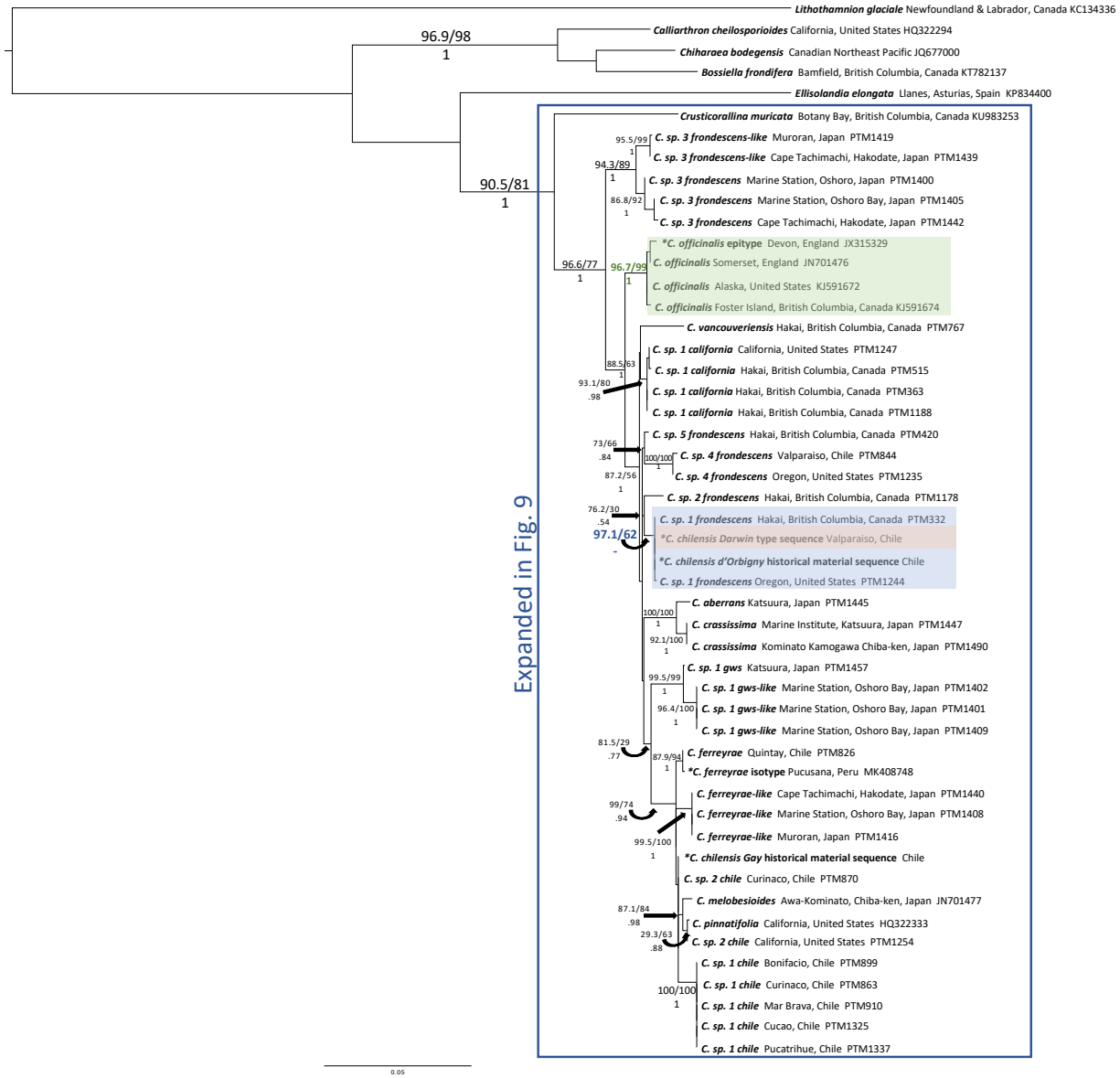


Figure 8. *rbc*L type tree. Entire phylogenetic *rbc*L tree including *Corallina chilensis* type specimen collected by Darwin. Asterisks designate 263 bp sequences of herbarium material from the 1800's, from the type and from specimens from d'Orbigny and Gay *C. chilensis;* and type specimens included in this tree. Top two branch support values are aLRT/maximum likelihood bootstrap percentages. The bottom value is the Bayesian posterior probability. The scale bar refers to substitutions per site and the blue box indicates the portion of the tree that is expanded in Fig. 9.

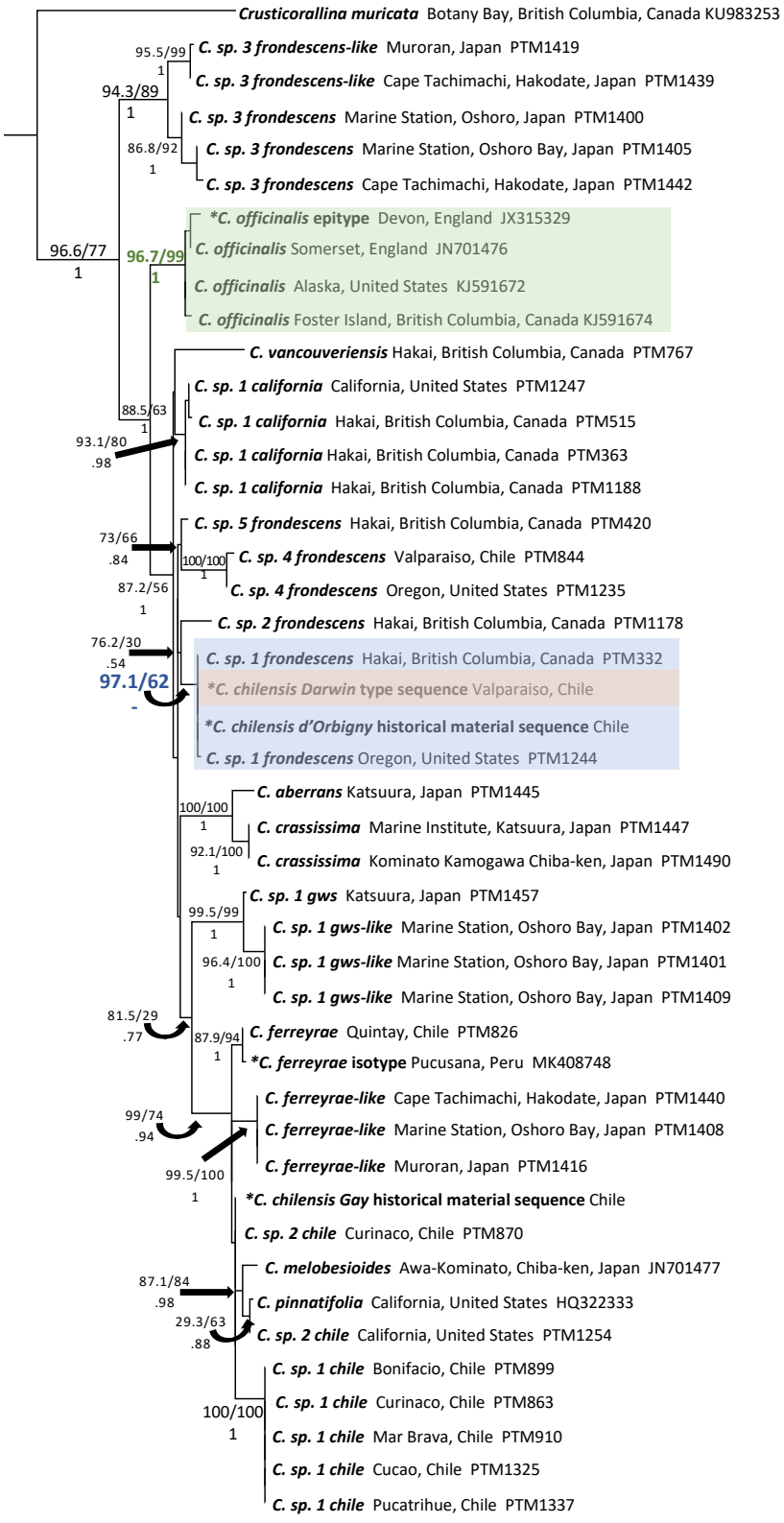


Figure 9. Expanded portion of the *rbc*L type tree from Fig. 8.

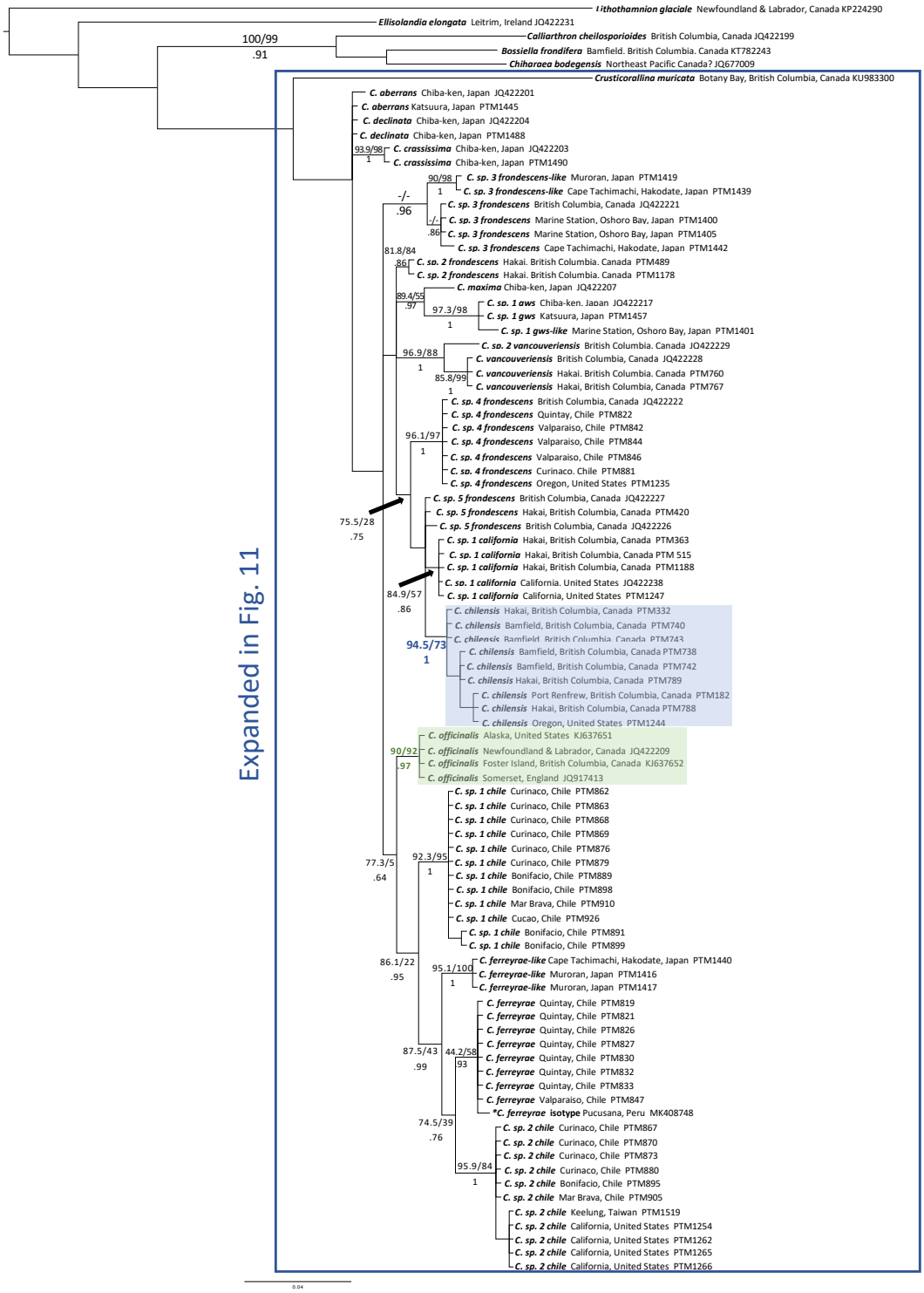


Figure 10. Entire phylogenetic tree of 91 *psb*A sequences from *Corallina* specimens and six outgroups. The top two branch support values are aLRT/maximum likelihood bootstrap percentages. The bottom number is the Bayesian posterior probability. The scale bar indicates substitutions per site, and the blue box is the portion of the tree expanded in Fig. 16. Asterisks denote type sequences.

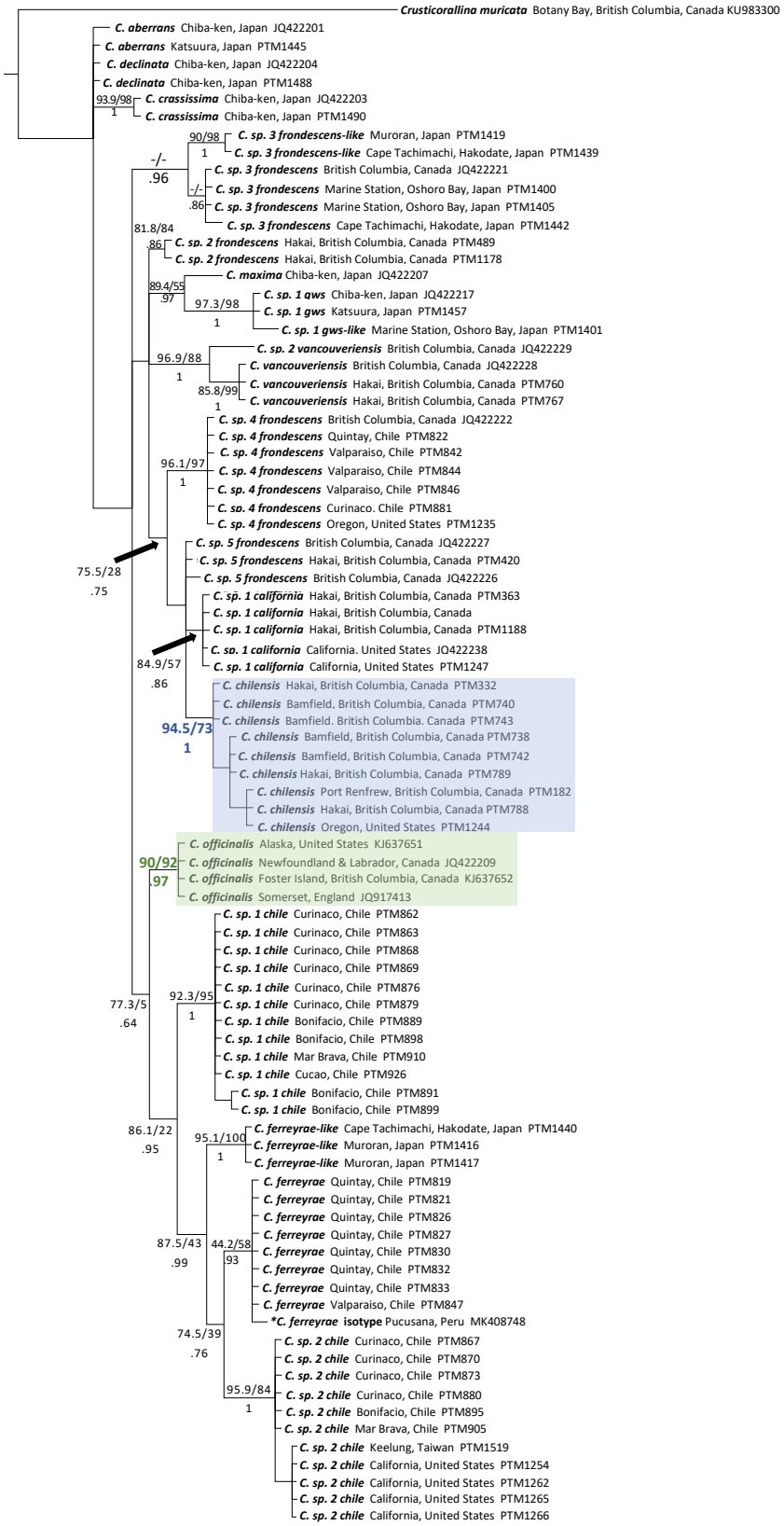


Figure 11. This has been expanded from the *psb*A tree in Fig. 10

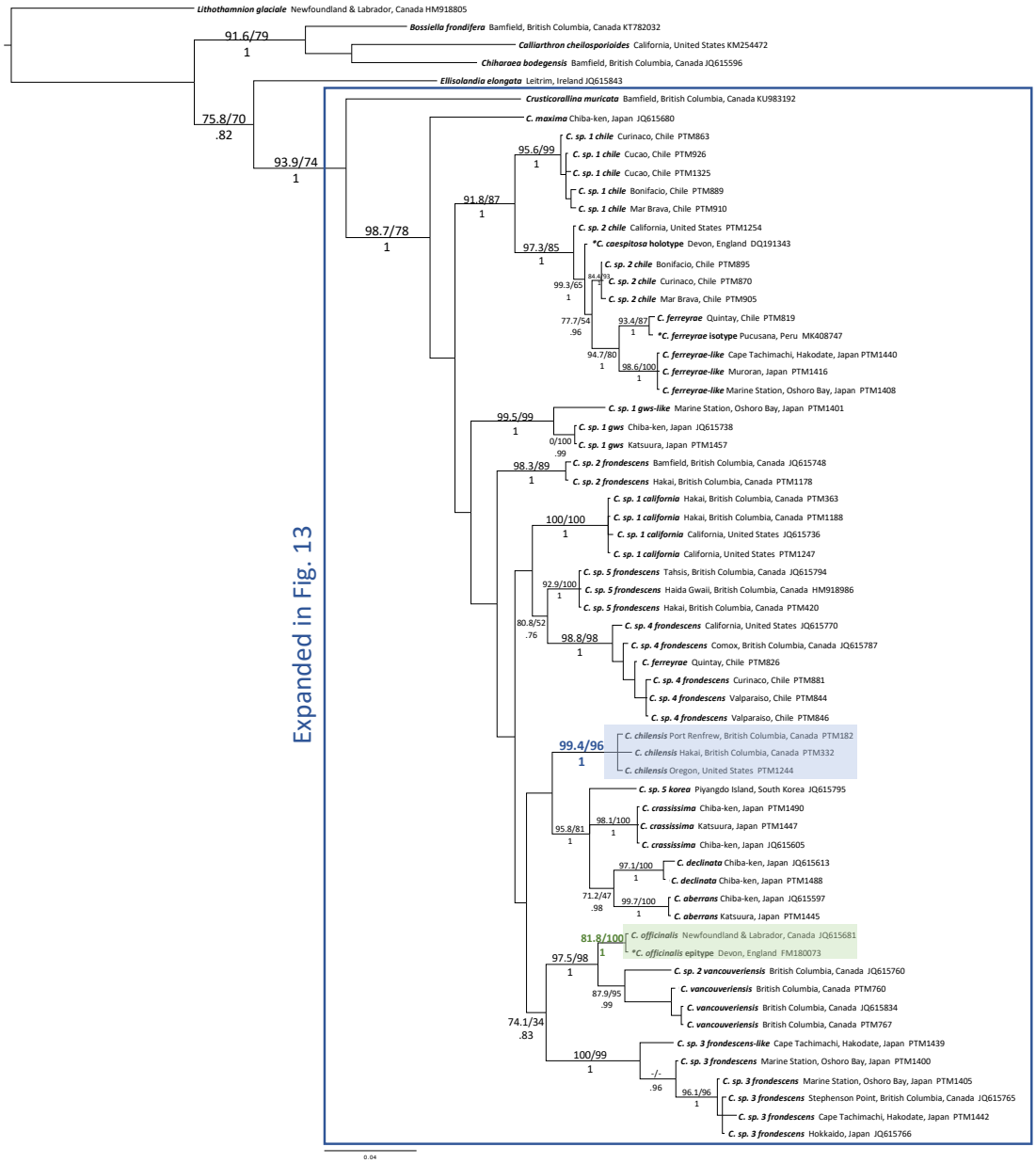


Figure 12. Entire phylogenetic tree of the *Corallina* genus consisting of 63 CO1 sequences including six outgroups. The top two branch support values are aLRT/Maximum Likelihood percentages (1000 bootstraps). The bottom number is the Bayesian posterior probability. The blue box indicates the portion of the tree that is expanded in Fig. 13. Asterisks denote type sequences.

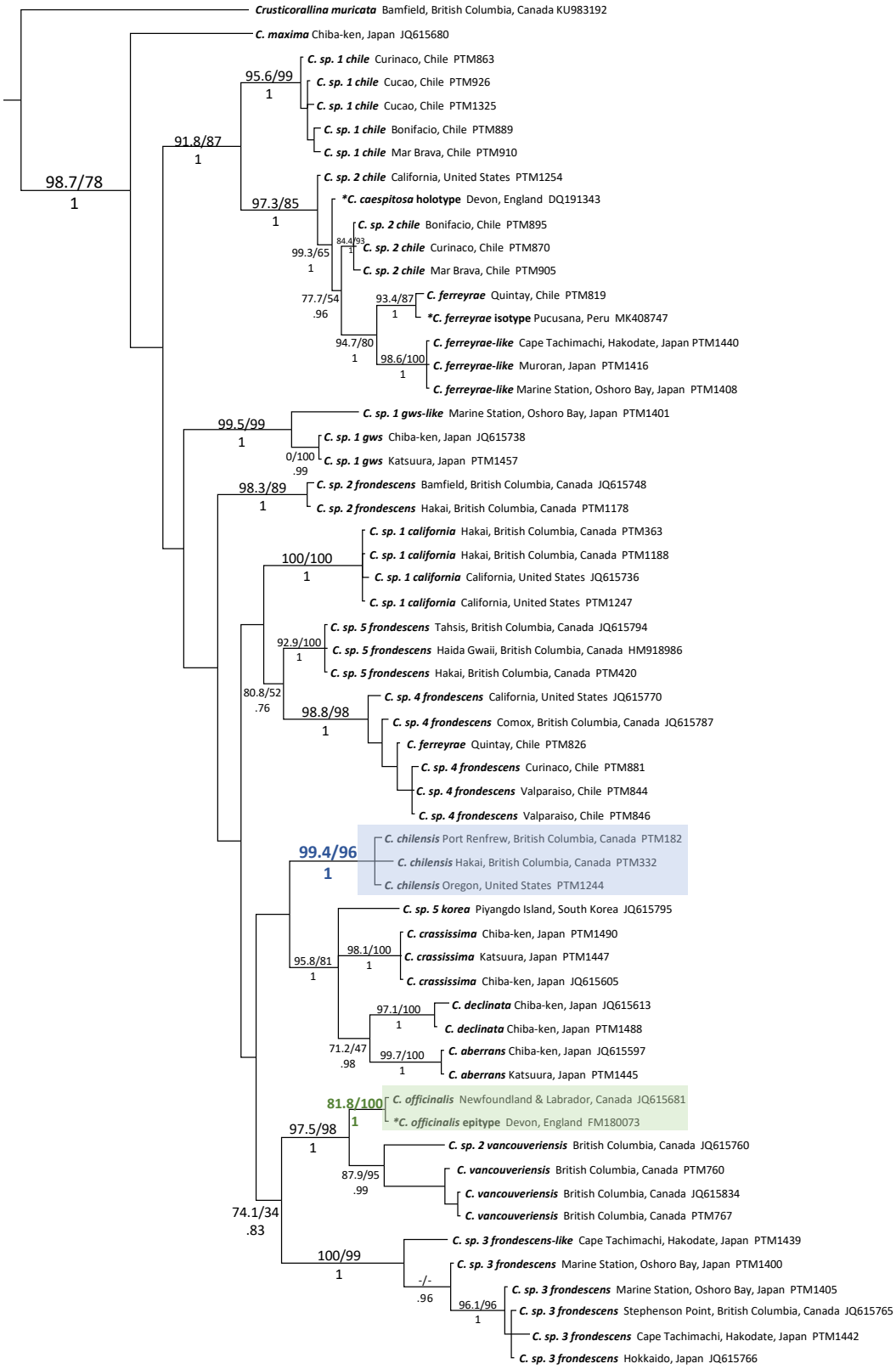


Figure 13. Expanded portion of the CO1 tree from Fig. 12.

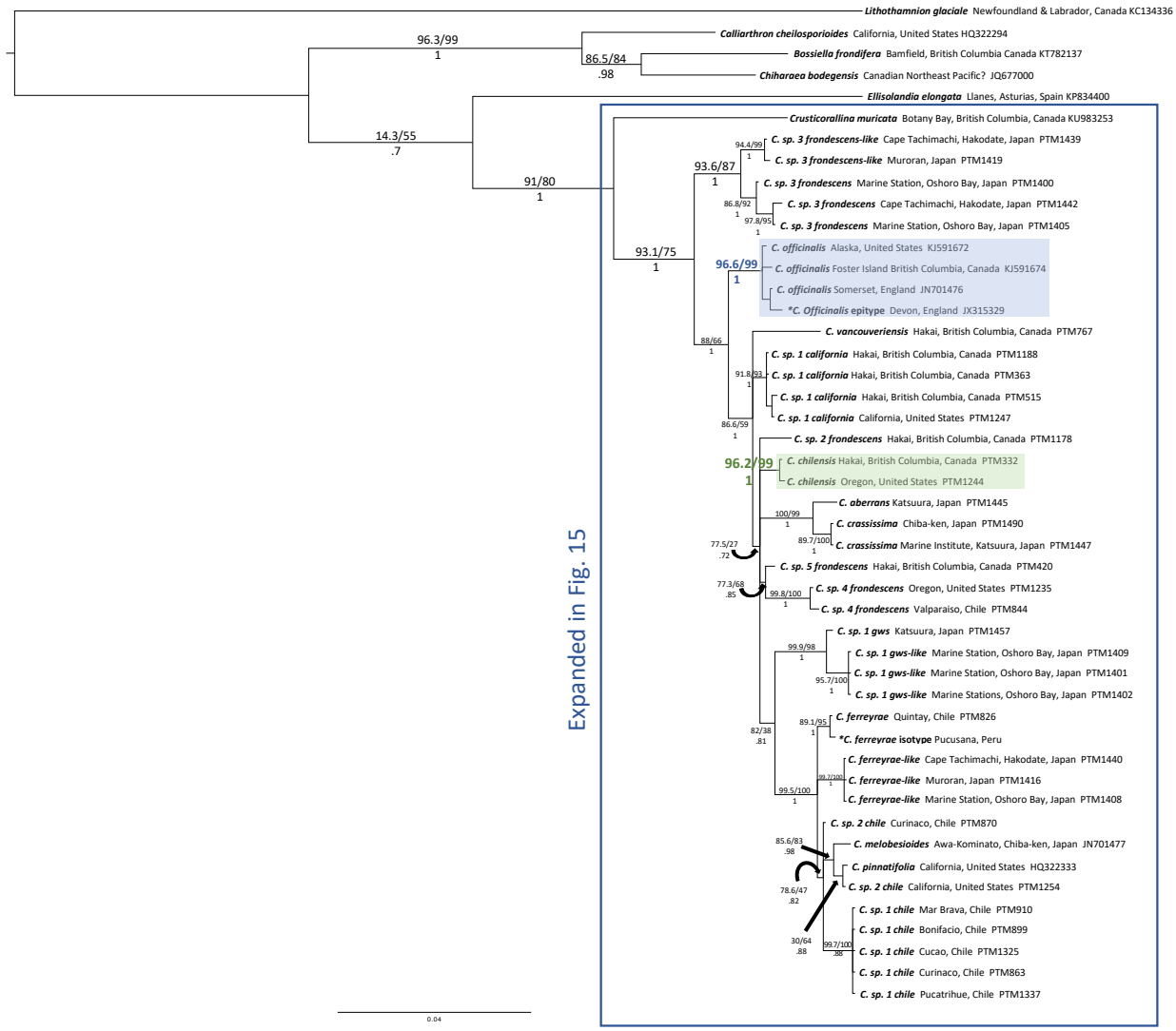


Figure 14. Entire phylogenetic tree of 47 *rbc*L sequences of *Corallina* and six outgroups. Sequences from the herbarium materials from the 1800's are not included in this tree. The top two branch support values are aLRT/Maximum Likelihood percentages (1000 bootstraps); the bottom number is the Bayesian posterior probability. The blue box indicates the portion of the tree that is expanded in Fig. 15. Asterisks denote type sequences.

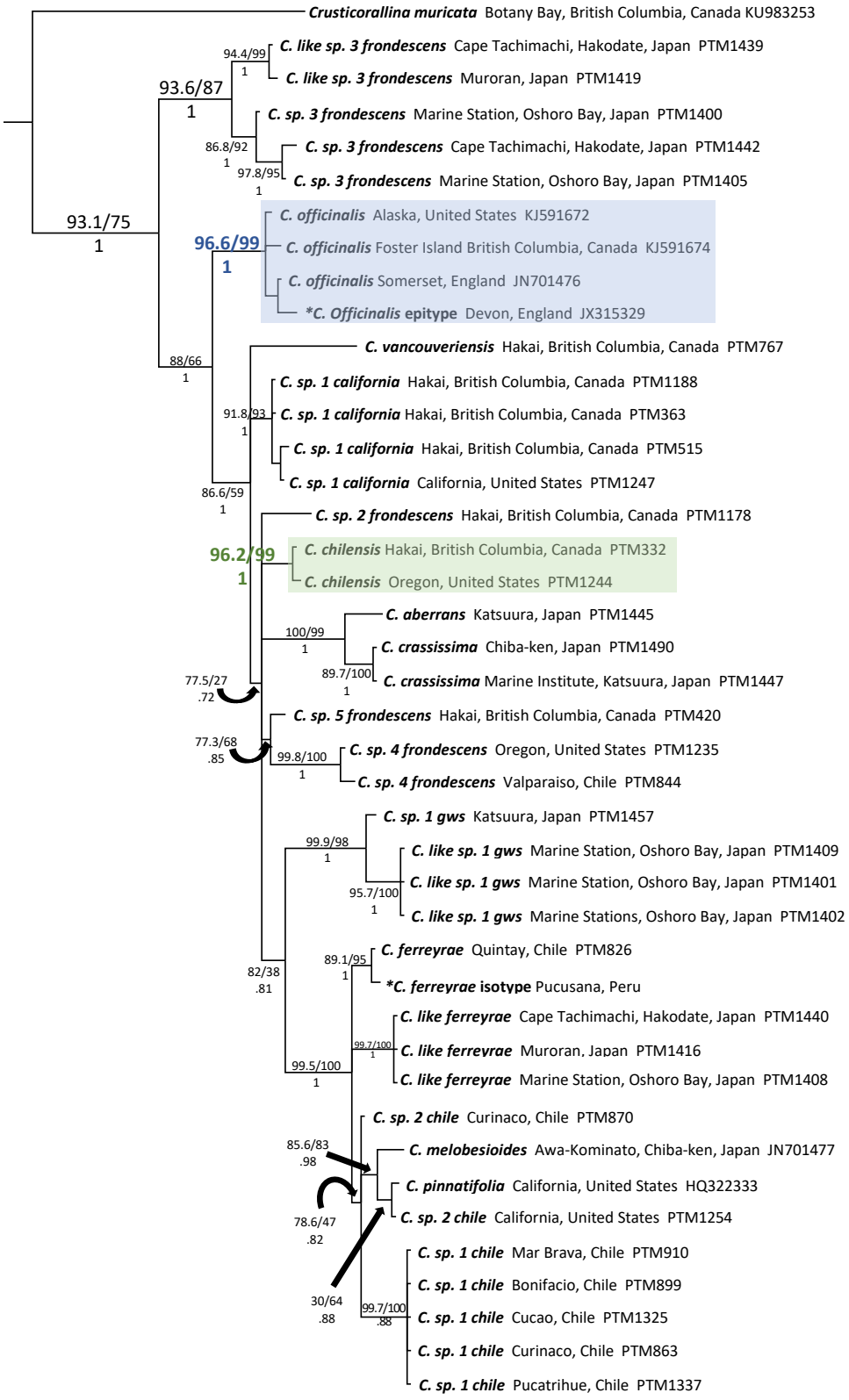


Figure 15. Expanded portion of the *rbc*L tree from Fig. 14.

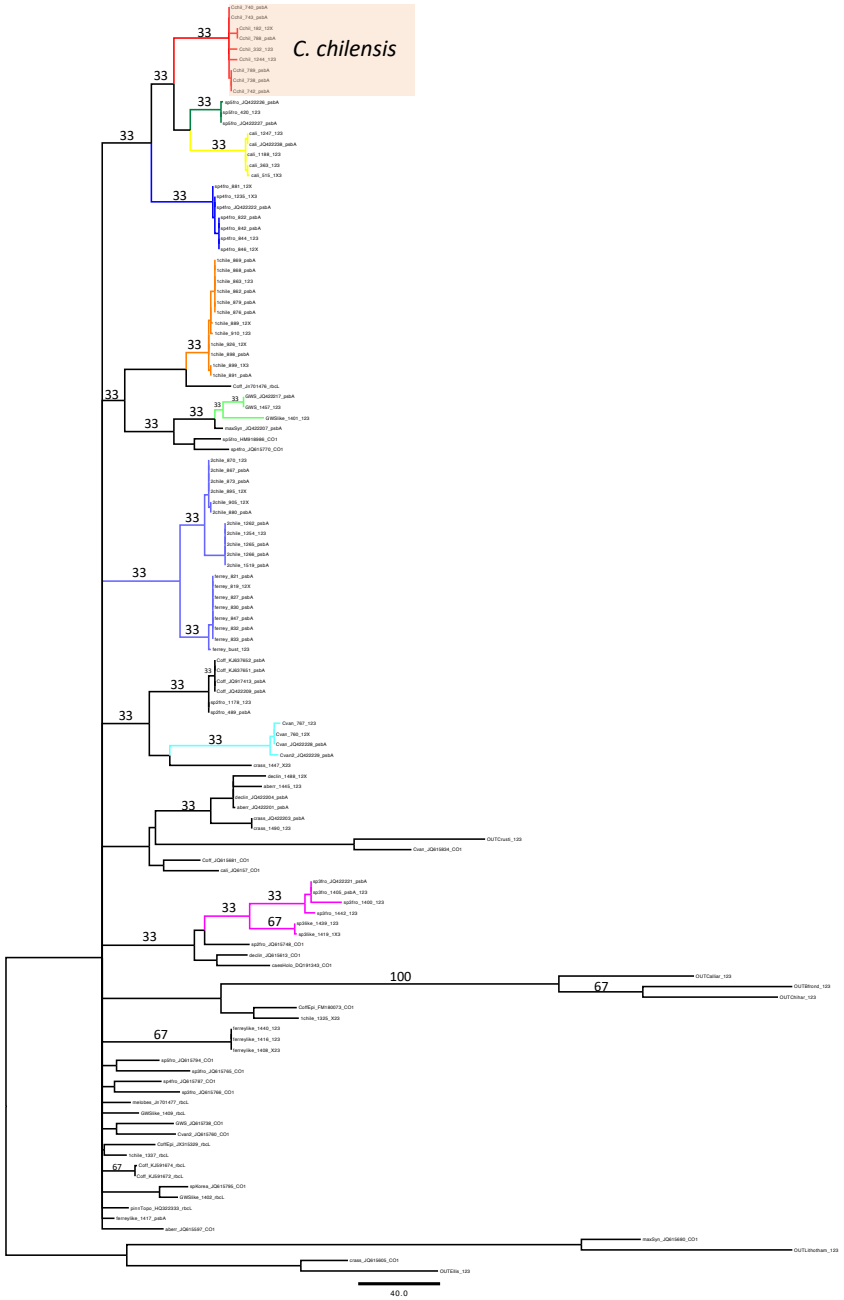


Figure 16. This *Corallina* 50% majority rule tree shows species-level clades that are shared across separate bootstrap consensus trees from *psb*A, CO1, and *rbc*L gene sequences. Percentages indicate whether the group was present in one (33%), two (67%), or three (100%) of the individual gene trees. The polytomies and instances of 33% support are due to conflict across loci, to limitations to the phylogenetic resolution possible from each locus, and to missing data from some loci for some taxa. Short or nonsense branches are relics of having had no data associated with taxa during intermediary steps in the analysis and should be disregarded. Relationships among species are not resolved consistently across the three loci.

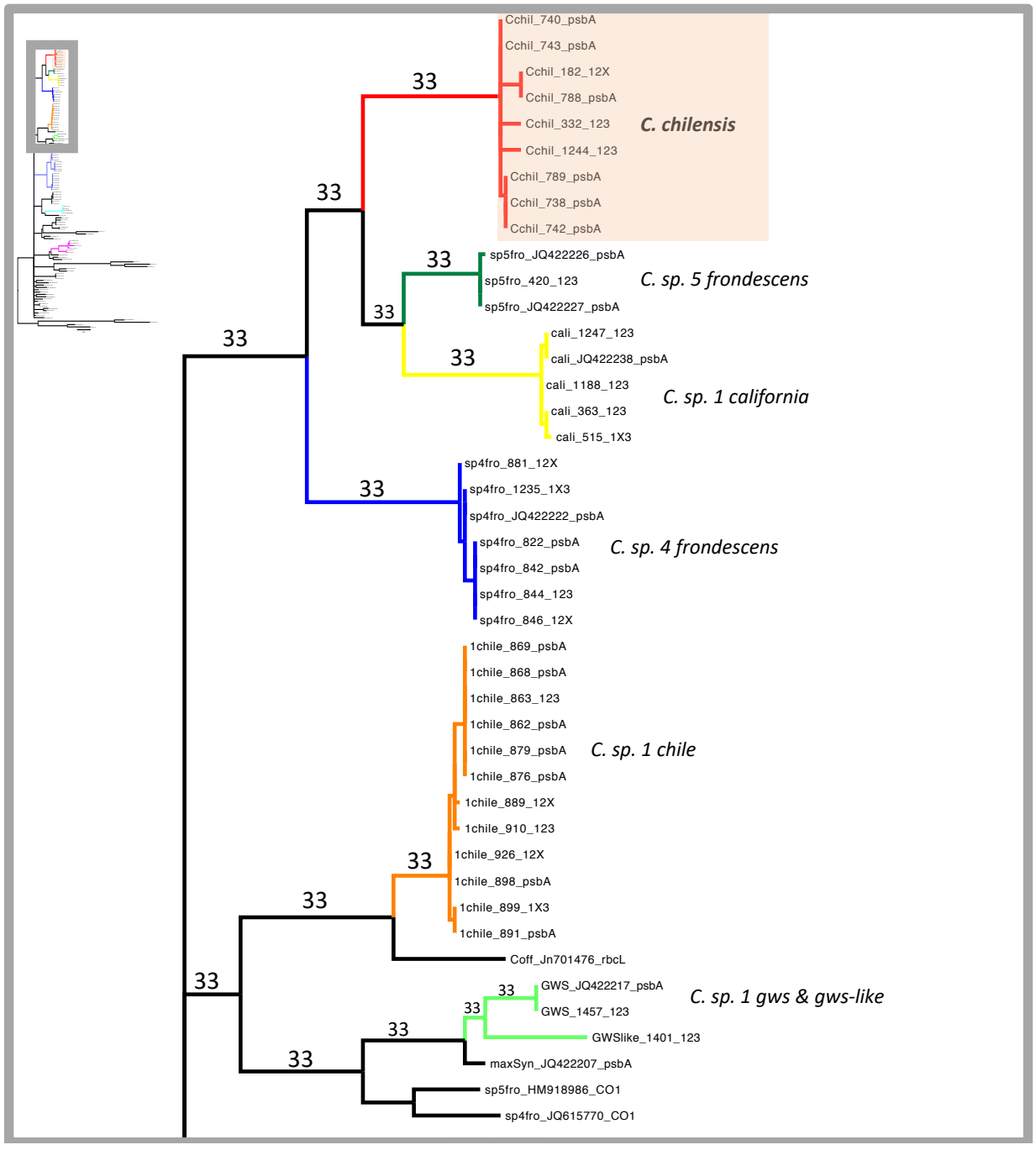


Figure 17. Expanded from Fig. 16.

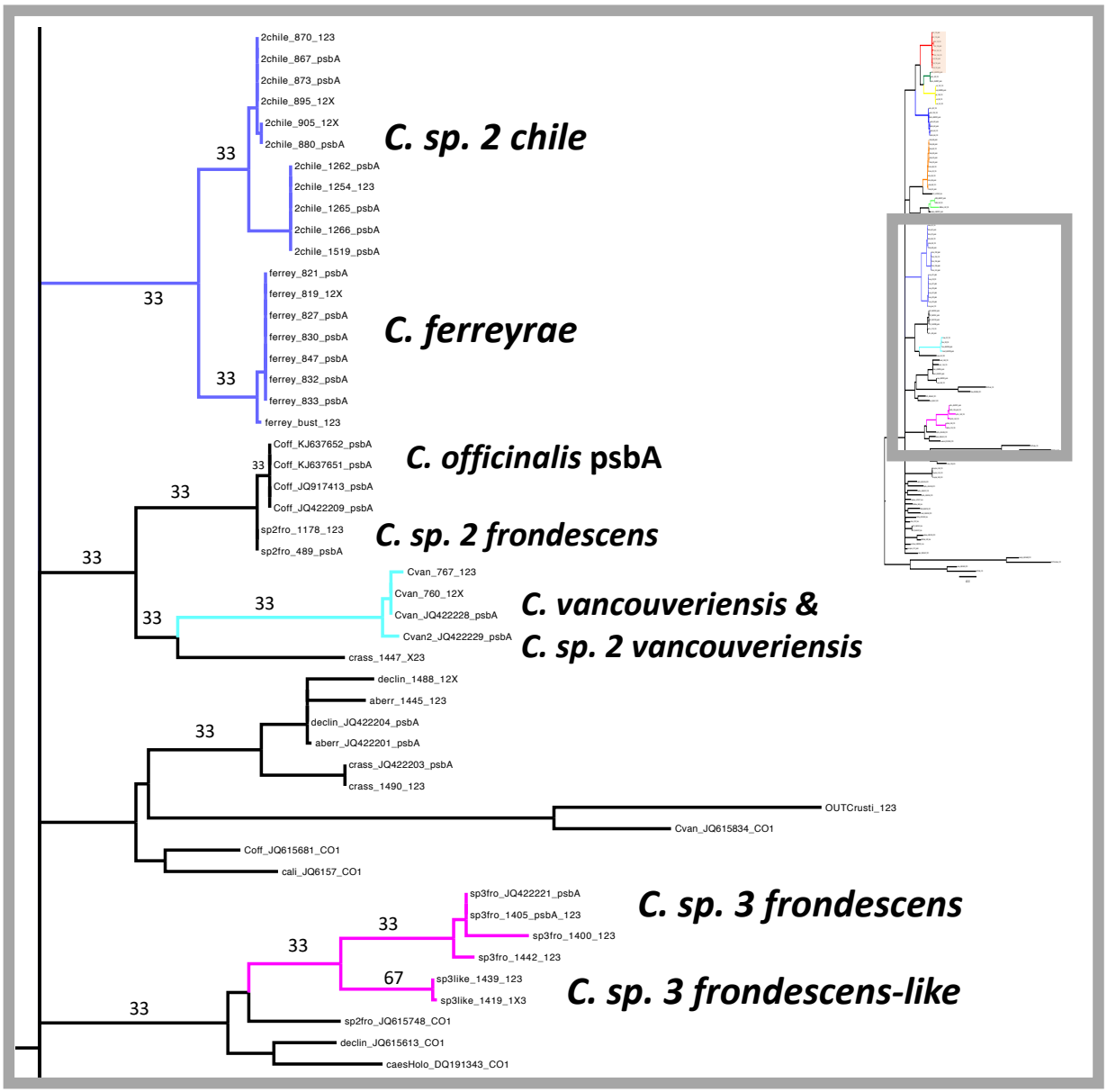


Figure 18. Expanded from Fig. 16.

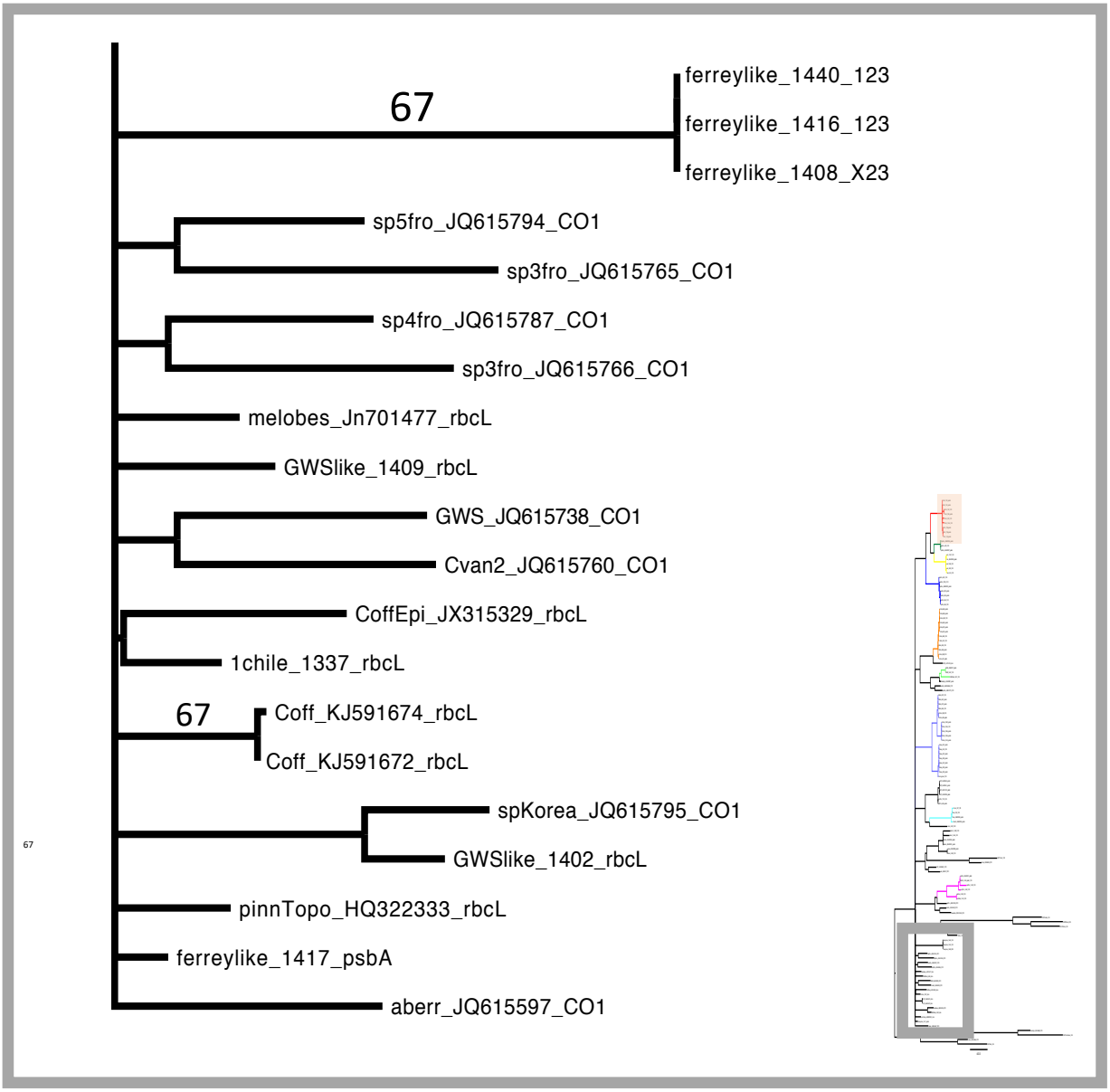


Figure 19. Expanded from Fig. 16.

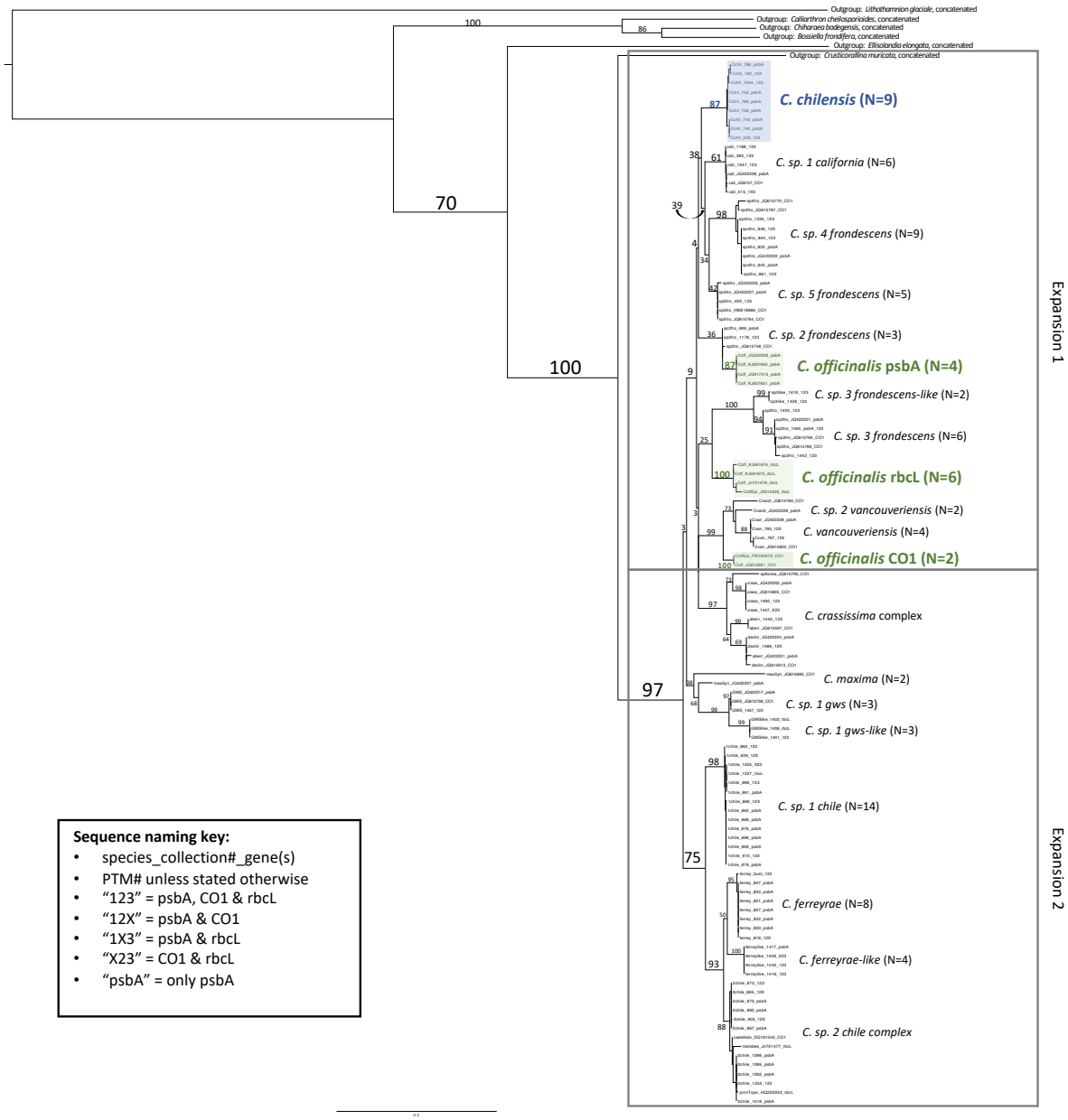


Figure 20. Indicating phylogenetic conflict among loci, individual gene sequences of *C. officinalis* appear in three locations in a maximum likelihood tree in which other taxa are represented by concatenated *psb*A/CO1/*rbc*L sequences. Numbers are bootstrap percentages. Outgroups are concatenated as specified in Table S3, Appendix II.

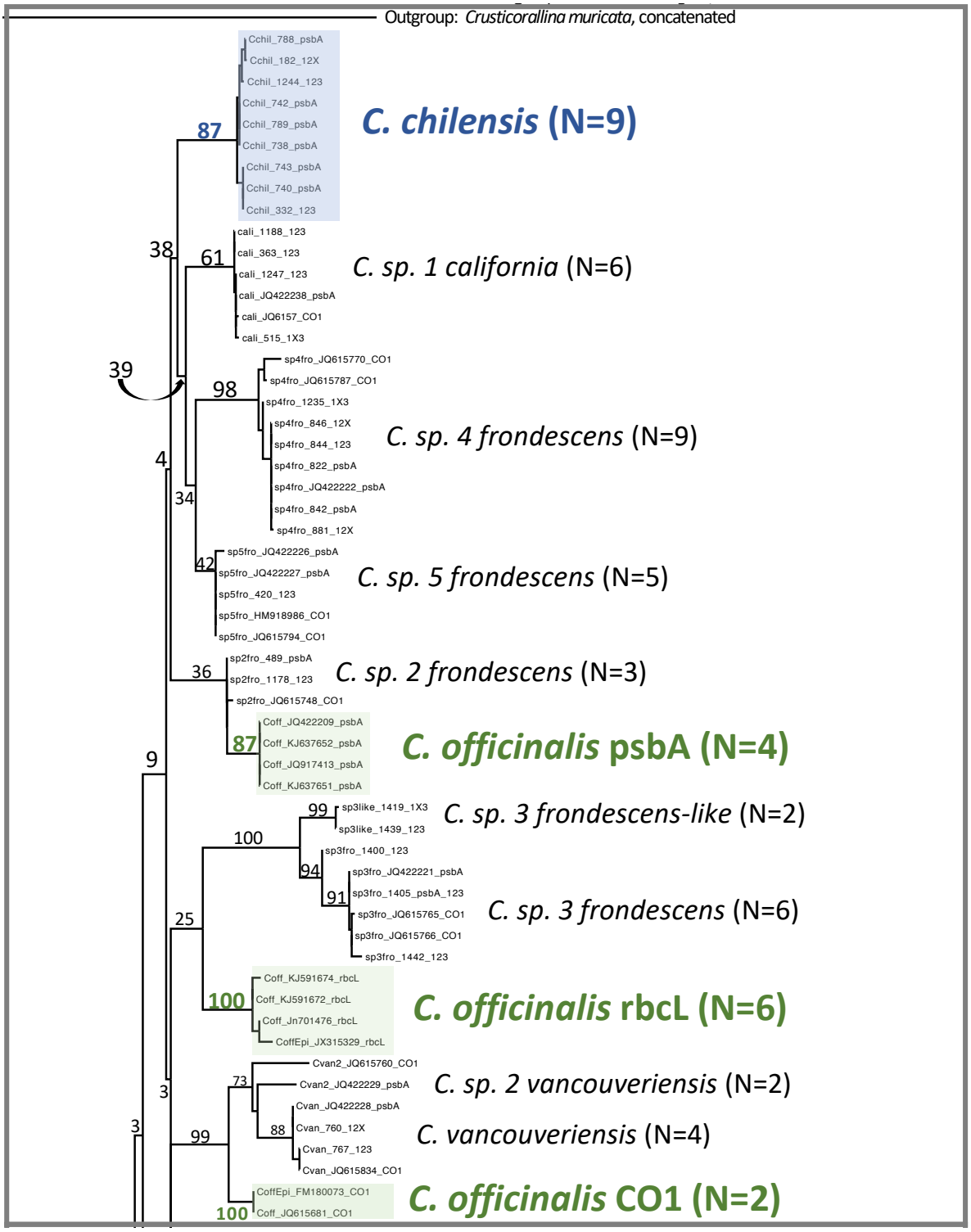


Figure 21. Expanded from Fig. 20.

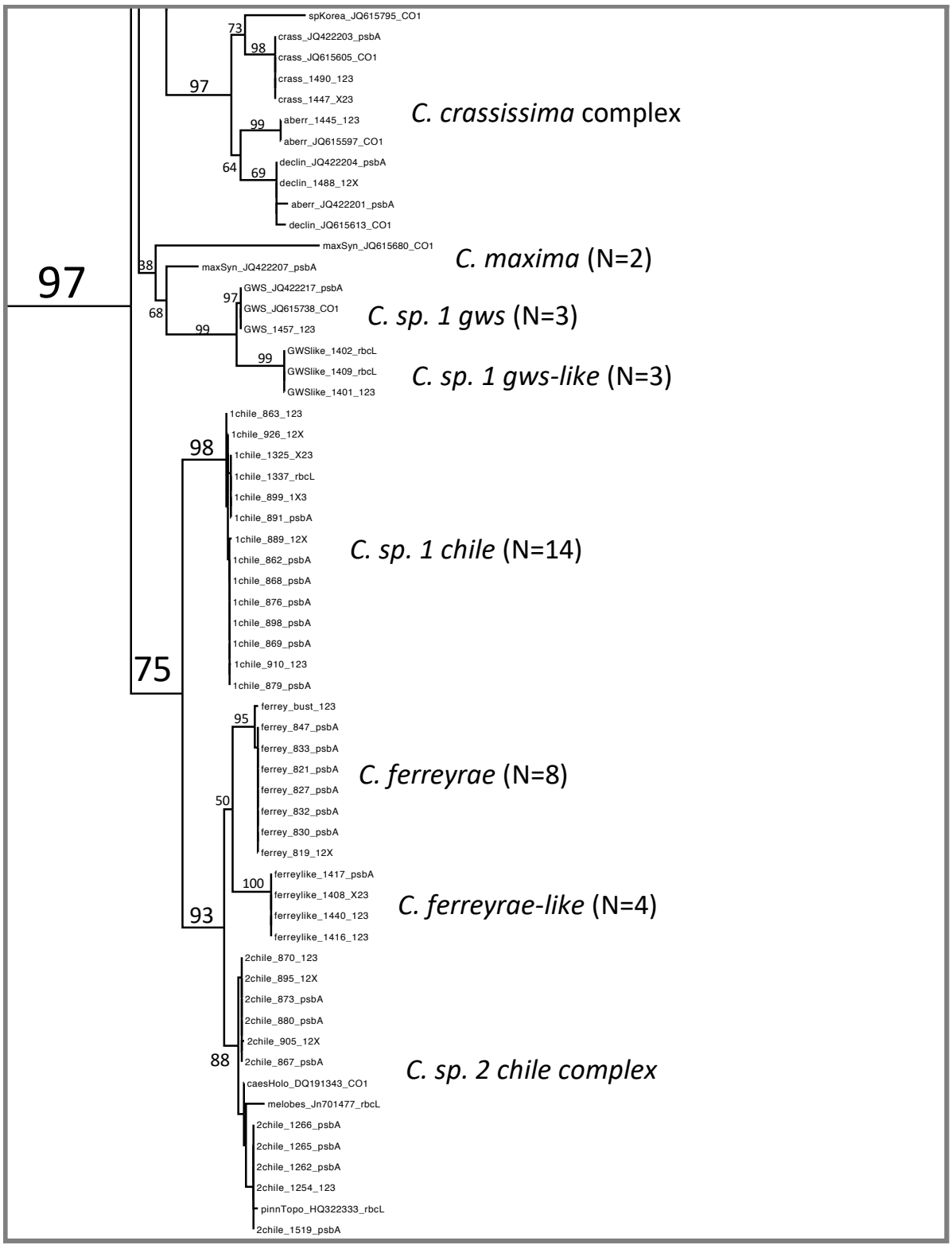


Figure 22. Expanded from Fig. 20.

1.3.1 *Corallina chilensis* is not a variety of *C. officinalis*

If Kützing were correct and *Corallina chilensis* were a variety of *C. officinalis*, *C. chilensis* would have to be monophyletic with *C. officinalis* in the gene trees (Figs. 10-15). Further, *C. officinalis* and *C. chilensis* sequences would be expected to show a high percent similarity in distance matrices (Appendix V), and the two would group together in the barcode gap analyses (Table 2).

In the preliminary ABGD analyses (Table 2), one would expect to see *C. chilensis* and *C. officinalis* delimited as separate species consistently across all three genes if *C. chilensis* were not a variety of *C. officinalis*. However, *C. chilensis* and *C. officinalis* were delimited as separate species with respect to CO1 and *rbc*L, although they were grouped as the same candidate species along with C. sp. 1 california, C. sp. 2 frondescens, and C. sp. 5 frondescens in the *psb*A analysis (Table 2).

Corallina chilensis likewise did not fit with the expectations for varietal status in the phylogenetic analyses. The *C. chilensis* clade was not monophyletic with the *C. officinalis* clade in any of the gene trees (Figs. 8-22). In the *psb*A tree, *C. chilensis* sequences formed a clade with strong branch support that was nested among species other than *C. officinalis* (Figs. 10-11), which occupied its own strongly supported clade (Figs. 10-11). *Corallina chilensis* occurred in a clade with C. sp. 1 california and C. sp. 5 frondescens whereas *C. officinalis* occurred within a clade containing C. sp. 1 chile, C. sp. 2 chile, *C. ferreryrae*, and C. ferreyrae-like (Figs. 10-11).

In the CO1 tree, *C. officinalis* sequences formed a clade with 81.8/100/1 branch support (aLRT percent value/bootstrap percent value/Bayesian posterior probability) (Figs. 12-13). *Corallina officinalis* was sister to *C. vancouveriensis* (97.5/98/1 branch support) in the CO1 tree

rather than sister to *C. chilensis* (Figs. 12-13). *Corallina chilensis* formed a clade with *C. crassissima*, *C. declinata*, *C. aberrans*, and C. sp. 5 korea in the CO1 tree (Figs. 12-13).

In the *rbc*L tree, *C. chilensis* and *C. officinalis* formed two separate strongly supported clades although it was difficult to determine their nearest sister relationships due to the polytomies (Figs. 14-15). However, in the concatenated tree (Figs. 20-22), *C. officinalis rbc*L sequences were sister to the C. sp. 3 frondescens clade. *Corallina officinalis psb*A sequences formed a clade with C. sp. 2 frondescens, and *C. officinalis* CO1 sequences were sister to the *C. vancouveriensis* complex (Figs. 20-22). *Corallina officinalis* was sister to three different species or species complexes within the concatenated tree, none of which were, or contained, *C. chilensis*. In the majority-rule tree (Figs. 16-19), *C. officinalis* did not form a clade, but *C. chilensis* formed a clade that was not sister to any *C. officinalis* sequences.

When analyzing raw pairwise distances, one would expect that if *C. chilensis* were a variety of *C. officinalis*, the two would demonstrate intraspecific levels of percent difference. Instead, upon analyzing raw pairwise distances, *C. chilensis* differed from *C. officinalis* by 0.94-1.06, 6.31-7.26, and 1.8-2.36% across *psb*A, CO1, and *rbc*L gene distance matrices respectively, levels consistent with interspecific variation (Table 3, Appendix V). The high percent differences between *C. chilensis* and *C. officinalis* across all three genes (Table 3, Appendix V) indicates that the two are distinct species from one another.

Table 3. Summary table (derived from full matrices in Appendix V) presenting the range of percent difference between *C. chilensis* and other known species or potentially closely related species within *Corallina*.

	***C. chilensis psb* A**	***C. chilensis* CO1**	***C. chilensis rbc* L**
C. chilensis	0-0.24	0.45-0.91	0.09-0.09
C. officinalis	0.94-1.06	6.31-7.26	1.8-2.36
C. vancouveriensis	1.06-1.18	7.72-8.62	2.08-2.18
C. crassissima	1.29-1.41	6.2-6.66	1.95-2.08
C. ferreyrae	0.94-1.06	8.62-9.08	1.72-1.9
C. sp. 2 frondescens	0.59-0.82	6.96-7.26	1.12-1.17
C. sp. 3 frondescens	1.29-1.53	8.45-9.53	2.98-3.43
C. sp. 4 frondescens	0.71-0.94	5.9-6.66	1.55-1.63
C. sp. 5 frondescens	0.24-0.59	5.9-6.05	0.81-0.81

Overall, lack of monophyly of *C. chilensis* with *C. officinalis,* separation of the two in ABGD delimitation, and separation of the two by a consistent pattern of pairwise distances was consistent with expectations under the assumption that they are separate species.

1.3.2 Specimens from the Northern Hemisphere matched the *C. chilensis* holotype & formed a clade in all trees.

Analysis of single loci and concatenated data from the three loci provided congruent phylogenetic support for a clade of specimens including the *C. chilensis* holotype collected by Darwin (#2151) in Valparaiso, Chile. The *C. chilensis* holotype (Fig. 4 and Appendix II, Table S1) occurred in a clade with 97.1/62/- support in the *rbc*L type tree (Figs. 8-9). The 263 bp *rbc*L sequence from the holotype was identical over its length to *rbc*L sequences from what had been referred to as Corallina sp. 1 frondescens (Appendix II, Table S1/PTM 332 UBC A89284, British Columbia, Canada) from the Northern Hemisphere (Figs. 8-9). I therefore refer to

"Corallina sp. 1 frondescens" as "*Corallina chilensis*" from this point forward, and I use sequences from voucher specimen PTM 332 to represent *C. chilensis* in subsequent analyses.

While the *C. chilensis* holotype was only included in one of the *rbc*L trees (Figs. 8-9), all other gene trees showed a clade of closely related sequences centered around *C. chilensis* specimen PTM 332 (Figs. 10-22). Nine *C. chilensis* specimens formed a clade with 94.5/73/1 branch support in the *psb*A phylogeny (Figs. 10-11). Three *C. chilensis* specimens formed a strongly supported clade in the CO1 phylogeny with 99.4/96/1 branch support (Figs. 12-13). In the *rbc*L phylogeny that did not include short 1800's herbarium sequences, the two *C. chilensis* specimens formed a strongly supported clade with 96.2/99/1 branch support (Figs. 14-15). The *C. chilensis* clade was likewise supported by the concatenated gene tree (Figs. 20-22), with 87% bootstrap support, and the majority rule tree in which *C. chilensis* sequences formed a clade (Figs. 16-19).

1.3.3 Other specimens thought to have been *C. chilensis* based on morphology

The *rbc*L sequence from the 1800's d'Orbigny herbarium material matched the sequence from the *C. chilensis* holotype (100% identical over 263 bp) (Figs. 8-9). However, the *rbc*L sequence from the 1800's Gay specimen (Fig. 5, collection unknown) differed by 0.76% (2 bp different over 263 bp) from the *C. chilensis* Darwin sequence (#2151). The sequence from the Gay specimen was identical to a sequence from a specimen (PTM870) called C. sp. 2 chile over 263 bp and was situated within the clade including *C. ferreyrae* and C. sp. 1 chile, with 99/74/.94 branch support (Figs. 8-9).

The two 2019 samples collected in Chile and identified in the field as *C. officinalis var. chilensis* did not match *C. chilensis* sequences when compared side by side, or via nucleotide

BLAST in GenBank. Based on *psb*A PTM 1985 (Appendix II, Tables S1 & S3) was only 0.06-0.18% (1-2 bp over 851 bp) different from C. sp. 2 chile, but was 1.23-1.35% (11-12 bp over 851 bp) different from *C. chilensis*. Also based on *psb*A, PTM 1984 (Appendix II, Table S1) appeared to be a species in another genus that has yet to be described. Specimen PTM 1984 was 8.83% different (71 bp different over 796 bp) from *C. chilensis* in the *psb*A gene.

The *psb*A sequence contributed by Paul Gabrielson from his Playa Cocholgue, Chile collection (NCU 656905) was identified as *C. chilensis* because it was 99.88% similar with PTM 332 across 851 basepairs (Appendix II, Table S1).

1.3.4 Analysis of conflict and congruence among gene trees

In the concatenated gene tree, which included all sequenced vouchers from Appendix II and Table S1 (except for PTM 826 (UBC A91600)) and the herbarium specimens from the 1800's), species-level groups were monophyletic, often with strong support (Figs. 20-22). In the majority rule of the bootstrap consensus trees from the three individual loci that likewise includes all sequenced vouchers (except for PTM 826 and 1800's herbarium specimen sequences) (Figs. 16-19), most sequences still clustered together by species name, indicating good resolution at the species level. However, relationships among *Corallina* species were unresolved, appearing as a polytomy of 19 clades (Figs. 16-19).

Inconsistency in reconstructing relationships by different loci was evident at various levels in the trees. Deep in the phylogeny, branching order among species varied across gene trees (Figs. 10-15). In the *psb*A tree, Corallina sp. 3 frondescens was sister to the clade containing the remainder of the *Corallina* genus (Figs. 10-11). In the CO1 tree, the *C. crassissima* complex clade was sister to the clade containing the remainder of the genus (Figs.

12-13). In the *rbc*L tree, the *C. ferreyrae* complex clade was sister to the remainder of the genus (Figs. 14-15).

Inconsistent topology among the terminal nodes was seen in the inconsistent topological arrangement of taxa within the *C. ferreyrae* clade across loci (Figs. 10-15). In the *psb*A tree, *C.* ferreyrae-like was sister to a clade containing *C. ferreyrae* sister to C. sp. 2 chile (Figs. 10-11). In the CO1 tree (Figs. 12-13), C. ferreyrae-like was sister to *C. ferreyra*. In the *rbc*L tree (Figs. 14-15), a polytomy was formed by *C. ferreyrae*, C. ferreyrae-like, and a clade containing C. sp. 2 chile and C. sp. 1 chile (Figs. 14-15).

I further explored the lack of resolution with respect to relationships for *C. officinalis*. *Corallina officinalis* had a different sister in each of the three gene trees (Figs. 10-15) as already mentioned in section 1.3.1. *Corallina officinalis* appeared as sister to the *C. ferreyrae* complex with 77.3/5/.64 (aLRT percent value/bootstrap percent value/Bayesian posterior probability) branch support in the *psb*A gene tree (Figs. 10-11). However, *C. officinalis* clustered sister to *C. vancouveriensis* with 97.5/98/1 branch support in the CO1 gene tree (Figs. 12-13). In the *rbc*L tree, *C. officinalis* clustered sister to the remainder of the genus with the exception of the C. sp. 3 frondescens complex with 88/66/1 branch support (Figs. 14-15). These are a couple of examples, but incongruence was widespread across individual gene trees (Figs. 10-15).

This incongruence was especially evident in the failure of *C. officinalis* sequences to form a single monophyletic group when sequences were not concatenated (Figs. 20-22). Instead, *C. officinalis* sequences formed three different clades by gene in the concatenated tree (Figs. 20-22).

I tested for congruence of the *psb*A, CO1 and *rbc*L genes of *C. officinalis* by aligning concatenated genes from all taxa except *C. officinalis.* I added the *psb*A, CO1 and *rbc*L genes of

C. officinalis as separate OTUs (Operational Taxonomic Units) rather than concatenating them. If the gene genealogies were congruent, I predicted that the individual genes from *C. officinalis* would, if resolved, form a monophyletic or paraphyletic group in the concatenated gene tree. Instead, *C. officinalis psb*A, CO1 and *rbc*L genes each formed a sister relationship with a different clade (Fig. 20-22). I defined conflict as incongruent branches with more than .6 posterior probability and more than 60% bootstrap or aLRT support. Since *C. officinalis* had a different sister species relationship in each gene tree with fair to strong branch support for all three *C. officinalis*/sister combinations, the three gene trees were clearly in conflict (Figs. 10-15).

Beyond individual examples, overall incongruence was remarkably widespread throughout the genus, as demonstrated by the collapsed branches in the majority rule consensus of individual gene bootstrap trees (Figs. 16-19). The percentages on the branches in the majority rule consensus tree indicated the frequency that the particular topology appeared across all three individual majority rule gene trees (e.g. 33% indicated that a branch only appeared in one of the three majority rule gene trees). Some of the low support values resulted from missing data in one or more genes (Figs. 16-19). However, much of the low support resulted from disagreement across the genes. There was only one instance where all three gene trees agreed and that was with respect to how three of the outgroups clustered (Fig. 16).

Short branches mostly near the bottom of the majority rule tree (Figs 16 & 19) or otherwise nonsensically paired with other taxa were an artefact of missing data (Figs. 16-19). I confirmed this by aligning questionable sequence pairs and counting basepair differences. For example, the CO1 sequence of *C. vancouveriensis* JQ6158 clustered with the outgroup *Crusticorallina muricata* (Figs16 & 18) partly because it lacked data in the *psb*A and *rbc*L gene trees. Thus, the positions of short branches were (and should be) generally disregarded.

1.3.5 Distribution of *C. chilensis*

It is evident from the recent confirmed range of *C. chilensis* based on DNA sequences that the species has a fairly continuous distribution in the Northeast Pacific and has been found in two localities in Chile. *Corallina chilensis* has never been reported from tropical waters in the East Pacific and appears to be absent from this region. The southernmost point of its confirmed range was Playa Cocholgue, Concepción, Chile; and the northernmost point of its confirmed range was Haida Gwaii, British Columbia, Canada (Figure 23.) All *C. chilensis* specimens included in this analysis were verified either via DNA comparison in phylogenetic trees or in GenBank (See Appendix II, Tables S1 & S3). The *C. chilensis* specimens included in this analysis from the Martone collection (Appendix II, Table S1) were collected in California and between Yaquina Head, Oregon, United States; and Calvert Island, British Columbia, Canada (Fig. 23). Samples were collected specifically from the Hakai Conservancy on Calvert Island (N = 15), near the Bamfield Marine Science Centre on Vancouver Island (N = 4), in Port Renfrew located on the southern outer coast of Vancouver Island (N = 2), and from Yaquina Head, Oregon (N = 1). Hind & Saunders (2013A) likewise collected *C. chilensis* (as C. frondescens) from British Columbia (N=87) and northern California (N=4). Although specifically sought between southern California and Valparaiso, Chile, only one known specimen of *C. chilensis* has been found or documented to date by the Martone Laboratory or collaborators (Hind & Saunders 2013A; Gabrielson, *pers. comm*.). This specimen was collected in the drift in 2008 by Paul Gabrielson, about 700 KM south of Valparaiso, Chile, in Playa Cocholgue, Concepción, Chile (NCU656905, Appendix II, Table S3).

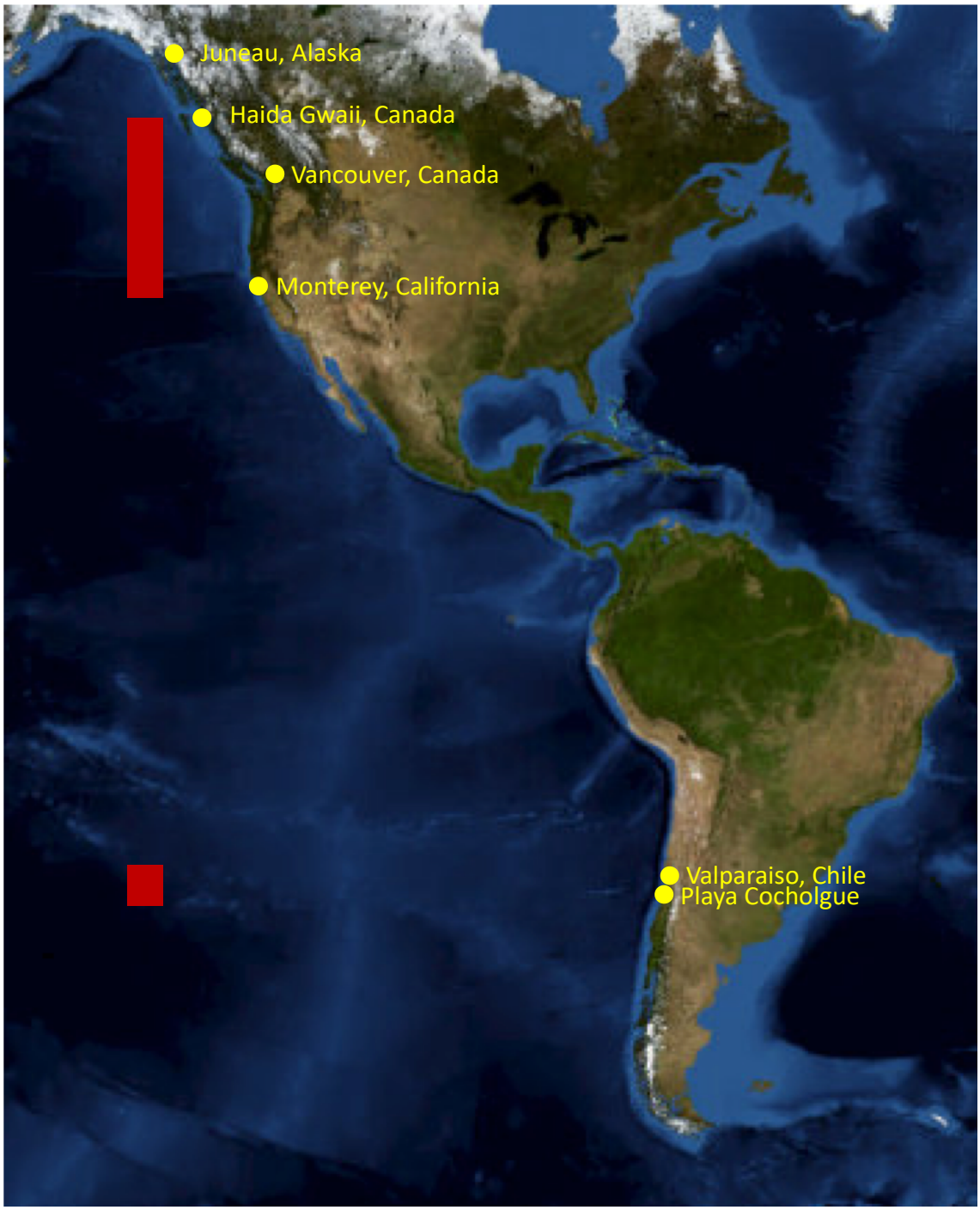


Figure 23. Recently confirmed range of *C. chilensis*. https://viewer.nationalmap.gov/advanced-viewer/

1.3.6 Morphological measurements

The morphology of *C. chilensis* populations in the Northeast Pacific was highly variable (Table 4), and DNA was used to confirm specimen identities (Appendix II, Table S3). Morphometric measurement analysis revealed that the tallest fronds in each sample (Fig. 7A) were slightly longer and wider than the randomly selected fronds (Fig. 7B) that were measured, but that the width to length proportions were nearly the same (Table 4). For the tallest frond per sample, maximum height was 16-116 mm (average = 51 mm, Table 4). For the randomly selected fronds, height was 14-95 mm (average = 41mm, Table 3). Frond crowns (Fig. 7C) were 11-87 mm long (average = 31mm, Table 3), and stems (Fig. 7C) were 0-28 mm long (average = 10 mm, Table 4). Secondary pinnate branches growing from the main axis (Fig. 7D) were 6-38 mm long (average = 15mm, Table 4). Nearly half of the measured samples had secondary branches that were 5-10 mm long with three outliers 30-40 mm long. Average values for secondary branch mid-intergenicular dimensions (Fig. 7F) were minimum 0.7 mm wide, maximum 1.4 mm wide, and 1.6 mm long (Table 4). Average values for mid-intergenicular dimensions on the main axis (Fig. 7E) were minimum 1 mm wide, maximum 1.6 mm wide, and 1.6 mm long (Table 4). The length of mid-intergenicula on the secondary branches were on average less than 0.1 mm different from the length of the intergenicula on the main axis (Fig. 7E-F, Table 4). Basal intergenicula on the main axis (Fig. 7G) averaged 1.2 mm wide with a median length of 1.3 mm (Table 4). Conceptacles (Fig. 7H) averaged 0.6 mm at their widest point, and were 1.5 mm long including subtending intergenicula (Table 4). A complete list of measurements may be found in Appendix III.

Table 4. Summary table of morphological measurements from *Corallina chilensis* specimens collected in the Northeast Pacific (N=22).

	Average (mm)	Range (mm)
Frond width, random	23.93	8.97 - 51.17
Frond length, random	41.32	14.24 - 95.2
Frond width, tallest	29.09	7.19 - 60.1
Frond length, tallest	50.59	15.98 - 115.31
Crown length	31.47	11.08 - 87.2
Stem length	9.85	0 - 28.39
Main axis mid intergeniculum, maximum width	1.62	1.08 - 2.05
Main axis mid intergeniulum, minimum width	0.97	0.62 - 1.44
Main axis mid intergeniculum, length	1.56	1.23 - 2.41
Basal intergeniculum, width	1.25	0.69 - 1.83
Basal intergeniculum, length	1.28	0.69 - 2.32
Secondary branch length	14.91	6.49 - 37.92
Secondary branch, mid intergeniculum, maximum width	1.38	0.89 - 2.49
Secondary branch, mid intergeniculum, minimum width	0.68	0.41 - 1.07
Secondary branch, mid intergeniculum, length	1.64	1.17 - 2.02
Conceptacle width	0.65	0.61 - 0.71
Conceptacle length	1.52	0.96 - 2.54

1.3.7 Morphological description of *C. chilensis* in the Northeast Pacific

Kützing described *Corallina officinalis* [*var.*] *chilensis* as tripinnate, having oblong wedged joints, “sterile” [non-reproductive] pinnules on both sides [of the branch], pointed cystocarps [conceptacles], red-violet color, [from] Chile (Kützing 1858, see Fig. 6 for original Latin text). This description likewise applied to *C. chilensis* collected from the Northeast Pacific. I have designated PTM 789 (UBC A91532) and PTM 333 (UBC A89285) from Calvert Island, British Columbia (Appendix II, Table S1) to serve as exemplar specimens representing Northern Hemisphere populations. I selected these two specimens because visually they were representative of all the specimens I examined; PTM 333 being symmetrical and orderly looking, while PTM 789 was asymmetrical and erratic in appearance.

Corallina chilensis fronds in the Northeast Pacific were typically 4 to 5 cm tall, but exhibited growth up to 12 cm. Crowns were nearly twice as long as stems regardless of frond height, lending some fronds a unique, feathered shape (Fig. 24 A, B & D; Fig. 27). These pinnate

feather-like fronds had unbranched “stems,” and exhibited regular opposite branching about 1/3 of the way up the length of the frond. Fronds typically exhibited pinnate or bipinnate branching patterns, but some exhibited tripinnate branching.

Branching was always distichous in *C. chilensis*. In some individuals the main axis was dichotomously divided early in development near the base. Any clumping appearance was due to layers of branching and multiple degrees of branching, but the branches were distichous. Individuals looked spindly or robust depending on branch thickness and gap size between branches (Fig. 24). Some had secondary branches that were so broad, little to no space was visible between the branches (Fig. 24, B & D). Other specimens had narrower branches, thus larger gaps between branches, giving fronds a sparse appearance (Fig. 24, A, E, & F). Many specimens appeared to be symmetrical and orderly looking (Fig. 24, A, B, & D). Incongruent development of secondary or tertiary branching and damaged branches may have contributed to giving other specimens an erratic, irregular look (Fig. 24, C, E-F). Terminal peripheral intergenicula ranged in form from thin and rod-shaped to broad and nearly palmate.

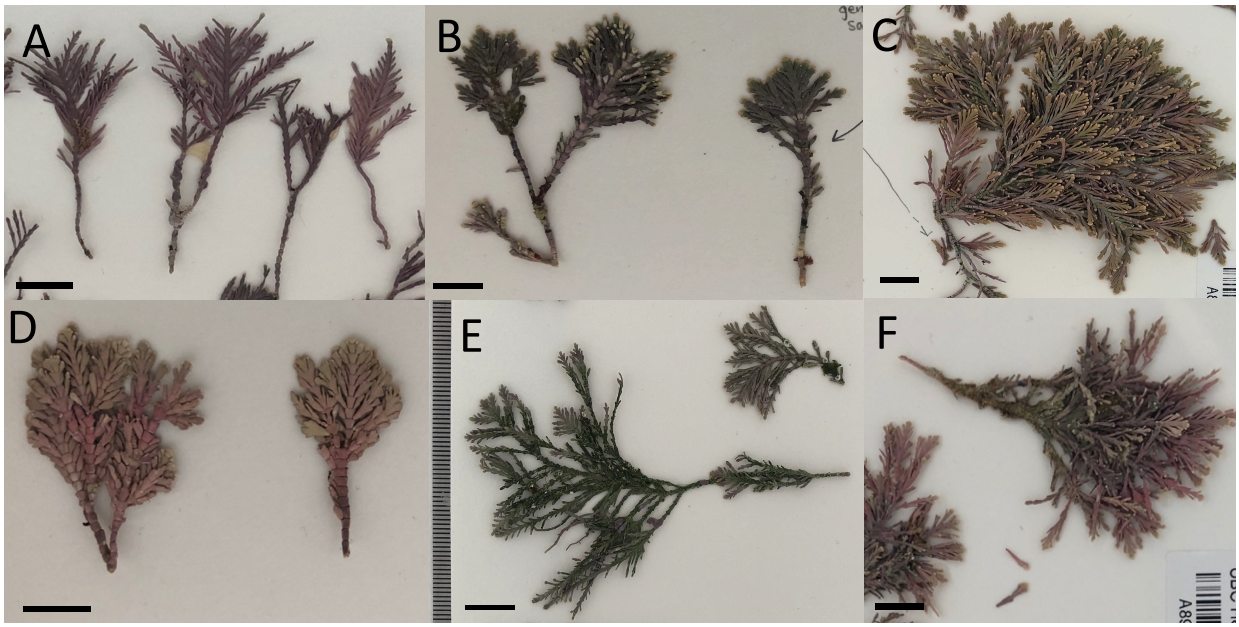


Figure 24. *Corallina chilensis* from Martone collections from British Columbia, Canada. Scale bars represent 1 cm. Specimens are morphologically variable despite growing in the same region. (A) PTM 789 (UBC A91532) Calvert Island, Fifth Beach. Mid intertidal, in tidepool. (B) PTM 487 (UBC A89808) Calvert Island, Fifth Beach channel, subtidal. (C) PTM 182 (UBC A88708) Botanical Beach, Port Renfrew, Vancouver Island. Very exposed, mid intertidal tidepool. (D) PTM 333 (UBC A89285) Calvert Island, Fifth beach, exposed point. Low intertidal. (E) PTM 629 (UBC A89961) Botany Bay, Port Renfrew, Vancouver Island. Mid intertidal tidepool. (F) PTM 326 (UBC A89279) Calvert Island, Fifth beach. Low intertidal tidepool.

Figure 25 shows *Corallina vancouveriensis* fronds side-by-side with *C. chilensis* fronds, including at higher magnifications. While it is difficult to generalize and there are exceptions to every description, the *C. chilensis* specimens that I observed had mid-intergenicula on secondary branches off the main axes that tended to taper downwards distinctively. These midaxis-intergenicula possessed clear minimum and maximum width points characteristic of *Corallina* intergenicula, including *C. vancouveriensis* intergenicula (Fig. 25, E & F). *Corallina chilensis* basal intergenicula on the main axis appeared to be symmetrically square in surface view and were about as long as they were wide, similar to *C. vancouveriensis* basal intergenicular shape (Fig. 25, A & B). However, with the exception terminal peripheral intergenicula and basal intergenicula, all *C. chilensis* intergenicula tended to be well over 1 mm in length, 1mm at their widest points, and over .5 mm at their narrowest points (Fig. 26).

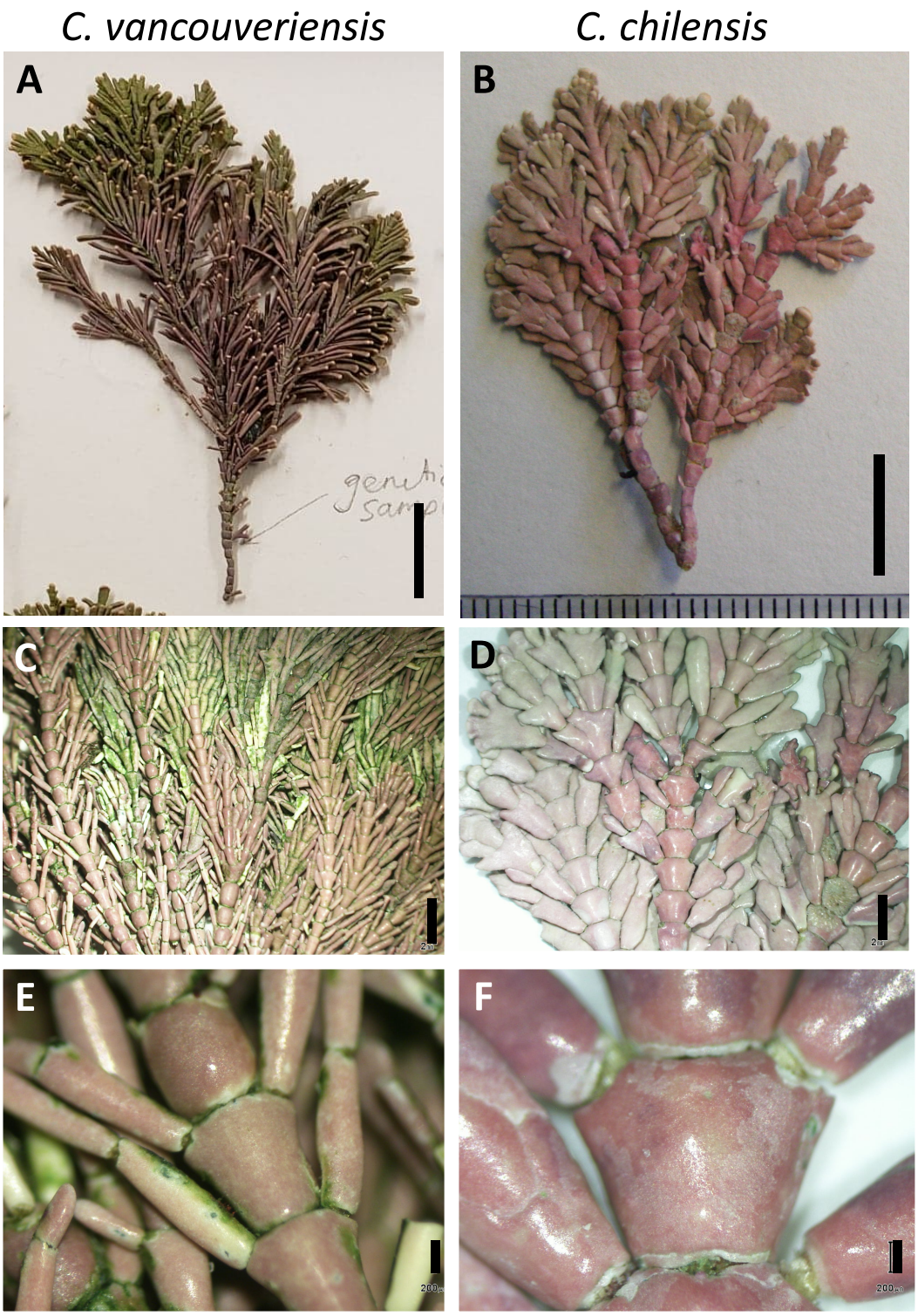


Figure 25. *Corallina vancouveriensis* and *C. chilensis* side-by-side comparison. (A) Macroscopic *C. vancouveriensis*, scale bar ~10mm, PTM 179 (UBC A88705). (B) *Corallina chilensis*, scale bar ~10mm, PTM 333 (A89285). (C) *Corallina vancouveriensis*, scale bar = 2mm. (D) *Corallina chilensis*, scale bar = 2mm. (E) *Corallina vancouveriensis*, scale bar = 200µm. (F) *Corallina chilensis*, scale bar = 200 µm.

Corallina chilensis had noticeably larger mid-main axis and mid-secondary branch intergenicula than *C. vancouveriensis* with respect to all three dimensions—length, maximum width, and minimum width—of intergenicula that were neither apical or basal (Fig. 26). This difference is so striking that it is even apparent in side-by-side photographs of the two species (Fig. 25). With respect to habitat, *C. chilensis* was often found in the low intertidal zone under *Phyllospadix* spp. or kelp whereas *C. vancouveriensis* was found either under kelp in the low intertidal or growing exposed on rocks and around rims of mid-intertidal pools.

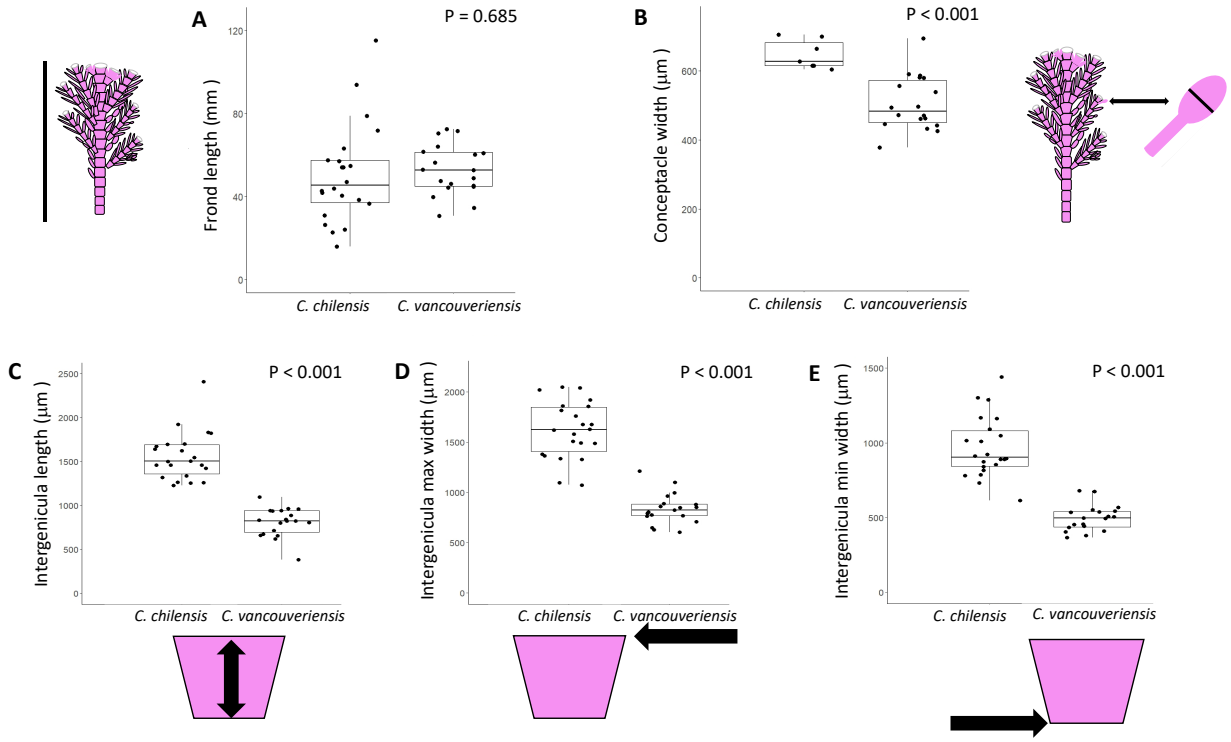


Figure 26. *Corallina chilensis* (N=22) and *C. vancouveriensis* (N=19) intergenicular dimensions. All measurements taken from intergenicula located midway up the main axis.

In summary, the *C. chilensis* populations measured from the Northeast Pacific (Appendix III, Table S4) are unified by their overall frond shape, distichous branching, and typically, opposite pinnate branching (Figs. 24, 25B & D) and are distinguished from *C. vancouveriensis* by their larger mid-axis intergenicula (Fig 25). However, specimens even within the same area may appear very different from each other due to differences in overall symmetry, frond width and height, branch thickness, differing degrees of pinnateness, and variability in shape of the small peripheral branches.

Figure 27. In situs photograph in which *C. chilensis* fronds were growing in an exposed location. North Beach, Calvert Island, British Columbia, Canada. July 26, 2017. PTM1588 (UBC A93226)(Appendix II, Table S1).

1.4 Discussion

1.4.1 Identity and rank of *C. chilensis*

DNA sequencing has been increasingly used over the past decade to discriminate and identify species of articulated corallines, particularly as it has become more evident that exclusively morphologically-based species are inadequate due to the presence of cryptic species, as well as morphologically variable speciation. This could be on account of convergent evolution or phenotypic plasticity. Even when morphological boundaries are known to broadly align with molecular-based species boundaries, analyzing DNA sequences increases the resolution into the relatedness of taxa. Because random mutations in the genome are not always reflected in the phenotype, by directly comparing base pair differences in the DNA itself, we gain an increased level of resolution to a degree that morpho-anatomic analyses simply are not capable of achieving. While DNA sequences may be compared along-side one another base by base to determine the percent difference between specimens or species, DNA sequences also may be incorporated into phylogenetic analyses used to detect reciprocal monophyly, a criterion confirming that any given population is a distinct "species." Distance matrices may be used to compare DNA sequences and may indicate divergence of intermediates even if newly emerging species do not yet demonstrate reciprocal monophyly in phylogenetic analyses or exhibit distinguishing morphological features. DNA sequences may also be analyzed using species discrimination programs such as Automatic Barcode Gap Discovery. Such programs delimit species based on greater variation between (interspecific) than within (intraspecific) any given set of diverging populations or species. Inclusion of DNA from multiple loci has the potential to strengthen evidence for speciation, given that no universal barcode has been demonstrated to effectively segregate coralline species (Broom 2008, Leliaert 2014). For this reason, I selected

three different markers from two different organelles (*psb*A-plastid, CO1-mitochondrial, and *rbc*L-plastid) that have been commonly used in previous analyses, as well as for the sake of comparison between my analysis and analyses completed by other researchers. Once species boundaries are determined, it is necessary to compare DNA from field-collected specimens with the DNA from type specimens in order to correctly apply species names. To this end, I included type specimen DNA from *Corallina chilensis* in my analysis to ensure correct name application.

This study established that a partial *rbc*L sequence from the holotype specimen of *C. chilensis*, basonym of *C. officinalis var. chilensis*, from Valparaiso, Chile was identical over its length with PTM 332 (UBC A89284, Appendix II, Table S1) from Hakai, British Columbia, Canada. PTM 332 in turn matched ~100 other specimens included in this study that were collected from the Northern Hemisphere (Fig. 23, Appendix II, Tables S1 & S3), more specifically from the Northeast Pacific from the Haida Gwaii archipelago in British Columbia through Laguna Beach, California, USA, and one other specimen (NCU656905, Appendix II, Table S3) collected from the Southern Hemisphere, from Playa Cocholgue, Concepción, Chile.

Overall, evidence that *C. chilensis* is a "separately evolving metapopulation" (De Queiroz 2007) and thus a distinctive species includes: (1) *C. chilensis* specimens form well-supported clades based on phylogenetic analyses of each of the three markers; (2) *C. chilensis* is molecularly distinct from other species within its genus based on sequence divergence values for each of the same markers (Table 3, and *see* Appendix V); (3) *C. chilensis* was shown to be an independent species from *C. officinalis* using CO1 and *rbc*L gene sequences in ABGD analyses (4) *C. chilensis* exhibits a biogeographic range and phenotype distinct from *C. officinalis* (Brodie et al. 2013, Hind et al. 2014A). (5) Northeast Pacific *C. chilensis* specimens examined were morphologically different from other species including congeneric species *C. vancouveriensis*.

Given the DNA-confirmed result that *C. chilensis* has been collected from several locations in the Northeast Pacific including in the same locale from which Yendo collected, and that it has been collected twice from Chile; Yendo (1902) was correct: *C. chilensis* is present in both hemispheres. Further, based on the evidence provided above, *C. chilensis* is a distinct species and cannot be considered a variety of *C. officinalis* as proposed by Kützing (1858) and accepted by numerous, although not all, subsequent researchers. While the main questions introduced in this thesis have been answered, these answers raise new questions that merit discussion.

1.4.2 Known global distribution of *Corallina*, and specifically *C. chilensis*

Members of the subfamily Corallinoideae are distributed world-wide (Guiry & Guiry 2020) and the *Corallina* genus as a whole exhibits an extensive global distribution (Broom et al. 2008, Walker et al. 2009, Brodie et al. 2013, Williamson 2015, Bustamante 2019, Guiry & Guiry 2020). Other genera in subfamily Corallinoideae are also widespread. For instance, *Bossiella* species are found in both the Northeast and Southeast temperate Pacific, but only one *Bossiella* species is known to span both hemispheres (Hind et al. 2014B, 2015, 2018). *Jania* has been confirmed (with DNA sequences) to be present in both the Atlantic and Pacific oceans, in South African waters, and surrounding Australia (Kim et al. 2007, Harvey et al. 2020).

Even individual species within *Corallina* are widespread. *Corallina officinalis* has been confirmed in the North Pacific and the North Atlantic including as far North as Iceland south to Spain (Yesson et al. 2018), also extending into the Southern Hemisphere (Broom et al. 2008). *Corallina ferreyrae* grows in the Northeast Pacific and in Chile, and may possibly inhabit the North Atlantic as well, depending on the criteria defining species (Appendix II, table S1; Walker

et al. 2009, Bustamante et al. 2019). Like these congeners, *C. chilensis* also exhibits an extensive range.

Corallina chilensis, identified only using morphology, was reported specifically in Valparaiso, Chile (Harvey 1849), Puerto del Hambre (Port Famine) in the Strait of Magellan (Montagne 1852), Magellanes province, Chile (Ardissone 1888), Bahia Orange, Chile (Hariot 1889), Tierra del Fuego, Chile (Foslie 1907), Beagle Channel, Southern Chile north through Lima (Ramírez & Santelices 1991), Berkeley Sound, Port Louis, Falklands (Foslie 1907), Norfolk Island, Australia (Harvey 1849), Port Renfrew, British Columbia, Canada (Yendo 1902A), South Africa (Silva et al. 1996), Hakodate, Japan (Yendo 1902B); and reported as "common" on the coast of California (Setchell & Gardner 1903), "common" from San Diego, California north to Vancouver Island, Canada (Smith 1944), "common" all along the coast of Mexico from Isla Magdalena, Baja Mexico Sur, north to British Columbia, Canada (Dawson 1953). Collectively these reports depict a nearly continuous range from the Falklands and southern Chile through Vancouver Island, Canada, with the exception of presence in the tropics. My study used DNA sequences to confirm the presence of *C. chilensis* in some, but not all, of these locations because not all historically sampled sites were resampled, or *C. chilensis* was not present at the sites that were sampled recently, in the past few decades. This study confirmed the presence of *C. chilensis* in the Northeast Pacific as far south as Laguna Beach, California. A specimen was also collected from Yaquina Head, Oregon, but the majority of specimens were collected from waters surrounding Vancouver and Calvert Islands, British Columbia, Canada. Sequences taken from Hind and Saunders (2013A) were from specimens collected from Haida Gwaii, Canada, and matched the *C. chilensis* sequences from my study. This indicates that *C. chilensis* is distributed at least as far North in the Northeast Pacific as Haida Gwaii, British

Columbia, Canada. Additional sampling is required to determine the northern most boundary for *C. chilensis*. With respect to Southern Hemisphere distribution, sequencing the Darwin material confirmed that *C. chilensis* was present in Valparaiso, Chile, in the early 1800's, and this research also confirmed that it is still present in Chile, ~700 km South of Valparaiso in Playa Cocholgue, Concepción.

Early *C. chilensis* species identifications were made based exclusively on morphology, and without direct comparison to the type specimen. Even if these collections had been morphologically compared with the type specimen, that still would not have guaranteed correct name application, as was the case with the Gay specimen which closely resembled the *C. chilensis* Darwin specimen, but was not conspecific when confirmed using DNA. While in some geographic locations it may be sometimes possible to discern *C. chilensis* from other neighboring corallines based on morphology, it may not be possible to do so in other geographical locations where different species are present and or cryptic with one another. Thus, without DNA confirmation, there is no way of verifying the identifications in the historical reports based only on morphological identifications, and therefore the historical range of *C. chilensis* is poorly documented.

Even if historical reports of *C. chilensis* presence between California and Valparaiso were inaccurate, this range is consistent with other algae that exhibit similar disjunct ranges. The articulated coralline *Bossiella orbigniana* ranges from Haida Gwaii Canada, south to Baja California Norte, Mexico (Hind et al. 2014B). *Callophyllis variegata* has likewise been confirmed (by DNA), and ranges from Monterey, California through Haida Gwaii, British Columbia, although its type specimen was from Valparaiso, Chile, and it was collected recently from Ancud Bay and Los Chonos Chiloé, Chile (Clarkston & Saunders 2013). Similarly,

Mastocarpus latissimus was confirmed in Chile, but otherwise ranges from Moss Landing, Monterey Co., California, north through Alaska (Lindstrom et al. 2011).

If more *C. chilensis* specimens were collected from the Southern Hemisphere, and if quality DNA could be extracted from old herbarium specimens, it would then be possible to conduct genetic studies on both Southeast and Northeast Pacific populations to understand the extent of their genetic separation, if they did indeed display any dissimilarity. If haplotypes were the same between the two populations, the indication would be that the two populations had only recently diverged, and that one was thus more recently (perhaps in the past few centuries) introduced to the opposite hemisphere. Alternatively, different haplotypes between the two populations would indicate that separation occurred over much deeper time. Knowing how recently the populations were separated could potentially provide clues as to how *C. chilensis* was distributed across its range. For instance, if the introduction of *C. chilensis* to the opposite hemisphere had been recent, in the past few centuries, maritime traffic could have been responsible for its introduction to locations far from its origin (Callahan et al. 2001, Ruiz et al. 2003, Mach et al. 2017, Goldsmit et al. 2018), anomalous cooling events might have impacted its distribution in the Southern Hemisphere (Thompson et al. 1986, 2003, Meyer 2009), or perhaps a rare event in which *C. chilensis* fronds could have become entangled in and transported via kelp raft might have occurred (Saunders 2014).

1.4.3 How to identify *C. chilensis* in British Columbia, Canada

I recommend first attempting to identify the genus of a given unknown geniculate coralline that could potentially be *C. chilensis*. Reproductive *C. chilensis* exhibits the typical *Corallina* shaped bulbous axial conceptacles, and non-reproductive specimens may still be

placed into *Corallina* based on the typical shape of mid intergenicula on the main secondary axes (see Abbott & Hollenberg 1976, Johansen 1981, Baba et al. 1988) distinguishing them from certain other genera, such as *Bossiella*, *Calliarthron*, *Chiharaea*, and *Johansenia*. (For a description of these other genera containing species that fall within the same range as *C. chilensis* from British Columbia, see Hind et al. 2014A and Hind et al. 2015 for descriptions of *Bossiella* species; Gabrielson et al. 2011 for *Calliarthron* species, Martone et al. 2012 and Hind & Saunders 2013B for *Chiharaea* species; Hind & Saunders 2013A for *Johansenia*.) This typical *Corallina* shape occurs because width is shorter than length and the intergenicula taper downwards decreasing in width. Variation in branching characteristics likely contributes to the overall variable appearance of the *C. chilensis* population from the same region. However, the consistent shape of the central intergenicula is a unifying feature across otherwise enigmatic morphological variation.

Many described *Corallina* species do not occur in the same geographic range as British Columbian *C. chilensis*, or are not likely to be mistaken for *C. chilensis* on account of distinct morphological differences (Walker et al. 2009, Martone et al. 2012, Hind & Saunders 2013A, Hind et al. 2014A, Bustamante 2019). The only other described and DNA-confirmed members of *Corallina* present in British Columbia growing in the same geographical range as *C. chilensis* are *C. vancouveriensis* and *C. officinalis*. *Corallina officinalis* only occurs in its '*Pachyarthron'* morphology within that range (Hind et al. 2014A) making it highly unlikely that it would be mistaken for *C. chilensis* because it looks similar to *Calliarthron*. This leaves mostly only *C. vancouveriensis* and *C. chilensis* to be mistaken for one another. There are some other provisionally named species that resemble and could possibly be confused with *C. vancouveriensis*, and this analysis did not compare species from other genera that could

potentially be mistaken for *Corallina* from time to time. While *C. chilensis* exhibits similar intergenicular shape to other *Corallina* species, some specimens may be reliably differentiated in the field without a microscope from neighboring *C. vancouveriensis* based on the immense intergenicula size difference. While frond length is an insignificant diagnostic characteristic, the best features to look for when differentiating between the two species is intergenicular length, maximum intergenicular width, minimum intergenicular width, and conceptacle width. Conceptacles branching from *C. chilensis* may appear small for *Corallina* conceptacles, but only because *C. chilensis* genicula are so large, creating the illusion that the conceptacles are smaller. On average, *C. chilensis* conceptacles are actually ~0.1 mm wider than *C. vancouveriensis* conceptacles.

1.4.4 Phylogenetic position of *C. chilensis* within *Corallina*

While all three gene trees support *C. chilensis* as a distinct species, they do not resolve the relationship of *C. chilensis* with other species in the genus. *Corallina chilensis* is sister to different *Corallina* species depending on the gene analyzed. Many of these ambiguities will be illustrated in detail in the Future Directions chapter of this thesis. While *C. chilensis* is clearly not the most closely related species (sister) to *C. officinalis*, the relationships between *C. chilensis* and other species in the genus are not well supported or consistent across trees.

In spite of their polytomies, the concatenated and majority rule trees show consistent groupings of sequences into species groups based on the inclusion of more data in the trees. In the concatenated analysis, *C. officinalis* sequences were left as separate operational taxonomic units rather than concatenating them, for the purpose of evaluating whether different loci sequenced from the same specimens occurred in the same clade with *C. chilensis*. Indicating

disagreement across the three individual gene trees, *C. officinalis* sequences from each locus had a different sister relationship in the concatenated tree. None of the three single-locus clades of *C. officinalis* formed a sister relationship with *C. chilensis*, indicating that *C. chilensis* cannot be considered a variety of *C. officinalis*.

Corallina chilensis was delimited as a distinct species in both the CO1 and *rbc*L ABGD analyses, but there was lack of evidence for separation in the *psb*A ABGD analysis. The context of this result should be taken into consideration. While *psb*A is used because it is easy to amplify, it is the least variable of the three loci that I used and it offered the least resolution of relationships at the species level. Additional alternative markers are required to provide better resolution for the purpose of species delimitation (Broom et al. 2008). Thus, the lack of evidence in the ABGD *psb*A analysis for delimitation of *C. chilensis* as a species separate from a group of four others including *C. officinalis* is probably a function of *psb*A's low information content (Zhan et al. 2020).

Pairwise percent differences further support the distinction of *C. chilensis* from other described or provisional (undescribed) species, exceeding thresholds listed in previous publications of *Corallina* and other genera in Corallinoideae (Martone et al. 2012, Hind & Saunders 2013A, Hind & Saunders 2013B, Hind et al. 2018). Interestingly, despite C. sp. 5 frondescens being highly similar to *C. chilensis* with respect to *psb*A and *rbc*L sequences, the two species exceeded previously described percent difference thresholds for species delimitation with respect to CO1 (Hind & Saunders 2013B, Hind et al. 2018).

1.4.5 Incongruence across coralline gene trees: an anomaly or more common than we think?

The relationships among species in individual gene trees were rife with topological incongruence. The purpose of the majority rule tree was to illustrate the great amount of conflict across individual trees and the breakdown of deeper structure. While *C. chilensis* formed a clade in the majority rule tree, *C. officinalis* sequences still consistently clustered in primarily three different locations although this result was confounded by the low support values and the presence of zero-length branches. However, the concatenated tree, consistent with the majority rule consensus tree shows *C. officinalis* sequences segregated by gene into different clades. *Corallina officinalis* provides one example of the widespread incongruence across the three *Corallina* gene trees. The abundance of polytomies throughout all phylogenetic analyses and disagreement indicated by collapse of backbone structure in the majority rule tree testify to the conflict among gene trees for almost every species within *Corallina*.

As in my study, incongruence has been noted in previous studies. Contradictory topologies are evident in previously published trees for *Corallina* (see Figs 1-3 in Hind & Saunders 2013A). However, it is difficult to determine how widespread such disagreement is within genera across Corallinoideae. Several genera have few known species, e.g., *Ellisolandia* (Hind & Saunders 2013A) and *Johansenia* (Hind & Saunders 2013A) each have only one species, and *Calliarthron* and *Alatocladia* each have only two species (Gabrielson et al. 2011). In the case of *Crusticorallina* with four species, the SSU gene tree and the concatenated *psb*A-CO1-*rbc*L gene tree have different topologies, but this may be attributable to the SSU gene's lack of resolving power. Low bootstrap support in the SSU tree suggests that some of the conflicting branching order may reflect stochastic variation rather than strong phylogenetic signal. Differences in taxon sampling, especially of outgroups may also have contributed to

conflict (Hind et al. 2016, see Figs 1-2). Upon examination of the individual gene trees, *psb*A and *rbc*L trees were in agreement, but the CO1 tree disagreed with the *psb*A and *rbc*L trees (Hind et al. 2016, see supplementary materials).

There is some evidence of incongruence within *Bossiella*, a genus with ~14-17 species, comparable in size to *Corallina*. However, individual trees from each marker are not always presented in publications on *Bossiella*, and even in the same publication, tree topologies are sometimes difficult to compare because they were generated using different methods (Hind et al. 2014, Hind et al. 2015, Hind et al. 2018). While the differences between the trees could be attributable to the different analyses and outgroups, a large polytomy and mediocre branch support in the concatenated tree in Hind et al. (2014) could also be related to disagreement between *psb*A and CO1 (see Figs. 1-2 in Hind et al. 2014). For future endeavors, I would recommend including rigorous analysis of individual genes and presentation of individual gene trees in publications, or including them in supplemental materials sections so that the degree of conflict between gene trees may be better ascertained.

Assuming that each plastid and mitochondrial genome consists of a single chromosome that is uniparentally inherited, I would expect individual plastid or mitochondrial gene genealogies to be congruent (Janouškovec et al. 2013, Muñoz-Gómez et al. 2017, Lee et al. 2018, Yoshida & Mogi 2019). So it was surprising to discover such strong and widespread discordance between two plastid genes (*psb*A and *rbc*L) presumably located on the same chromosome. Incongruence has recently been more thoroughly documented among non-algal taxa (Moncalvo et al. 2006, Bell & Hyvönen 2009, Cranston et al. 2009, Moyer et al. 2009, Pelser et al. 2010, Jarvis et al. 2014) but there are few explicit references to it with respect to individual gene tree topologies in the red algal literature (Lee et al. 2018, Zhan et al. 2020). For

Rhodophyta, this is likely due to limited genomic sampling (Janouškovec et al. 2013, Lee et al. 2016, Muñoz-Gómez et al. 2017). The findings from the handful of studies that have extensively examined red algal genomes cited high genomic diversity, evidence of horizontal gene transfer, transposons (Janouškovec et al. 2013, Muñoz-Gómez et al. 2017), and one study cited evidence that ancient red algal plasmids spread as parasitic genetic elements, a.k.a. "selfish genes" (Lee et al. 2016). Given that such means of potential genomic flexibility have been detected among the few taxa that have been studied, undetected conflict may be more prevalent than currently thought. An apparent lack of conflict could reflect unrecognized conflict in previous studies.

Incongruence indicates differences in evolutionary histories among gene trees due to one or more phenomena. Some studies have attempted to assess incongruencies and determine their causes (Pelser et al. 2010), but approach and methodology are still under debate (Mossel & Vigoda 2005, Cranston et al. 2009, Pelser et al. 2010). Possible causes include incomplete lineage sorting, especially among early-diverged lineages after a rapid radiation; movement of genes between species perhaps via hybridization, introgression, or horizontal gene transfer; or the duplication and subsequent extinction of gene copies (Maddison 1997, Liu & Pearl 2007, Cranston et al. 2009, Moyer et al. 2009, Pelser et al. 2010, Bell & Hyvönen 2010, Jarvis et al. 2014, Lee et al. 2016, Lee et al. 2018). Hybridization and incomplete lineage sorting are difficult to distinguish (Pelser et al. 2010), and future work involving many more replicates and loci across as many species as possible would be required to tease the two influences apart (Maddison & Knowles 2006, Moyer et al. 2009, Janouškovec et al. 2013, Jarvis et al. 2014).

While some studies have used concatenated analyses despite incongruence among gene trees (Hind & Saunders 2013A, Cranston et al. 2009, Jarvis et al. 2014), other researchers advise against concatenated species trees when there is conflict among individual gene trees because

concatenating genes in phylogenetics has the potential to obscure distinct evolutionary histories, yielding misleading results (Mossel & Vigoda 2005, Liu & Pearl 2007). Instead of concatenating, Mossel & Vigoda (2005) and Liu & Pearl (2007) recommend reconstructing phylogeny based on each individual locus when there are conflicting signals, an approach that I used in creating three separate gene trees as well as the majority rule tree where multiple genes were included in the same tree, but sequences were not concatenated.

1.4.6 Conclusions

In conclusion, the *rbc*L sequence of a holotype, Darwin's *Corallina chilensis* specimen from the Southern Hemisphere, was an identical genetic match with a sequence from an entity provisionally called C. sp. 1 frondescens in the Northern Hemisphere. Going forward, this entity should not be referred to as *C. officinalis var. chilensis* but rather as *C. chilensis* because that is the oldest applicable name, and because *C. chilensis* is not a variation of *C. officinalis*. I have confirmed that *C. chilensis* is present in both hemispheres, including near the British Columbia location where Yendo once collected. Yendo was therefore likely to have been correct, back in 1902, in his report that *C. chilensis* (as *C. officinalis var. chilensis*) was present in British Columbia.

1.4.7 Future directions with respect to *C. chilensis*

Study of additional specimens would be required to update the description of Southern Hemisphere *C. chilensis* populations and to reconstruct the full extent of *C. chilensis*' range. In this thesis I provide a morphological description based on the *C. chilensis* populations of the Northeast Pacific because only two additional collections have been found of *C. chilensis* from the Southern Hemisphere. Only three specimens from the Southern Hemisphere have been

confirmed (using DNA) as *C. chilensis* to date, which is too small a sample size upon which to base a description. More collections are needed from the Southern Hemisphere, particularly from the subtidal which has not yet been extensively sampled. It is especially important to update the description of the Southern Hemisphere *C. chilensis* populations given how the original morphological description of *C. chilensis* may have been based in part on the 1800's Gay collection, which as I have shown, does not represent *C. chilensis*.

While I have completed a fairly comprehensive search of the literature regarding *C. chilensis*, more research has the potential to establish the historical range of the species and to determine if the species' range or abundance has shifted since the early 1800's. The historical ranges could be inferred by obtaining and mapping the reported collection localities of *C. chilensis* from herbarium records. Reports could be traced to specimens, where possible. To establish the identity of herbarium specimens as bona fide *C. chilensis*, DNA from them should be extracted and sequenced. Sites where bona fide *C. chilensis* was collected historically (e.g. from Southern Chile and the Falkland Islands in the 1800's) should then be thoroughly resampled, to assess whether *C. chilensis* is now present or absent at those locations.

Chapter II
Future Directions in *Corallina*

2.1 Introduction

Corallina chilensis is just one of many species in *Corallina*. A number of older *Corallina* species names need re-evaluation based on molecular analysis. Some of these existing names may even apply to contemporary species that currently have only provisional names.

Corallina species, including provisional species, were recognized by Hind & Saunders (2013A) primarily based on a CO1 neighbor-joining analysis with a minimum threshold of 3.3% difference in CO1 and a three-gene concatenated tree that had no strongly supported nodes within the genus (see Hind & Saunders 2013A, Figs. 1-2). The CO1 percent difference threshold of 3.3% from Hind & Saunders 2013A was updated to 4.5-5.8% in Hind et al. (2018).
The lack of support in the Hind & Saunders (2013A) concatenated tree was likely a result of the incongruence across individual genes that I also detected.

Multiple lines of evidence should be used, where possible, when delimitating species. For this thesis, and to augment and improve upon Hind and Saunders (2013A), I conducted three types of analyses in an attempt to provide additional evidence for species boundaries within *Corallina*. I conducted phylogenetic analyses on *psb*A, CO1, and *rbc*L genes independently, as well as on multigene alignments (See Figs. 16-22 for majority rule & concatenated trees). ABGD barcoding (Table 2) and percent distance matrices (see Appendix V) were also completed as preliminary analyses, but provide supplemental support and corroborate the phylogenetic findings. Hind et al. (2018) percent difference thresholds were used as a guideline, but not as a

cutoff for species delimitation. Percent differences between sequences that fall below Hind et al. (2018) may indicate conspecificity or merely that the species are closely related. In this chapter, I review the results for other *Corallina* species besides *C. chilensis.*

2.2 Examination of currently accepted *Corallina* species

AlgaeBase (Guiry & Guiry, Retrieved May 7, 2020) lists 204 valid species names, an additional 68 infraspecific names, with 28 of these taxonomically accepted as species in *Corallina*. I included sequences corresponding to 9 of these species names in my analyses: *C. officinalis*, *C. vancouveriensis*, *C. ferreyrae*, *C. aberrans*, *C. crassissima*, *C. declinata*, *C. maxima*, *C. melobesioides*, and *C. pinnatifolia*. All three of the gene sequences were only available for some species. Some species are represented by only one or two of the genes, or by only one or a few specimens (Appendix II, Table S1).

2.2.1 Evaluation of the generitype *C. officinalis* Linnaeus

Sequences from the *C. officinalis* epitype selected by Brodie et al. (2013) were included in my study. Consistent with its delimitation as a separate species, *C. officinalis* sequences were monophyletic in each individual gene tree (Figs. 10-15) and they were also monophyletic when concatenated and included in a tree of concatenated genes (see Hind & Saunders 2013A, Fig. 1, concatenated tree.) The relationship of *C. officinalis* to other species was unresolved, due to the conflicting positions of the species in different gene trees (Figs. 10-15, 20-22).

2.2.2 Evaluation of accepted species *C. vancouveriensis* Yendo

Yendo originally described *C. vancouveriensis* in his 1902 publication on seaweeds he collected from Vancouver Island, Canada. Unfortunately his collections have not been located,

but a specimen in the University of California herbarium (UC) has been designated as a lectotype and has been sequenced (Unpublished data, Gabrielson, *pers. comm*.). I did not have access to this lectotype for my study.

My analyses support recognition of *C. vancouveriensis* as a distinct species. The individual *psb*A and CO1 gene trees demonstrated strong branch support for *C. vancouveriensis* on its own or in combination with C. sp. 2 vancouveriensis (Figs. 10-13). Likewise, *C. vancouveriensis* formed a clade in the majority rule tree (Figs. 16-19) and had fair branch support in the concatenated tree (Figs. 20-22). All three ABGD analyses delimited *C. vancouveriensis* as a separate species (Table 2).

2.2.3 Evaluation of accepted species *C. crassissima*, *C. declinata*, and *C. aberrans* (Yendo) K.R.Hind & G.W. Saunders

For some of the species that Yendo described as new to science, Yendo cited multiple localities without designating a holotype. Hind and Saunders (2013A) designated lectotypes from among Yendo's illustrations for *C. crassissima*, *C. declinata*, and *C. aberrans* corresponding with basonyms *Amphiroa crassissima, Amphiroa declinata, and Amphiroa abberans* respectively (Yendo 1902B). The published DNA sequences that I included in my analyses were from Hind and Saunders (2013A), but are not topotype material because it is unknown which of the syntype localities corresponded with each of the designated lectotypes.

This complex as a whole was monophyletic across all gene trees (Figs. 10-15) with strong support except for in the *psb*A tree (Figs. 10-11). The CO1 and *rbc*L trees (Figs. 12-15) differentiated between closely related species within the complex, but the *psb*A tree (Figs. 10-11) lacked the resolving power to differentiate between species. This is likewise reflected in the distance matrices (Table 5). The monophyly of the complex indicates that it represents at least

one distinct species within *Corallina*. The taxon sampling was incomplete for *C. declinata*, which lacked *rbc*L data and limits my ability to assess its status as a species. Knowledge of morphological and ecological factors could also perhaps provide additional lines of evidence for the distinction of species within this complex.

Table 5. Percent differences across all three genes and concatenated analyses. Percent difference ranges from Hind et al. (2018) included for ease of comparison.

	C. crassissima						Hind et al. 2018	
C. crassissima	psbA	0					psbA	0.7-1.3
	CO1	0					CO1	4.5-5.8
	rbcL	0	*C. aberrans*				rbcL	1.6-1.9
C. aberrans	psbA	0.47-0.59	psbA	0.12				
	CO1	4.54	CO1	0				
	rbcL	0.82	rbcL	/	*C. declinata*			
C. declinata	psbA	0.47	psbA	0-0.12	psbA	0		
	CO1	4.84-5.22	CO1	4.54-5.06	CO1	0.47		
	rbcL	/	rbcL	/	rbcL	/	*C. officinalis*	
C. officinalis	psbA	0.82	psbA	0.82-0.94	psbA	0.82	psbA	0
	CO1	6.12-7.41	CO1	6.68-7.12	CO1	6.68-7.44	CO1	0
	rbcL	2.62-3.83	rbcL	3-3.98	rbcL	/	rbcL	0.15-0.59

2.2.4 Evaluation of accepted species *C. ferreyrae* E.Y. Dawson, O.C.Acleto, & N.Foldvik

DNA was extracted and sequenced from a *C. ferreyrae* isotype specimen, by Bustamante et al. (2019), from which I obtained sequences for all three of my gene analyses. My analyses support *C. ferreyrae* as a distinct species within *Corallina*. *Corallina ferreyrae* isotype sequences fell within monophyletic groups in all three gene trees (Figs. 10-15) with strong support except for in *psb*A (Figs. 10-11), in which it was only moderately supported with 44.2/58/.93 branch support. *Corallina ferreyrae* sequences likewise formed monophyletic groups in each of the majority rule and concatenated trees (Figs. 16-22), with strong branch support in the concatenated tree (Figs. 20-22). Discounting the outlier PTM 826 (likely contamination) from

the CO1 analysis, all three ABGD analyses (Table 2) indicated that *C. ferreyrae* was its own distinct species.

2.2.5 Evaluation of accepted species *C. maxima* (Yendo) K.R. Hind & G.W. Saunders

Corallina maxima is associated with the basionym *Cheilosporum maximum* (Yendo 1902B). The two *C. maxima* sequences included in my analyses, one *psb*A sequence (JQ422207) and one CO1 sequence (JQ615680) (Appendix II, Table S1), were both from the same Japanese representative specimen from Hind and Saunders (2013A). There was no DNA available from the designated lectotype specimen, as it was an illustration of a specimen from an unknown locale (Yendo 1902B). Even without *rbc*L data, the divergence of its *psb*A and CO1 sequences consistently indicated that *C. maxima* is genetically distinct from other species in my dataset. *Corallina maxima* was distinguished at the species level in both *psb*A and CO1 ABGD analyses (Table 2). The two sequences appeared on longer branches, particularly in the concatenated tree (Figs. 20-22), and the clade that they formed was not well supported, due to the absence of overlapping data that serves in binding the clade together. *Corallina maxima* was sister to C. sp. 1 gws in the *psb*A tree (Figs. 10-11), but sister to the remainder of the genus in the CO1 tree (Figs. 12-13) indicating incongruence as also seen among other members of the genus. More sampling of specimens, with sequencing of loci including *rbc*L would be advisable for delimiting *C. maxima* and for understanding within-species variation (Figs. 10-13, 16-22).

2.2.6 Evaluation of accepted species *C. melobesioides* (Segawa) P.T.Martone, S.C.Lindstrom, K.A.Miller, P.W.Gabrielson

Martone et al. (2012) synonymized genus *Yamadaia* with *Corallina*. In their paper, their reference herbarium specimen from which DNA was extracted was collected from near the type locale, but was not the type specimen indicated in Segawa (1955) (Guiry & Guiry 2020).

Only one *rbc*L sequence of Japanese *C. melobesioides* was included in these analyses (UBC voucher A62034, Appendix II, Table S1). The *rbc*L ABGD analysis (Table 2) delimited it as a species, and it appeared to be closely related to *C. pinnatifolia* in the *rbc*L tree with fair branch support (85.6/83/.98) (Figs. 14-15). Interestingly, it clustered with *C. pinnatifolia* among other taxa in the concatenated tree with 88% bootstrap support (Figs. 20-22), although on a longer branch than the other taxa within the cluster. This will be further discussed below in section 2.3.5. This small amount of evidence raises a possibility that *C. melobesioides* is synonymous with *C. pinnatifolia*, which could be tested with more data.

2.2.7 Evaluation of accepted species *C. pinnatifolia* (Manza) E.Y.Dawson

Formerly referred to as *Joculator pinnatifolius* (basonym), the holotype associated with *C. pinnatifolia* was collected by F.M. Reed in 1934 (UC #545769 UC) and has not been sequenced (Dawson 1953). The Gabrielson et al. (2011) *rbc*L sequence I used in my analyses was extracted from Laguna Beach, California, specimen UBC A88590 (Appendix II, Table S1).

Corallina pinnatifolia was distinct from other known species in the *rbc*L ABGD analysis (Table 2), clustered in isolation in the majority rule tree (Figs. 16-19), yet formed a monophyletic group with 88 bootstrap support with *C. melobesiodies* in the concatenated tree (Figs. 20-22) among other provisionally named species to be discussed below in section 2.3.4. *Corallina pinnatifolia* is closely related to, or perhaps conspecific with C. sp. 2 chile, the *C. caespitosa*

holotype, and possibly *C. melobesioides* (Figs. 10-22, Appendix II, Table S1). If these names were to be synonymized, *C. pinnatifolia* would have priority over the others given that it is the oldest name.

2.2.8 Summary of evidence supporting or rejecting currently accepted species designations

In summary, of the 9 species accepted by AlgaeBase that I included in my analyses, there were at least 6 species that could be distinguished with my data. The evidence in my analyses specifically supports *C. officinalis*, *C. vancouveriensis*, *C. ferreyrae*, and *C. maxima* as delimited species. *Corallina aberrans* and *C. declinata* are closely related, possibly conspecific, and possibly also conspecific with *C. crassissima*. Similarly, *C. melobesioides* and *C. pinnatifolia* are closely related and may be the same species. More sequence data from more specimens and an examination of ecological and morphological evidence is required to determine explicit boundaries within these potential complexes.

2.3 Provisionally identified *Corallina* species

Prior to this study, Hind and Saunders (2013A) gave provisional identifiers to seven species that have yet to be confirmed or described as new. These were: C. sp. 2 vancouveriensis, C. sp. 1 gws, C. sp. 1 california, C. sp. 2 frondescens, C. sp. 3 frondescens, C. sp. 4 frondescens, and C. sp. 5 frondescens. I further added C. sp. 1 gws-like, C. sp. 3 frondescens-like, C. ferreyrae-like, C. sp. 1 chile, and C. sp. 2 chile.

2.3.1 C. sp. 3 frondescens & C. sp. 3 frondescens-like

The C. sp. 3 frondescens complex formed a well-supported clade across all phylogenetic analyses (Figs. 10-22) with the exception of the *psb*A tree (Figs. 10-15), which did not have

strong support, possibly because of long branch attraction with outgroup *Ellisolandia* in the Maximum Likelihood/aLRT *psb*A tree.

My study supported delimiting C. sp. 3 frondescens-like as a closely related species separate from C. sp. 3 frondescens (Hind & Saunders 2013A). Corallina sp. 3 frondescens and C. sp. 3 frondescens-like sequences clustered together across all individual gene trees in the same clade with strong support. The concatenated tree (Figs. 20-22) also grouped the clade containing C. sp. 3 frondescens-like sequences as sister to the clade containing C. sp. 3 frondescens sequences with strong branch support.

Other than the ABGD *psb*A analysis, which did not separate the two, (Table 2), the majority of the phylogenetic and ABGD evidence strongly supported delimiting C. sp. 3 frondescens-like as a species separate from C. sp. 3 frondescens. The two were distinguishable in both CO1 and *rbc*L ABGD analyses (Table 2). The C. sp. 3 frondescens-like sequences clustered together exclusively in two out of three genes, a rare occurrence in the majority rule analysis (Figs. 16-19). Again, more sampling, more sequencing, and in-depth morphological analysis could provide additional support for either conspecificity or separation. Meanwhile, based on the preponderance of the evidence, I interpret C. sp. 3 frondescens and C. sp. 3 frondescens-like as two separate species that have diverged recently.

A potential 3rd species in this group was indicated by the CO1 ABGD analysis that designated a single voucher, PTM 1400, UBC A92938 as a different species from the other C. sp. 3 frondescens sequences (Table 2, Appendix II, Table S1). As this is a single sequence from one sample, more substantial evidence, from more gene sequences and from additional collections from where PTM 1400 was found would be required for confirmation that it is indeed a distinct species across other genes besides CO1. The sequence from specimen PTM 1400

accounts for the over two percent difference range (3.03-5.3% difference) within C. sp. 3 frondescens in the CO1 distance matrix (Table 6). Corallina sp. 3 frondescens PTM 1400/UBC A92938 likewise stood apart from all other C. sp. 3 frondescens sequences in the concatenated analysis (Figs. 20-22), but was included as a longer branch within the cluster in the majority rule tree where sequences were not concatenated (Figs. 16-19).

Table 6. Percent differences across all three genes and concatenated analyses. Percent difference ranges from Hind et al. (2018) included for ease of comparison.

			Hind et al. 2018	
			psbA	0.7-1.3
			CO1	4.5-5.8
			rbcL	1.6-1.9
	C. sp. 3 frondescens			
C. sp. 3 frondescens-like	psbA	0.41-0.59		
	CO1	3.03-5.3		
	rbcL	0.72-1.2	C. sp. 3 frondescens-like	
C. officinalis	psbA	1.06-1.18	psbA	1.29
	CO1	8.32-8.42	CO1	7.42-8.02
	rbcL	2.62-3.24	rbcL	2.77-3.39
C. vancouveriensis	psbA	1.41-1.65	psbA	1.76
	CO1	7.66-8.85	CO1	8.47-8.77
	rbcL	3.55-3.71	rbcL	3.46-3.55

2.3.2 C. sp. 1 gws & C. sp. 1 gws-like

Corallina sp. 1 gws formed a sister group to C. sp. 1 gws-like with strong support across all three gene and concatenated trees (Figs. 10-15, 20-22), supporting both as a new and distinct *Corallina* species. Following a similar pattern, ABGD analysis of CO1 and *rbc*L also supported the separate species while the ABGD analysis of *psb*A did not resolve them as different (Table 2 & Figs. 10-11). Both taxa were distinct from *C. officinalis* based on percent difference across all distance matrices (Table 7) well above the comparable ranges described by Hind et al. (2018). Corallina sp. 1 gws-like was not very dissimilar from C. sp. 1 gws when comparing percent

differences (Table 7), well below Hind et al. 2018 percent difference thresholds across all three genes.

The evidence described above overwhelmingly supports the species distinction of C. sp. 1 gws, one of the provisionally identified species suggested by Hind & Saunders (2013A). However, C. sp. 1 gws-like may be too closely related to C. sp. 1 gws to be a distinct species, and could simply reflect the wide range of genetic variation within the species (Figs. 10-22).

Table 7. Percent differences across all three genes and concatenated analyses. Percent difference ranges from Hind et al. (2018) included for ease of comparison.

	C. sp. 1 gws		C. sp. 1 gws-like		*C. officinalis*	
C. sp. 1 gws	psbA	0				
	CO1	0				
	rbcL	/				
C. sp. 1 gws-like	psbA	0.24	psbA	/		
	CO1	3.18	CO1	/		
	rbcL	0.52	rbcL	0		
C. officinalis	psbA	1.18	psbA	1.41	psbA	0
	CO1	8.02-8.16	CO1	9.23-9.46	CO1	0
	rbcL	0.15-0.59	rbcL	2.85-3.24	rbcL	0.15-0.59

Hind et al. 2018	
psbA	0.7-1.3
CO1	4.5-5.8
rbcL	1.6-1.9

2.3.3 C. sp. 2 vancouveriensis

Corallina sp. 2 vancouveriensis was also identified in Hind and Saunders (2013A) who reported it as morphologically cryptic with *C. vancouveriensis*. However, there are still no *rbc*L sequences for C. sp. 2 vancouveriensis and only one *psb*A and one CO1 sequence available. Corallina sp. 2 vancouveriensis was sister to *C. vancouveriensis* in the *psb*A and CO1 trees (Figs. 12-13), but without branch support. Corallina sp. 2 vancouveriensis appeared distinct from *C. vancouveriensis* in the *psb*A and CO1 distance matrices (Table 8). The status of the species cannot be evaluated due to lack of overlapping data.

Table 8. Percent differences across all three genes and concatenated analyses. Percent difference ranges from Hind et al. (2018) included for ease of comparison.

	C. sp. 2 vancouveriensis						Hind et al. 2018	
C. sp. 2 vancouveriensis	psbA	/					psbA	0.7-1.3
	CO1	/					CO1	4.5-5.8
	rbcL	/	*C. vancouveriensis*				rbcL	1.6-1.9
C. vancouveriensis	psbA	0.71-0.71	psbA	0				
	CO1	4.08-4.39	CO1	0-0.45				
	rbcL	/	rbcL	/	*C. officinalis*			
C. offcinalis	psbA	1.53	psbA	1.18	psbA	0		
	CO1	3.53-4.24	CO1	3.9-4.08	CO1	0		
	rbcL	/	rbcL	2.74-3.1	rbcL	0.15-0.59		

2.3.4 Taxa surrounding *C. ferreyrae* E.Y. Dawson, O.C. Acleto, & N. Foldvik

Bustamante (2019) sequenced the Peruvian isotype specimen of *C. ferreyrae* (Peru, Voucher #UC 1404138), and determined that it was conspecific with Northern Atlantic *C. caespitosa* (R.H.Walker, J.Brodie & L.M.Irvine). The older binomial name *C. ferreyrae* has priority over the newer name, *C. caespitosa*. Bustamante (2019) reported that "A BLAST analysis of cox1, *psb*A, and *rbc*L gene markers of *C. ferreyrae* found exact matches to sequences of *C. caespitosa* R.H. Walker, J. Brodie, and L.M. Irvine (Walker et al. 2009)". However, Walker (2009) only used 18S rRNA and CO1 sequences in their study. While Williamson et al. (2015) later sequenced *rbc*L, *psb*A must not have been used in the analyses. Incomplete overlap of data between research groups and a broader definition of "species" in Bustamante et al. (2019) could account for the discrepancy between my study and Bustamante's findings. I included sequences from Walker et al. (2009) and Bustamante et al. (2019) as well as sequences from other specimens collected from South America (Appendix II, Table S1). Across all three gene trees in my analysis (Figs. 10-15), *C. ferreyrae* sequences consistently formed a clade with C. sp.1 chile, C. sp. 2 chile, and C. ferreyrae-like, which appear to be four different species in my analysis.

C. sp. 1 chile

Corallina sp. 1 chile was monophyletic with strong branch support across all phylogenetic tree analyses (Figs. 10-22). It was delimited as a species across all three ABGD analyses (Table 2), and exhibited high percent difference from *C. ferreyrae* and related species with respect to *psb*A and CO1 gene sequences (Table 9). Interestingly, there was low percent difference between C. sp. 1 chile and other closely related species with respect to *rbc*L (Table 8). This disagreement between *psb*A and *rbc*L is also clearly reflected by *psb*A and *rbc*L tree topologies (Figs. 10-11, 14-15). Despite the similarity of *rbc*L sequences, the majority of evidence, and with a healthy sample size (N=14), strongly supports C. sp. 1 chile as a distinct species and C. sp. 1 chile therefore needs to be given a concrete name, morphological assessment, and described as new to science. This species is a good example of where one might come to a different conclusion if they based their species delimitation on only one gene or only one line of evidence.

C. ferreyrae-like

I applied the provisional name "C. ferreyrae-like" to differentiate specimens that had been previously identified as *C. ferreyrae*, based on a BLAST search of one gene. Phylogenetically, C. ferreyrae-like consistently formed a sister group to *C. ferreyrae* sequences with strong branch support for the exclusively C. ferreyrae-like clade (Figs. 10-15). Interestingly, *C. ferreyrae* was collected from Peru, whereas all C. ferreyrae-like specimens were from Japan. Corallina ferreyrae-like sequences formed a monophyletic group with very strong branch support in all three gene trees (Figs. 10-15). It also formed a clade in the majority rule tree with 67% branch value (Figs. 16-19), an unusually high consistency for this particular genus. Corallina

ferreyrae-like was delimited as a species in every ABGD analysis (Table 2) and with a few exceptions was dissimilar from other neighboring species in the distance matrices (Table 9). According to my analyses, C. ferreyrae-like is distinct from *C. ferreyrae* and may perhaps be correlated with a Japanese type specimen, as all three C. ferreyrae-like specimens (Appendix II, Table S1) were collected in Japan. If not correlated with a type, it should undergo morphological analysis, receive a concrete name, and be described as new to science.

2.3.5 C. sp. 2 chile complex within the *C. ferreyrae* clade

This complex is composed of provisional species C. sp. 2 chile, the *C. caespitosa* holotype (Walker et al. 2009), *C. melobesioides*, and *C. pinnatifolia* (HQ322333). *Corallina caespitosa,* which was found to occur in both hemispheres and both the Atlantic and Pacific oceans (Walker et al. 2009), was recently synonymized with the older existing name *C. ferreyrae* because it corresponded with a Peruvian *C. ferreyrae* specimen in a genetic analysis (Bustamante et al. 2019). Bustamante et al. (2019) were correct in applying the older name *C. ferreyrae* for sequences that correlated with the type specimen. However, Bustamante et al. (2019) applied a broad species concept. If the clade containing *C. ferreyrae* is split into the four narrower species supported in my analysis, then *C. caespitosa* has to be synonymized under *C. pinnatifolia*, the oldest name for the C. sp. 2 chile clade (Manza 1937, Dawson 1953).

Especially considering the sample size (N=11), my analyses indicated that the C. sp. 2 chile complex represents at least one distinct species. The majority rule tree (Figs. 16-19) and the concatenated tree (Figs. 20-22) supported C. sp. 2 chile as a clear clade, receiving 88% bootstrap support from the concatenated alignment (Figs. 20-22). Corallina sp. 2 chile formed a clade with strong branch support in the psbA tree (Figs. 10-11). In the CO1 tree however, three clades of

sequences of C. sp. 2 chile or *C. caespitosa* appeared paraphyletic to *C. ferreyrae*, and C. ferreyrae-like (Figs. 10-11). The paraphyly of C. sp. 2 chile in the CO1 tree seems to reflect biogeography (See Figs. 12-13). That is, the United States C. sp. 2 chile specimen is sister to the *C. caespitosa* holotype from England, which is in turn sister to a monophyletic strongly supported cluster of three Chilean C. sp. 2 chile specimens. More samples would be required to test for geographical structure in species diversity. Similarly in the *rbc*L tree, C. sp. 2 chile sequences were likewise split into two clades that were sister to C. sp. 1 chile (Figs. 14-15). Based on distances (Table 9), C. sp. 2 chile is also similar to C. sp.1 chile with respect to *rbc*L.

Table 9. Percent differences across all three genes and concatenated analyses. Percent difference ranges from Hind et al. (2018) included for ease of comparison.

	C. ferreyrae (Bustamante)						Hind et al. 2018	
C. ferreyrae (Bustamante)	psbA	/					psbA	0.7-1.3
	CO1	/					CO1	4.5-5.8
	rbcL	/	C. ferreyrae-like				rbcL	1.6-1.9
C. ferreyrae-like	psbA	0.82	psbA	0				
	CO1	3.03	CO1	0				
	rbcL	0.82	rbcL	0	C. sp. 1 chile			
C. sp. 1 chile	psbA	1.06-1.18	psbA	0.94-1.06	psbA	0-0.12		
	CO1	6.05-6.51	CO1	6.96	CO1	0.15-0.45		
	rbcL	0.97	rbcL	1.2	rbcL	0	C. sp. 2 chile	
C. sp. 2 chile	psbA	0.82	psbA	0.94-1.06	psbA	1.06-1.29	psbA	0-0.12
	CO1	3.03-3.48	CO1	3.03-3.48	CO1	4.39-5.89	CO1	0-1.37
	rbcL	0.37-0.68	rbcL	0.6-0.75	rbcL	0.6-0.9	rbcL	0.3

2.3.6 C. sp. 1 california

Corallina sp. 1 california appeared monophyletic across all phylogenetic analyses, with moderate branch support in the *psb*A tree (Figs. 10-12), strong support in the CO1 and *rbc*L trees (Figs. 12-15), and 61 percent bootstrap support in the concatenated tree (Figs. 20-22).

The CO1 ABGD analysis is the only ABGD analysis that grouped C. sp. 1 california independently from other species (Table 2). The *psb*A ABGD analysis did not resolve C. sp. 1

california separately from *C. officinalis*, *C. chilensis*, C. sp. 2 frondescens and C. sp. 5 frondescens (Table 2). The *rbc*L ABGD analysis grouped C. sp. 1 california with *C. chilensis* and C. sp. 5 frondescens. The distance matrices likewise showed that C. sp. 1 california is similar to these species with respect to *psb*A and *rbc*L (Table 10). However, the percent difference between C. sp. 1 california and its closely related species is unusually high with respect to CO1.

Given that C. sp. 1 california forms a well-supported clade across the phylogenetic analyses, it is likely to be a distinct species new to science, despite the lack of resolution in ABGD and percent distance analyses.

2.3.7 C. sp. 4 frondescens

Corallina sp. 4 frondescens sequences formed strongly supported clades across all phylogenetic trees (Figs. 10-22), including with N = 9 sample size in the concatenated tree (Figs. 20-22). It likewise resolved independently in the ABGD analyses (Table 2). The division that appears in the CO1 ABGD analysis and presence of multiple clades in the CO1 phylogeny is likely on account of biogeographical variation (Table 2, Figs. 12-13). Corallina sp. 4 frondescens exhibited high percent difference in at least one gene from all other species to which it was closely related (Table 10). Collectively the evidence in my analyses confirms Hind & Saunders (2013A) recognition of C. sp. 4 frondescens as a distinct species, and it is in need of morphological description and a concrete name.

2.3.8 C. sp. 2 frondescens

Corallina sp. 2 frondescens formed a moderately supported clade in the *psb*A tree (Figs. 10-11) and a strongly supported clade in the CO1 tree (Figs. 12-13). There was only one sequence for the *rbc*L gene. The CO1 and *rbc*L ABGD analyses (Table 2) delimited C. sp. 2

frondescens as a distinct species from all other taxa while the *psb*A ABGD analysis included it in a broader group (Table 2). The difference between C. sp. 2 frondescens and other species was most obvious from the CO1 distance matrix (Table 10). Corallina sp. 2 frondescens was similar to C. sp. 1 california and C. sp. 5 frondescens in the *psb*A distance matrix (Table 10) and somewhat similar to C. sp. 1 california, *C. chilensis*, and C. sp. 5 frondescens in the *rbc*L distance matrix (Fig. 10). While evidence is mixed and more *rbc*L sequence replicates would be desirable, overall my analyses indicate that C. sp. 2 frondescens is likely an independently evolving population; a distinct species that requires a concrete name and description.

2.3.9 C. sp. 5 frondescens

Corallina sp. 5 frondescens sequences formed a strongly supported clade in the CO1 gene tree (Figs. 12-13). It also appeared to be a distinct clade in the *rbc*L tree (Figs. 14-15), although it lacked branch support because there was only one sequence (N=1). Corallina sp. 5 frondescens created a well formed clade in the majority rule tree (Figs. 16-19) and in the concatenated tree, but only with 42 percent bootstrap support (Figs. 20-22). The cluster of three C. sp. 5 frondescens sequences in the *psb*A tree did not have any branch support (Figs. 10-11).

Corallina sp. 5 frondescens was delimited as a separate species only in the CO1 ABGD analysis (Table 2). Corallina sp. 5 frondescens was most distant from other similar species based on the CO1 gene in distance matrices (Fig. 9). Corallina sp. 5 frondescens appears to be an independently evolving population judging by its isolation or branch length in the phylogenetic analyses (Figs. 10-22), but additional evidence is recommended to confirm distinct species status, especially considering the weak support in the concatenated tree and lack of branch support in the *psb*A and *rbc*L gene trees.

Table 10. Percent differences across all three genes and concatenated analyses. Percent difference ranges from Hind et al. (2018) included for ease of comparison.

	C. chilensis								Hind et al. 2018	
C. chilensis	psbA	0-0.24							psbA	0.7-1.3
	CO1	0.45-0.91							CO1	4.5-5.8
	rbcL	0.09	C. sp. 1 california						rbcL	1.6-1.9
C. sp. 1 california	psbA	0.35-0.47	psbA	0						
	CO1	7.41-8.02	CO1	0-0.15						
	rbcL	0.9-1.08	rbcL	0.15	C. sp. 2 frondescens					
C. sp. 2 frondescens	psbA	0.59-0.82	psbA	0.47	psbA	0				
	CO1	6.96-7.26	CO1	6.51-6.66	CO1	0.3				
	rbcL	1.12-1.17	rbcL	1.12-1.27	rbcL	/	C. sp. 4 frondescens			
C. sp. 4 frondescens	psbA	0.71-0.94	psbA	0.59	psbA	0.47-0.59	psbA	0		
	CO1	5.9-6.66	CO1	6.51-6.66	CO1	5.9-6.2	CO1	0-1.82		
	rbcL	1.55-1.63	rbcL	1.33-1.55	rbcL	1.63-1.75	rbcL	0.17	C. sp. 5 frondescens	
C. sp. 5 frondescens	psbA	0.24-0.59	psbA	0.12-0.24	psbA	0.35-0.47	psbA	0.12-0.24	psbA	0-0.12
	CO1	5.9-6.05	CO1	4.99-5.14	CO1	5.14	CO1	4.54-4.69	CO1	0
	rbcL	0.81	rbcL	0.64-0.73	rbcL	1.05	rbcL	1.13-1.18	rbcL	/

2.4 Conclusion

Conclusions regarding the number of species in *Corallina* may vary across studies depending on the gene or combination of genes used to delimit species, and the type of analyses implemented. For instance, some new species proposed by Hind and Saunders (2013A) were appropriate based solely on CO1 percent difference and the CO1 ABGD analysis, but with more data from *psb*A and *rbc*L matrices combined with ABGD analyses, I have expanded the conclusions. ABGD analysis was sensitive to the variation from gene to gene. The most variable locus, CO1, resulted in the narrowest species delimitations. The *psb*A gene was least variable, and provided the broadest delimitations.

Percent difference ranges of existing published species may be used as guidelines to maintain consistency across studies, but the ranges may also need reevaluation as newer data become available. Some of the proposed species in Hind and Saunders (2013A) were indistinguishable from each other in *psb*A and *rbc*L, but differed from other species by >5% in CO1 sequences. If I had just used the CO1 gene to differentiate species, many of the proposed species would be considered distinct based on this single line of evidence, whereas *C.*

vancouveriensis would not be considered distinct from *C. officinalis* (Table 8). If on the other hand I differentiated species by using *psb*A or *rbc*L gene analyses and not CO1, *C. vancouveriensis* would be distinct, but many of the proposed species would be indistinct from one another. This is the reason it is necessary to use multiple genes and multiple lines of evidence when determining species boundaries.

While my study provided additional evidence that confirmed suggestions from previous studies, given the discrepancies and incongruence across analyses, even using three genes and three lines of evidence (phylogenetic analyses, ABGD analyses, percent distance matrices) likely barely scratched the surface with respect to species delimitation. Clearly, there are still many remaining research opportunities within genus *Corallina* alone, whether confirming existent species or describing new species; and this genus is only one of the many genera in need of taxonomic reform.

For the next steps forward, I first propose an effort to consolidate a small amount of material from as many *Corallina* type specimens known and available. Ideally DNA could be extracted from as many types as possible, and published so that all research groups could use them to guide the application of names to species. Secondly, I propose obtaining more samples for each species in *Corallina* from across any given species' entire known geographic range. Ideally high-throughput sequencing would be used, combined with ecological metadata, and morphological analysis, so that hundreds of loci may be compared using more sophisticated delimitation techniques to create phylogenetic trees that more closely represent actual species trees. Obtaining robust species trees might likewise enable us to identify additional barcoding genes (Zhan et al. 2020). However, even merely increasing the sample size of each species to at least N=10 (Carsten 2013) and sequencing 3-5 genes for every species would be a good first

step. That would provide better resolution with respect to species boundaries and the range of intraspecific variation. Once we know the extent of variation within each species and there are clearer species boundaries, we will then be able to more accurately determine species biogeographical ranges.

References

Abbott, I.A., Hollenberg, G.J. 1976. Marine algae of California. Stanford, California. Stanford University Press.

Anisimova, M. Gascuel, O. 2006. Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. *Systematic Biology* 55: 539-552.

Areschoug, J.E. 1852. Ordo XII. Corallineae. In Agardh, J. G. [Ed.] Species genera et ordines algarum... Volumen secundum: algas florideas complectens. C.W.K. Gleerup, Lund, Sweden, 506–576.

Ardissone, F. 1888. Le alghe della Terra del Fuoco raccolte dal prof. Spegazini. Rendiconti del Reale Istituto Lombardo di Scienze e Lettere. 2(21): 208-215.

Armstrong, S. L. 1988. Mechanical properties of the tissues of the brown algae *Hedophyllum sessile* (C. Ag.) Setchell: variability with habitat. *Journal of Experimental Marine Biology and Ecology* 114: 143-151.

Armstrong, S.L. 1989. The behavior in flow of the morphologically variable seaweed *Hedophyllum sessile* (C. Ag.) Setchell. *Hydrobiologia* 183: 115-122.

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. *Botanica Marina* 3: 15-22.

Bell, N.E., Hyvönen, J. 2010. Phylogeny of the moss class Polytrichopisda (Bryophyta): Generic-level structure and incongruent gene trees. *Molecular Phylogenetics and Evolution* 55: 381-398.

Bergsten, J., Bilton, D.T., Fujisawa, T., Elliott, M., Monaghan, M.T., Balke, M., Hendrich, L., Geijer, J., Herrmann, J., Foster, G.N., Ribera, I., Nilsson, A.N., Barraclough, T.G., Vogler, A.P. 2012. The effect of geographical scale of sampling on DNA barcoding. *Systematic Biology* 61(5): 851-869.

Blanchette, C.A. 1997. Size and survival of intertidal plants in response to wave action: a case study with *Fucus gardneri*. *Ecology* 78(5): 1563-1578.

Blanchette, C.A., Miner, B.G., and Gains, S.D. 2002. Geographic variability in form, size and survival of *Egregia menziesii* around Point Conception, California. *Marine Ecology Progress Series* 239: 69-82.

Boller, M.L., Carrington, E. 2006. The hydrodynamic effect of shape and size change during reconfiguration of a flexible macroalga. *Journal of Experimental Biology* 209(10): 1894-1903.

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 Algologie* 34(1): 49-56.

Broom, J.E., Hart, D.R., Farr, T.J., Nelson, W.A., Neill, K.F., Harvey, A.S. 2008. Utility of *psb*A and nSSU for phylogenetic reconstruction in the Corallinales based on New Zealand taxa. *Molecular Phylogenetics and Evolution 46*(3): 958-973.

Bustamante, D.E., Calderon, M.S., Hughey, J.R. 2019. Conspecificity of the Peruvian *Corallina ferreyrae* with *C. caespitosa* (Corallinaceae, Rhodophyta) inferred from genomic analysis of the type specimen. *Mitochondrial DNA Part B* 4(1): 1285-1286.

Caragnano, A., Foetisch, A., Maneveldt, G.W., Millet, L., Liu L.C., Lin, S.M., Rodondi, G., Payri, C.E. 2018. *Phycological Society of America* 54: 391-409.

Carstens, B.C., Pelletier, T.A., Reid, N.M., Satler, J.D. 2013. How to fail at species delimitation. *Molecular Ecology* 22: 4369-4383.

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. *European Journal of Phycology* 48(1): 27-46.

Collado-Vides, L. 2002. Morphological plasticity of *Caulerpa prolifera* (Caulerpales, Chlorophyta) in relation to growth form in a coral reef lagoon. *Botanica Marina* 45(2): 123-129.

Cranston, K.A., Hurwitz, B., Ware, D., Stein, L., Wing, R.A. 2009. Species trees from highly incongruent gene trees in rice. *Systematic Biology* 58(5): 489-500.

Dawson, E.Y. 1953. Marine red algae of Pacific Mexico. Part 1. Bangiales to Corallinaceae subf. Corallinoidae. *Allan Hancock Pacific Expeditions* 17: 1-239, Plates 1-33.

Dawson, E.Y., Acleto, O.C., Foldvik, N. 1964. The seaweeds of Peru. Behefte zur Nova Hedwigia. 13: 1-111.

Denny, M., Mach, K., Tepler, S., Martone, P.T. 2013. Indefatigable: an erect coralline alga is highly resistant to fatigue. *Journal of Experimental Biology* 216 (20): 3772-3780.

Denny, M.W., Thomas, D.L., Koehl, M.A.R. 1985. Mechanical limits to size in wave-swept organisms. *Ecological Monographs* 55(1): 69-102.

DeQueiroz, K.D. 2007. Species Concepts and Species Delimitation. *Systematic Biology* 56(6): 879-886.

DeWitt, T.J., Scheiner, S.M. (Editors) 2004. Phenotypic plasticity: functional and conceptual approaches. Oxford University Press.

Ellis, J. 1755. An essay towards a natural history of the corallines, and other marine productions of the like kind, commonly found on the coasts of Great Britain and Ireland. London. (Self-published, privately printed)

Farr, T., Broom, J., Hart, D., Neill, K., Nelson, W. 2009. Common coralline algae of northern New Zealand: an identification guide. *NIWA Information Series* 70: 1–249.

Foslie, M. 1907. Antarctic and Subantarctic Corallinaceae. In: Wissenschaftliche Ergebnisse der Suedpolar-Expedition 1901-1903 unter Leitung von Dr. Otto Nordenskjoeld. (Eds) Volume 4 (1) pp. 1-16 Stockholm: Lithographisches Institut des Generalstabs.

Foslie, M. 1908. Algologiske notiser V. *Kongelige Norske Videnskabers Selsskabs Skrifter* 7: 1-20.

Freshwater, W.D., Rueness, J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on rbcL nucleotide sequence analysis. *Phycologia* 33: 187-194.

Gabrielson, P.W. 2008A. Molecular sequencing of Northeast Pacific type material reveals two earlier names for Prionitis lyallii, Prionitis sternbergii and Prionitis jubata (Halymeniaceae, Rhodophyta). *Phycologia* 47: 89–97.

Gabrielson, P.W. 2008B. On the absence of previously reported Japanese and Peruvian species of Prionitis (Halymeniaceae, Rhodophyta) in the Northeast Pacific. *Phycological Research* 56: 105–114.

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(3): 298-316.

Gabrielson, P.W., Lindstrom, S.C. 2018. Keys to the seaweeds and seagrasses of southeast Alaska, British Columbia, Washington, and Oregon. Island Blue/Printorium Bookworks. Victoria, British Columbia.

Gaylord, B., Blanchette, C.A., Denny, M.W. 1994. Mechanical consequences of size in wave-swept algae. *Ecological Monographs* 64(3): 287-313.

Gerard, V.A., Mann, K.H. 1979. Growth and production of *Laminaria longicuris* (Phaeophyta) populations exposed to different intensities of water movement. *Journal of Phycology* 15: 33-41.

Goldsmit, J., Archambault, P., Chust, G., Villarino, E., Liu, G., Lukovich, J.V., Barber, D.G., Howland, K.L. 2018. Projecting present and future habitat suitability of ship-mediated aquatic invasive species in the Canadian Arctic. Biological Invasions 20: 501-517.

Graham, L.E., Wilcox, L.W., Graham, J.M. 2009. Algae, 2nd edition, Benjamin Cummings, San Francisco.

Guiry, M.D. & Guiry, G.M. 2020. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway.

Guenther, R.J., Martone, P.T. 2014. Physiological performance of intertidal coralline algae during a simulated tidal cycle. *Journal of Phycology* 50: 310-321.

Hariot, P. 1889. Algues. In: Mission Scientifique du Cap Horn. 1882-1883. Tome V. Botanique. Cryptogamie. (Hariot, P., Petit, P., Muller De Argovie, J., Bescherelle, E., Massolongo, C. & Franchet, A. Eds). Gauthier-Villars et Fils, Imprimieurs Libraires, Paris.

Harvey, W.H. 1849. Nereis Australis, algae of the Southern Ocean. Reeve Brothers, London.

Harvey, A.S., Broadwater, S.T., Woelkerling, W.J., Mitrovski, P.J. 2003. Choreonema (Corallinales, Rhodophyta): 18S rDNA Phylogeny and Resurrection of the Hapalidiaceae for the Subfamilies Choreonematoideae, Austrolithoideae, and Melobesioideae. *Journal of Phycology* 39: 988-998.

Harvey, A. S., Woelkerling, W. J., Farr, T. J., Neill, K. F., Nelson, W. A. 2005. Coralline algae of central New Zealand: an identification guide to common 'crustose' species. *NIWA Information Series* 57: 1–145.

Harvey, A.S., Woelkerling, W.J., Reviers, B. 2020. A taxonomic analysis of Jania (Corallinaceae, Rhodophyta) in south-eastern Australia. *Australian Systematic Botany* 33: 221-277.

Hind, K., Gabrielson, P., Jensen, C., Martone, P.T. 2018. Evolutionary reversals in *Bossiella* (Corallinales, Rhodophyta): first report of a coralline genus with both geniculate and non-geniculate species. *Journal of Phycology* 54: 788-798.

Hind, K.R., Gabrielson, P.W., Jensen, C., Martone, P.T. 2016. *Crusticorallina gen. nov.*, a non-geniculate genus in the subfamily Corallinoideae (Corallinales, Rhodophyta). *Journal of Phycology* 52: 929-941.

Hind, K.R., Gabrielson, P.W., Lindstrom, S.C., Martone, P.T. 2014A. Misleading morphologies and the importance of sequencing type specimens for resolving coralline taxonomy (Corallinales, Rhodophyta): *Pachyarthron cretaceum* is *Corallina officinalis*. *Journal of Phycology* 50(4): 760-764.

Hind, K.R., Gabrielson, P.W., Saunders, G.W. 2014B. Molecular-assisted alpha taxonomy reveals pseudocryptic diversity among species of *Bossiella* (Corallinales, Rhodophyta) in the eastern Pacific Ocean. *Phycologia* 53: 443-456.

Hind, K.R., Miller, K.A., Young, M., Jensen, C., Gabrielson, P.W., Martone, P.T. 2015. Resolving cryptic species of *Bossiella* (Corallinales, Rhodophyta) using contemporary and historical DNA. *American Journal of Botany* 102(11): 1912-1930.

Hind, K.R., Saunders, G.W. 2013A. A molecular phylogenetic study of the tribe Corallineae (Corallinales, Rhodophyta) with an assessment of genus-level taxonomic features and descriptions of novel genera. *Journal of Phycology* 49(1): 103-114.

Hind, K.R., Saunders, G.W. 2013B. Molecular markers from three organellar genomes unravel complex taxonomic relationships within the coralline algal genus *Chiharaea* (Corallinales, Rhodophyta). *Molecular Phylogenetics and Evolution* 67: 529-540.

Hippler, D., Buhl, D., Witbaard, R., Richter, D.K., Immenhauser, A. 2009. Towards a better understanding of magnesium-isotope ratios from marine skeletal carbonates. *Geochimica et Cosmochimica Acta* 73(20): 6134-6146.

Holzinger, A., Karsten, U. 2013. Desiccation stress and tolerance in green algae: Consequences for ultrastructure, physiological and molecular mechanisms. *Frontiers in Plant Science* 4: 1-18.

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 assessment." *Botany* 90: 1191–1194.

Hughey, J. R., Silva, P. C., Hommersand, M. H. 2001. Solving taxonomic and nomenclatural problems in Pacific Gigartinaceae (Rhodophyta) using DNA from type material. *Journal of Phycology* 37: 1091–1109.

Hughey, J.R., Silva, P.C., Hommersand, M.H. 2002. ITS1 sequences of type specimens of Gigartina and Sarcothalia and their significance for the classification of South African Gigartinaceae (Gigartinales, Rhodophyta). *European Journal of Phycology* 37: 209-216.

Hunt, L.J.H., Denny, M.W. 2008. Desiccation protection and disruption: A trade-off for an intertidal marine alga. *Journal of Phycology* 44(5): 1164-1170.

Janouškovec, J., Liu, S.L., Martone, P.T., Carré, W., Leblanc, C., Collén, J., Keeling, P.J. 2013. Evolution of red algal plastid genomes: Ancient architectures, introns, horizontal gene transfer, and taxonomic utility of plastid markers. *PLoS ONE* 8(3): e59001.

Janot, K., Martone, P.T. 2018. Bending strategies of convergently-evolved, articulated coralline algae. *Journal of Phycology* 54: 305-316.

Janot, K., Martone, P.T. 2016. Convergence of joint mechanics in independently evolving, articulated coralline algae. *Journal of Experimental Biology* 219: 383-391.

Jarvis, C.E., Barrie, F.R., Allan, D.M., Reveal, J.L., 1993. A list of Linnaean generic names and their types. *Regnum Vegetabile* 127: 1-100.

Jarvis, E.D., Mirarab, S., Aberer, A.J., Li, B., Houde, P., Li, C.,…Zhang, G. 2014. Whole-genome analyses resolve early branches in the tree of life of modern birds. *Science* 246(6215): 1320-1331.

Jeong, S.Y., Won, B.Y., Hassel, K., Cho, T.O. 2019. Revision of *Phymatolithon purpureum* (Hapalidiales, Rhodophyta) based on ultrastructural and molecular data. *European Journal of Phycology* 54(3): 326-341.

Johansen, H.W. 1969. Morphology and systematics of coralline algae with special reference to *Calliarthron*. University of California Publications in Botany 49: 1-98.

Johansen, H.W. 1981. Coralline algae, a first synthesis. Boca Raton, Florida, CRC Press.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermiin, L.S. 2017. ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. *Nature Methods* 14: 587-589.

Katoh, S. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*. 30: 772-780.

Kim, J.H., Guiry, M.D., Oak, J.H., Choi, D.S., Kang, S.H., Chung, H., Choi, H.G. 2007. Phylogenetic relationships within the tribe Janieae (Corallinales, Rhodophyta) based on molecular and morphological data: a reappraisal of Jania. *Journal of Phycology* 43: 1310-1319.

Koehl, M.A.R., Silk, W.K., Liang, H. 2008. How kelp produce blade shapes suited to different flow regimes: A new wrinkle. *Integrative and Comparative Biology* 48(6): 834-851.

Kucera, H., Saunders, G.W. 2012. A survey of Bangiales (Rhodophyta) based on multiple molecular markers reveals cryptic diversity. *Journal of Phycology* 48: 869-882.

Kützing, F.T. 1858. Tabulae phycologicae oder Abbildunger der Tange. Volume 8. Nordhausen.

Kützing, F.T. 1849. Species algarum. Leipzig. F.A. Brockhaus.

Lee, J., Kim, K.M., Yang, E.C., Miller, K.A., Boo, S.M., Bhattacharya, D., Yoon, H.S. 2016. Reconstructing the complex evolutionary history of mobile plasmids in red algal genomes. *Scientific Reports* 6(1): 23744.

Lee, J.M., Song, H.J., Park, S.I., Lee, Y.M., Jeong, S.Y., Cho, T.O., Kim, J.H…Yoon, H.S. 2018. Mitochondrial and plastid genomes from coralline red algae provide insights into the incongruent evolutionary histories of organelles. *Genome Biology and Evolution* 10(11): 2961-2972.

Le Gall, L., Payri, C.E., Bittner, L., Saunders, G.W. 2010. Multigene phylogenetic analyses support recognition of the Sporolithales ord. nov. *Molecular Phylogenetics and Evolution* 54: 302-305.

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. *Journal of Phycology* 46: 274-389.

Leliaert, F., Verbruggen, H., Vanormelingen, P., Steen, F., Lopez-Bautista, J.M., Zuccarello, G.C., De Clerck, O. 2014. DNA-based species delimitation in algae. *European Journal of Phycology* 49(2): 179-196.

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(2): 97-108.

Lindstrom, S.C., Hughey, J.R., Martone, P.T. 2011. New, resurrected and redefined species of Mastocarpus (Phyllophoraceae, Rhodophyta) from the northeast Pacific. Phycologia 50(6): 661-683.

Linnaeus, C. 1758. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Volume 1, 10th edition. Laurentii Salvii, Stockholm.

Liu, L., Pearl, D.K. 2007. Species trees from gene trees: Reconstructing Bayesian posterior distributions of a species phylogeny using estimated gene tree distributions. *Systematic Biology* 56(3): 504-514.

Maneveldt, G.W., Gabrielson, P.W., Kangwe, J. 2017. Sporolithon indopacificum sp. nov. (Sporolithales, Rhodophyta) from tropical western Indian and western Pacific oceans: First report, confirmed by DNA sequence data, of a widely distributed species of Sporolithon. *Phytotaxa* 326: 115-128.

Mach, M.E., Levings, C. D., Chan, K. M. A. 2017. Nonnative species in British Columbia eelgrass beds spread via shellfish aquaculture and stay for the mild climate. *Estuaries and Coasts* 40(1): 187-199.

Maddison, W.P. 1997. Gene trees in species trees. *Systematic Biology* 46(3): 523-536.

Maddison, W.P., Knowles, L.L. 2006. Inferring phylogeny despite incomplete lineage sorting. *Systematic Biology* 55(1): 21-30.

Maneveldt, G.W., Keats, D.W. 2008. Effects of herbivore grazing on the physiognomy of the coralline alga *Spongites yendoi* on associated competitive interactions. *African Journal of Marine Science* 30(3): 581-593.

Manza, A.V. 1937. The genera of the articulated corallines. *Proceedings of the National Academy of Science of the United States of America* 28: 44-48.

Martone, P.T. 2007. Kelp versus coralline: cellular basis for mechanical strength in the wave-swept alga *Calliarthron* (Corallinaceae, Rhodophyta). *Journal of Phycology* 43(5): 882-891.

Martone, P.T. 2006. Size, strength, and allometry of joints in the articulated coralline *Calliarthron*. *Journal of Experimental Biology* 209(9): 1678-1689.

Martone, P.T., Denny, M.W. 2008A. To bend a coralline: effect of joint morphology on flexibility and stress amplification in an articulated calcified seaweed. *Journal of Experimental Biology* 211(21): 3421-3432.

Martone, P.T., Denny, M.W. 2008B. To break a coralline: mechanical constraints on the size and survival of a wave-swept seaweed. *Journal of Experimental Biology* 211(21): 3433-3441.

Martone, P.T., Lindstrom, S.C., Miller, K.A., Gabrielson, P.W. 2012. *Chihareae* and *Yamadaia* (Corallinales, Rhodophyta) represents reduced and recently derived articulated coralline morphologies. *Phycological Society of America* 48: 859-868.

Melbourne, L.A., Hernandez-Kantun, J.J., Russell, S., Brodie, J. 2017. There is more to maerl than meets the eye: DNA barcoding reveals a new species in Britain, Lithothamnion erinaceum sp. nov. (Hapalidiales, Rhodophyta). *European Journal of Phycology* 52(2): 166-178.

Merrill, E.D. 1930. The Fifth International Botanical Congres. *The Scientific Monthly* 31(6) 565-568.

Miller, M.A., Pfeiffer, W., Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*. New Orleans, LA 1-8.

Meyer, I., Wagner, S. 2009. Chapter 16: The Little Ice Age in Southern South America: Proxy and Model Based Evidence. In Past Climate Variability in South America and Surrounding Regions. From the Last Glacial Maximum to the Holocene. Edited by Francoise Vimeux, Florence Sylvestre, Myriam Khodri. Springer.

Moncalvo, J.M., Nilsson, R.H., Koster, B., Dunham, S.M., Bernauer, T., Matheny, P.B…Vilgalys, R. 2006. The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. *Mycologia* 98(6): 937-948.

Monro, K., Poore, A.G.B. 2005. Light quantity and quality induce shade-avoiding plasticity in a marine macroalgae. *Journal of Evolutionary Biology* 18(2): 426-435.

Montagne, C. 1852. Botanica. Tomo octavo. Flora Chileana. Plantas cellulares. Tomo segundo. Algas. In: Historia fisica y politica de Chile segun documentos adquiridos en esta republica durante doce años de residencia en ella y publicata bajo los auspicios del supremo gobierno.... (Gay, C. Eds) 8: 228-256. Paris & Santiago: Paris: en casa del autor. Chile: en el museo de historia natural de Santiago.

Mossel, E., Vigoda, E. 2005. Phylogenetic MCMC algorithms are misleading on mixtures of trees. *Science* 309: 2207-2209.

Moyer, G.R., Remington, R.K., Turner, T.F. 2009. Incongruent gene trees, complex evolutionary processes, and the phylogeny of a group of North American minnows (*Hybognathus* Agassiz 1855). *Molecular Phylogenetics and Evolution* 50: 514-525.

Muñoz-Gómez, S.A., Mejía-Franco, F.G., Durnin, K., Colp, M., Grisdale, C.J., Archibald, J.M., Slamovits, C.H. 2017. The new red algal subphylum Proteorhodophytina comprises the largest and most divergent plastid genomes known. *Current Biology* 27: 1677-1684.

Nash, M.C., Adey, W., Hurd, C. 2017. Multiple phases of mg-calcite in crustose coralline algae suggest caution for temperature proxy and ocean acidification assessment: lessons from the ultrastructure and biomineralization in Phymatolithon (Rhodophyta, Corallinales). *Journal of Phycology* 53(5): 970-984.

Nelson, W.A., Sutherland, J.E., Farr, T.J., Hart, D.R., Neill, K.F., Kim, H.J., Yoon, H.S. 2015. Multi-gene phylogenetic analyses of New Zealand coralline algae: Corallinapetra Novaezelandiae gen. et sp. nov. and recognition of the Hapalidiales ord. nov. *Journal of Phycology* 51(3): 454-468.

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2014) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32: 268–274.

Padilla, D.K. 1984. The importance of form: differences in competitive ability, resistance to consumers and environmental stress in an assemblage of coralline algae. *Journal of Experimental Marine Biology and Ecology* 79: 105-127.

Papenfuss, G.F. 1964. Catalogue and bibliography of Antarctic and Sub-Antarctic benthic marine algae. In: Antarctic Research Series. Volume 1. Bibliography of the Antarctic Seas. (Lee, M.O. Eds). 1-76. Washington D.C., American Geophysical Union.

Pelser, P.B., Kennedy, A.H., Tepe, E.J., Shidler, J.B., Nordenstam, B., Kadereit, J.W., Watson, L.E. 2010. Patterns and causes of incongruence between plastid and nuclear Senecioneae (Asteraceae) phylogenies. *American Journal of Botany* 97(5): 856-873.

Puillandre, N., Lambert, A., Brouillet, S., Achaz, G. 2011. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology* 21(8): 1864-1877.

Rambaut A. FigTree version v1.4.4 http://tree.bio.ed.ac.uk/software/figtree/ 2018.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*.

Ramus, J. 1972. Differentiation of the green alga *Codium fragile*. *American Journal of Botany*. 59: 478-482.

Ramírez, M.E., Santelices, B. 1991. Catálogo de las algas marinas bentónicas de la costa temperada del Pacífico de Sudamérica. *Monografías Biológicas* 5: 1-437.

Richards, J.L., Sauvage, T., Schmidt, W.E., Fredericq, S., Hughey, J.R., Gabrielson, P.W. 2017. The coralline genera *Sporolithon* and *Heydrichia* (Sporolithales, Rhodophyta) clarified by sequencing type material of their generitypes and other species. *Journal of Phycology* 53: 1044-1059.

Ronquist, F., M. Teslenko, P. van der Mark, D.L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M.A. Suchard, and J.P. Huelsenbeck. 2012. MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology* 61: 539-542.

Rösler, A., Perfectti, F., Peña, V., Braga, J.C., Gabrielson, P. 2016. Phylogenetic relationships of Corallinaceae (Corallinales, Rhodophyta): Taxonomic implications for reef-building corallines. *Journal of Phycology* 52(3): 412-431.

Ruiz, G.M., Carlton, J.T. 2003. Invasive Species Vectors and Management Strategies. Island Press.

Saunders, G.W., Hommersand, M.H. 2004. Assessing red algal supraordinal diversity and taxonomy in the context of contemporary systematic data. *American Journal of Botany* 91(10): 1494-1507.

Saunders, G.W., McDevit, D.C. 2012. Acquiring DNA sequence data from dried archival red algae (Florideophyceae) for the purpose of applying available names to contemporary genetic species: a critical assessment. *Botany* 90: 191-203.

Saunders, G.W. 2014. Long distance kelp rafting impacts seaweed geography in the north-east Pacific: the kelp conveyor hypothesis (Letter). *Journal of Phycology* 50(6): 968-974.

Schmitz, F. 1889. Systematische Übersicht der bisher bekannten Gattungen der Florideen. *Flora oder Allgemeine botanische Zeitung* 72: 435-456.

Segawa, S. 1955. Systematic anatomy of the articulated corallines (supplementary report). The structure and reproduction of Yamadaia melobesioides Segawa. *Botanical Magazine, Tokyo* 68: 241-247.

Setchell, W.A. 1943. *Mastophora* and the Mastophoreae: genus and subfamily of Corallinaceae. *Proceedings of the National Academy of Science of the United States of America* 29: 127-135.

Setchell, W.A., Gardner, N.L. 1903. Algae of northwestern America. *University of California Publications in Botany* 1: 165-418.

Silva, P.C., Johansen, H.W. 1986. A reappraisal of the order Corallinales (Rhodophyceae). *British Phycological Journal* 21: 245-254.

Silva, P.C., Basson, P.W. , Moe, R.L. 1996. Catalogue of the benthic marine algae of the Indian Ocean. *University of California Publications in Botany* 79: 1-1259.

Silvestro, D., Michalak, M. 2012. raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity and Evolution*. 12: 335-337.

Sissini, M.N., Oliverira, M.C., Gabrielson, P.W., Robinson, N.M., Okolodkov, Y.B., Rodriguez, R.R. 2014. *Mesophyllum erubescens* (Corallinales, Rhodophyta) – So many species in one epithet. *Phytotaxa* 190(1): 302-319.

Skottsberg, C. 1923. Botanische Ergebnisse der schwedischen Expedition nach Patagonien und dem Feuerlande 1907-1909. IX. Marine algae. 2. Rhodophyceae. *Kungliga Svenska Vetenskapsakademiens Handlingar, Ny Följd* 63(8): 1-70.

Smith, G.M. 1944. Marine algae of the Monterey Peninsula. Stanford: Stanford University Press.

Smith, A.M., Sutherland, J.E., Kregting, L., Farr, T.J., Winter, D.J. 2012. Phylomineralogy of the Coralline red algae: Correlation of skeletal mineralogy with molecular phylogeny. *Phytochemistry* 81: 97-108.

Spalding, H.L., Conklin, K.Y., Smith, C.M. O'Kelly, C.J., Sherwood, A.R., Verbruggen, H. 2016. New Ulvaceae (Ulvophyceae, Chlorophyta) from mesophotic ecosystems across the Hawaiian archipelago. *Journal of Phycology* 52(1): 40-43.

Spencer, M.A., Irvine, L.M., Jarvis, C.E. 2009. Typification of Linnaean names relevant to algal nomenclature. *Taxon* 58(1): 237-260.

Swofford, D.L. 2002. PAUP.* Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Thompson, L.G., Mosley-Thompson, E., Dansgaard, W., Grootes, P.M. 1986. The Little Ice Age as Recorded in the Stratigraphy of the Tropical Quelccaya Ice Cap. *Science, New Series* 234 (4774): 361-364.

Thompson, L.G., Mosley-Thompson, E., Davis, M.E., Lin, P.N., Henderson, K., Mashiotta, T.A. 2003. Tropical glacier and ice core evidence of climate change on annual to millennial time scales. *Climactic Change* 59: 137-155.

Turland, N. J., Wiersema, J. H., Barrie, F. R., Greuter, W., Hawksworth, D. L., Herendeen, P. S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T. W., McNeill, J., Monro, A. M., Prado, J., Price, M. J., Smith, G. F. (eds.) 2018. International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile* 159. Glashütten: Koeltz Botanical Books. DOI https://doi.org/10.12705/Code.2018

Tyrell, B., Johansen, W.H. 1995. Reproductive and regenerative strategies of *Lithothrix aspergillum* (Corallinales, Rhodophyta) in southern California. *Phycologia* 34(1): 39-44.

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(12) 1-21.

van der Merwe, E., Miklasz, K., Channing, A., Maneveldt, G., and Gabrielson, P.W. 2015. DNA sequencing resolves species of *Spongites* (Corallinales, Rhodophyta) in the Northeast Pacific and South Africa, including *S. agulhensis* sp. Nov. *Phycologia* 54(5): 471-490.

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). *Journal of Phycology* 45: 287-297.

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(1): 2-11.

Yang, E.C., Boo, S.M., Bhattacharya, D., Saunders, G.W., Knoll, A.H., Fredricq, S. Graf, L., Yoon, H.S. 2016. Divergence time estimates and the evolution of major lineages in the florideophyte red algae. *Scientific Reports* 6: 1-11.

Yendo, K. 1902A. Corallinae verae of Port Renfrew. *Minnesota Botanical Studies* 2: 711-722.

Yendo, K. 1902B. Enumeration of corallinaceous algae hitherto known from Japan. *Botanical Magazine, Tokyo* 16: 185-196.

Yesson, Y., Jackson, A., Russell, S., Williamson, C.J. Brodie, J. 2018. SNPs reveal geographical population structure of Corallina officinalis (Corallinaceae, Rhodophyta). European Journal of Phycology 53(2): 180-188.

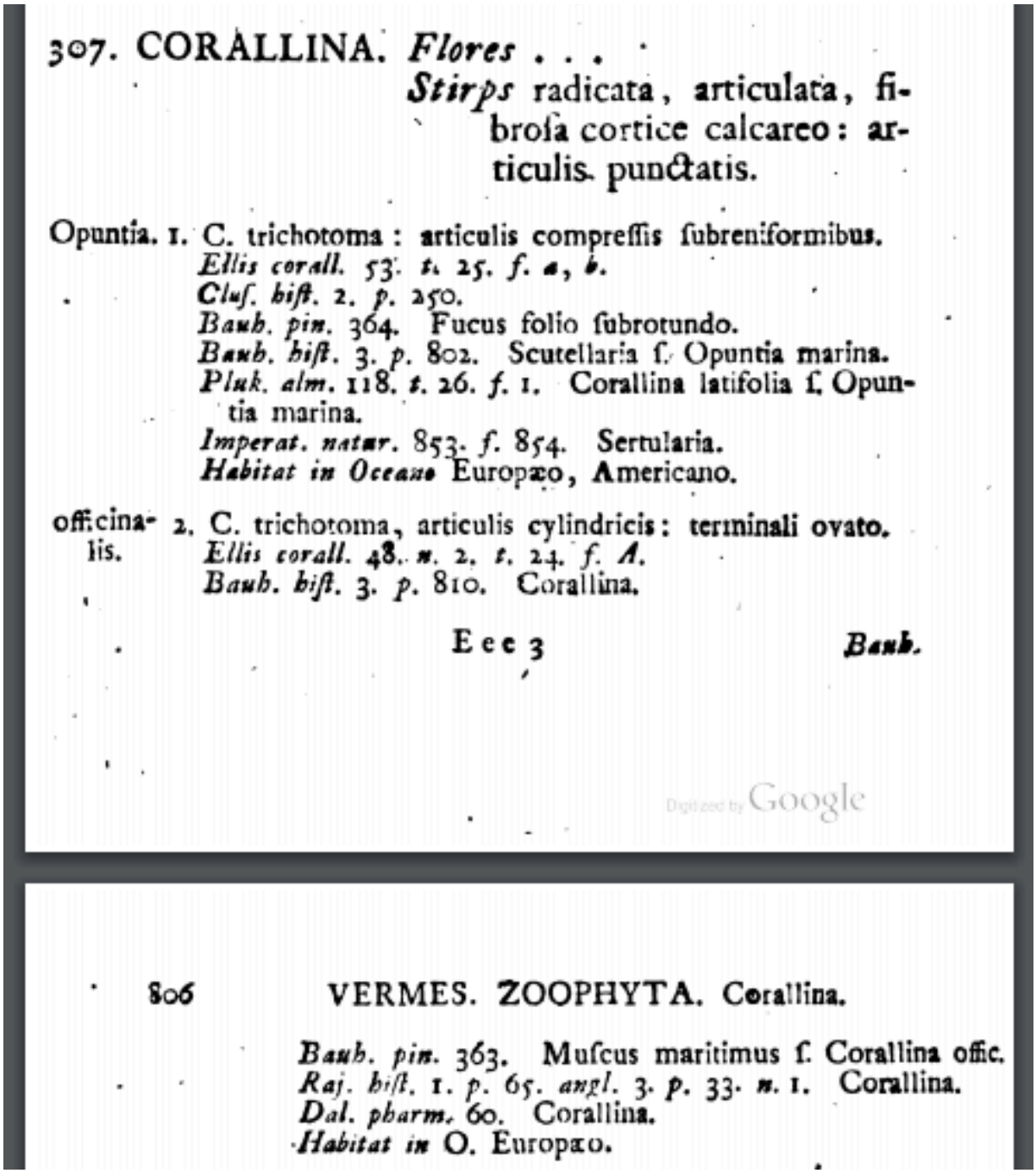
Yoshida, Y., Mogi, Y. 2019. How do plastids and mitochondria divide? *Microscopy* 68(1): 45-56.

Zhan, S.H., Shih, C.C., Liu, S.L. 2020. Reappraising plastid markers of the red algae for phylogenetic community ecology in the genomic era. *Ecology and Evolution* 10: 1299-1210.

Zasshi, S. 1921. Kichisaburo Yendo. *The Botanical Magazine* 35(415): 126-130.

Appendices

Appendix I: Historical materials



307. CORALLINA. *Flores . . .*
Stirps radicata, articulata, fibrosa cortice calcareo: articulis punctatis.

Opuntia. 1. C. trichotoma: articulis compressis subreniformibus.
Ellis corall. 53. *t.* 25. *f. a, b.*
Clus. hist. 2. *p.* 250.
Bauh. pin. 364. Fucus folio subrotundo.
Bauh. hist. 3. *p.* 802. Scutellaria s. Opuntia marina.
Pluk. alm. 118. *t.* 26. *f.* 1. Corallina latifolia s. Opuntia marina.
Imperat. natur. 853. *f.* 854. Sertularia.
Habitat in Oceano Europæo, Americano.

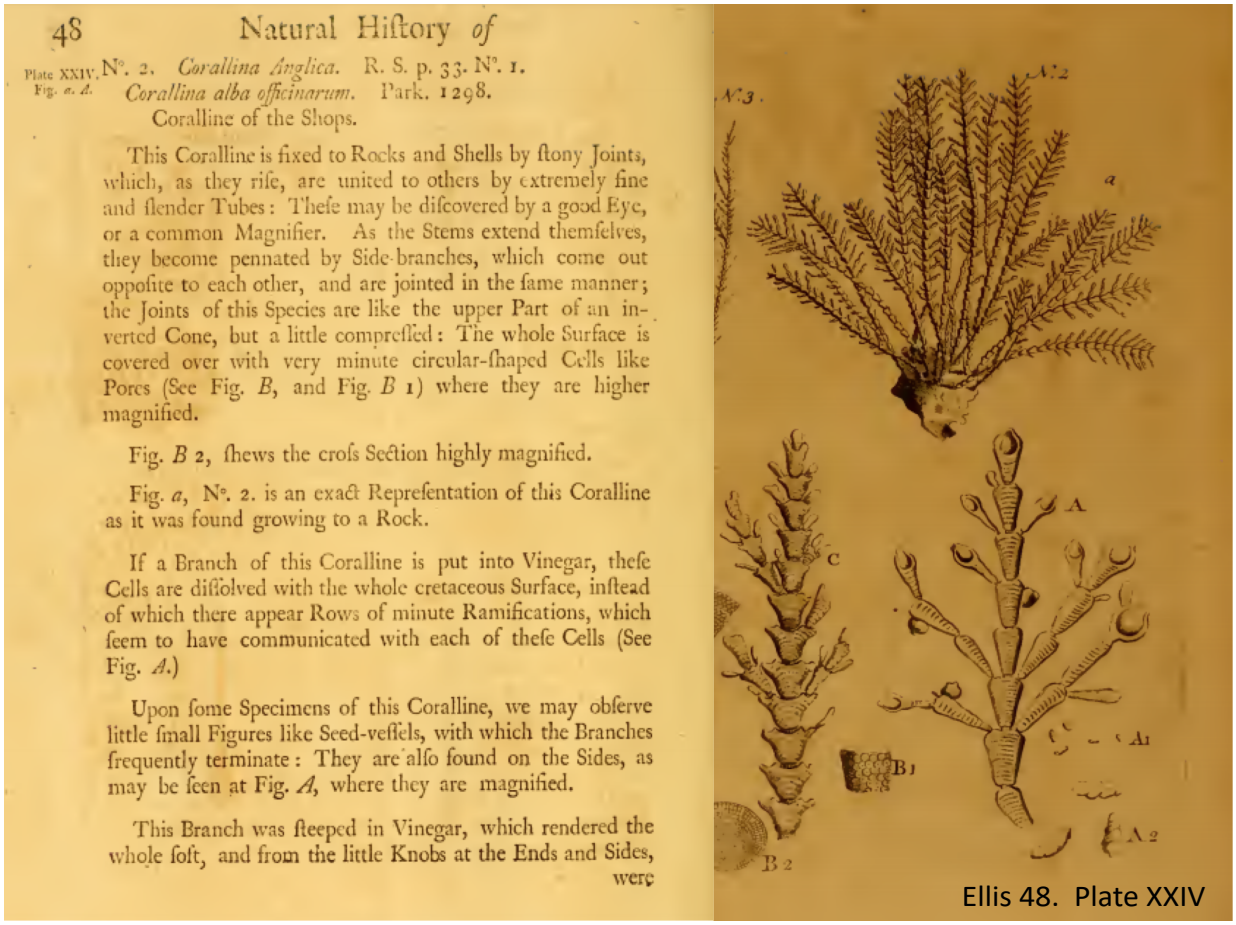
officinalis. 2. C. trichotoma, articulis cylindricis: terminali ovato.
Ellis corall. 48. *n.* 2. *t.* 24. *f. A.*
Bauh. hist. 3. *p.* 810. Corallina.

Eee 3 *Bauh.*

806 VERMES. ZOOPHYTA. Corallina.

Bauh. pin. 363. Muscus maritimus s. Corallina offic.
Raj. hist. 1. *p.* 65. *angl.* 3. *p.* 33. *n.* 1. Corallina.
Dal. pharm. 60. Corallina.
Habitat in O. Europæo.

Figure S1. Linnaeus (1758) binomial naming and description of *Corallina officinalis*.



48 Natural Hiſtory *of*

Plate XXIV. Fig. *a. A.* N°. 2. *Corallina Anglica.* R. S. p. 33. N°. 1.
Corallina alba officinarum. Park. 1298.
Coralline of the Shops.

This Coralline is fixed to Rocks and Shells by ſtony Joints, which, as they riſe, are united to others by extremely fine and ſlender Tubes: Theſe may be diſcovered by a good Eye, or a common Magnifier. As the Stems extend themſelves, they become pennated by Side-branches, which come out oppoſite to each other, and are jointed in the ſame manner; the Joints of this Species are like the upper Part of an inverted Cone, but a little compreſſed: The whole Surface is covered over with very minute circular-ſhaped Cells like Pores (See Fig. *B*, and Fig. *B* 1) where they are higher magnified.

Fig. *B* 2, ſhews the croſs Section highly magnified.

Fig. *a*, N°. 2. is an exact Repreſentation of this Coralline as it was found growing to a Rock.

If a Branch of this Coralline is put into Vinegar, theſe Cells are diſſolved with the whole cretaceous Surface, inſtead of which there appear Rows of minute Ramifications, which ſeem to have communicated with each of theſe Cells (See Fig. *A*.)

Upon ſome Specimens of this Coralline, we may obſerve little ſmall Figures like Seed-veſſels, with which the Branches frequently terminate: They are alſo found on the Sides, as may be ſeen at Fig. *A*, where they are magnified.

This Branch was ſteeped in Vinegar, which rendered the whole ſoft, and from the little Knobs at the Ends and Sides, were

Figure S2. Ellis (1755) description and illustration of what Linnaeus would name "*Corallina officinalis.*" This is the illustration that Linnaeus cited in his description of *C. officinalis* in Fig. S1.

Corallinaceae.

Choreonema *nov. nom.* (= Endosiphonia *Ardissone* 1883).
Ch. Turetii (*Bornet*).

Melobesia *Lamouroux* 1812 (= Agardhia *Meneghini* 1838; incl. *Hapalidium *Kützing* 1843 (=Lithocystis *Harvey* 1848(?); =Plectoderma *Reinsch* 1875)).
*M. farinosa *Lamouroux*.

Mastophora (*Decaisne*) *Harvey* 1847.
M. plana (*Sonder*) *Harvey*.

***Lithophyllum** *Philippi* 1837.
*L. lichenoides (*Ellis et Solander*) *Philippi*.

***Lithothamnion** *Philippi* 1837 (= Spongites *Kützing* 1841).
*L. fasciculatum (*Lamarck*) *Areschoug*.

Amphiroa *Lamouroux* 1812.
A. rigida *Lamouroux*.

***Cheilosporum** (*Decaisne*) *Areschoug* 1852 (incl. *Arthrocardia (*Decaisne*) *Areschoug* 1852).
Ch. sagittatum (*Lamouroux*) *Areschoug*.

Corallina (*Tournefort*) *Lamouroux* 1812 (= Titanephlium *Nardo* 1834; incl. Jania *Lamouroux* 1812).
C. officinalis *Linné*.

Figure S3. Schmitz (1889) designation of *Corallina* as a genus.

3. Corallina chilensis, *Dne.;* fronde brevi dense cæspitosa apice pinnata v. bipinnata, pinnis crebris subfasciculatis, articulis infimis caulinisque cuneatis compressis diametro sesquilongioribus, superioribus obovatis latioribus longioribusque sæpe palmatis vel apice profunde laciniatis, ramulorum gracilibus cylindraceis simplicibus vel apice trifidis, ceramidiis ovatis terminalibus. *Dne. in Herb. Paris. ined.*

Hab. Chili, *Herb. Paris.* Valparaiso (No. 2151) and Port Famine (No. 1840), *Mr. Darwin.* Norfolk Island, *Herb. Hooker.* (*v. s. in Herb. T. C. D. comm. cl. Darwin.*)

1–2 inches high, bi-tri-pinnate above, the pinnæ long, erecto-patent, the upper ones gradually shorter. *Articulations* of the stem and branches once and half as long as broad, cuneate, simple, the upper ones longer and more expanded towards the apex, very irregular in shape, often laciniate or crenate; the apical ones, especially, frequently palmate.

Nearly allied to *C. palmata*, Kg. and to *C. Deshayesii*, Mont. The Port Famine specimens have a *starved* look, and probably grew near high-water mark. Those from Valparaiso are more developed, and serve for the type of the species. The plant also occurs in a mixed bundle received from Mr. Darwin and marked "S. America." The Norfolk Island specimens in Herb. Hooker are slightly different.

Figure S4. Description of *Corallina chilensis* by Harvey (1849).

2. *Corallina chilensis.*

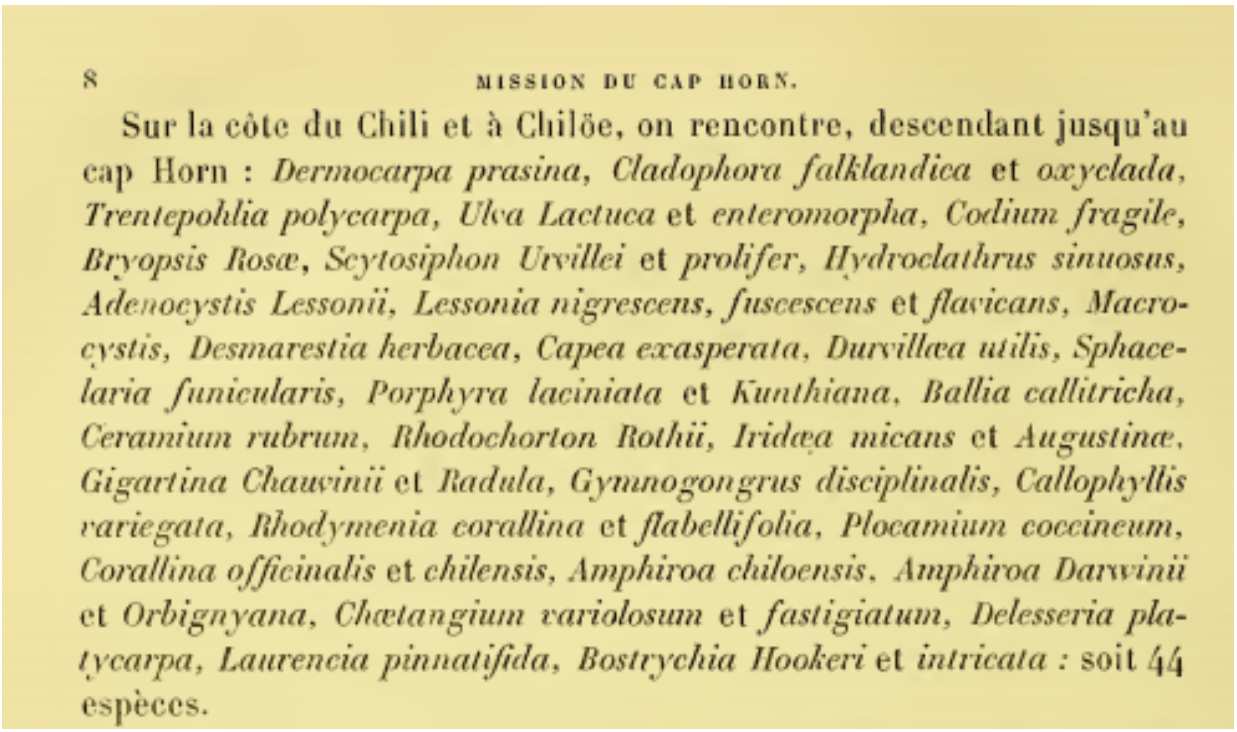
C. fronde brevi, dense cæspitosa, apice pinnata vel bipinnata, pinnis crebris subfasciculatis, articulis infimis caulinisque cuneatis compressis, diametro sesquilongioribus, superioribus obovatis latioribus longioribusque, sæpe palmatis vel apice profunde laciniatis, ramulorum gracilibus cylindraceis simplicibus vel apice trifidis; conceptaculis ovoideis terminalibus.

C. CHILENSIS Decaisne *in* Harv., l. c. — Aresch., l. c.

Fronda de una á dos pulgadas de alto, dos ó tres veces pennada en su parte superior. Pínulas bastante largas, enderezadas, numerosas y pareciendo como fasciculadas, haciéndose mas y mas cortas superiormente. Artículos de la fronda y de las pínulas de primer órden vez y media tan largos como anchos, en forma de cuña, sencillos; los superiores mas largos y mas dilatados hácia su vértice, bastante irregulares en su contorno, con frecuencia laciniados ó solamente almenados. Artículos extremos frecuentemente palmados.

Esta especie, que no he visto, es íntimamente aliada, segun el señor Harvey, al *C. palmata* Kütz. y á mi *C. Dehayesii* de la *Flora de Argelia*. Ha sido cojida en la costa de Chile, en Valparaiso, y en Puerto del Hambre en el estrecho de Magallanes, por Darwin.

Figure S5. Montagne (1852) report of *Corallina chilensis*.



8 MISSION DU CAP HORN.

Sur la côte du Chili et à Chilöe, on rencontre, descendant jusqu'au cap Horn : *Dermocarpa prasina, Cladophora falklandica* et *oxyclada, Trentepohlia polycarpa, Ulva Lactuca* et *enteromorpha, Codium fragile, Bryopsis Rosæ, Scytosiphon Urvillei* et *prolifer, Hydroclathrus sinuosus, Adenocystis Lessonii, Lessonia nigrescens, fuscescens* et *flavicans, Macrocystis, Desmarestia herbacea, Capea exasperata, Durvillæa utilis, Sphacelaria funicularis, Porphyra laciniata* et *Kunthiana, Ballia callitricha, Ceramium rubrum, Rhodochorton Rothii, Iridæa micans* et *Augustinæ, Gigartina Chauvinii* et *Radula, Gymnogongrus disciplinalis, Callophyllis variegata, Rhodymenia corallina* et *flabellifolia, Plocamium coccineum, Corallina officinalis* et *chilensis, Amphiroa chiloensis, Amphiroa Darwinii* et *Orbignyana, Chætangium variolosum* et *fastigiatum, Delesseria platycarpa, Laurencia pinnatifida, Bostrychia Hookeri* et *intricata* : soit 44 espèces.

Figure S6. Ardissone (1888) inventory of species (including *Corallina officinalis var. chilensis*) in Chiloé, Chile.

7. **Corallina officinalis** var. **chilensis** Kütz. Pl. LIV., Fig. 1; Pl. LVI., Fig. 15.

Fronde erecta, 5–10 cm. alta, inferne teretiuscula, superne flabellata bi-tripinnata: articulis inferioribus compressiusculis,

* Harvey: Nereis Austr., p. 99.
† Yendo: Cor. verae Japan. (Journ. of Sci. Coll. Tokyo, vol. XVI., art. III.)

mediis superioribusque oblongo-cuneatis compressis, pinnarum sterilium linearibus vel lanceolatis ancipitibus, ultimis compressis obovatis; conceptaculio pedunculatis subcompressis sæpe corniculatis. Color rubro-violaceus.

Corallina officinalis chilensis Kütz., Tab. phyc., VIII., p. 32, *taf. 66, Fig. 1.*

Cor. officinalis L. f. *δ* Yendo. Cor. veræ Japan., Pl. VII., Fig. 13 (Journ. Sc. Coll. Tokyo, Vol. XVI.).

The sterile specimens of this variety have been collected at Hakodate, a port in the northern part of Japan. As they lacked the conceptacle I was not able to satisfactorily determine the species and included them under the *Cor. officinalis* L. The specimens collected at Port Renfrew are fortunately fertile and accord very well with the description and figures of Kützing's Tab. Phyc. and at the same time correspond with the Hakodate specimens.

As I before noted (*l. c.*), this plant is a somewhat variable form to be counted under the species *Cor. officinalis* L.

Not very common; low-water mark, also in pools at the depth of 2–3 ft. below the surface.

Figure S7. Yendo (1902A) report of *Corallina officinalis var. chilensis.*

Corallina officinalis f. **Chilensis** (Decaisne) Kuetzing.

On rocks in the upper sublitoral and in deep pools in the litoral zone. Port Renfrew, B. C., *Yendo* (1902, p. 718).

We have seen no specimens of the type of this form, as represented by Kuetzing (1858, pl. 66 f. 1) from our territory, but it is not uncommon in various localities on the coast of California. The very simple condition represented by Kuetzing and by Yendo (1902, pl. 54, f. 1) is not so abundant as conditions with the branches and branchlets more numerous and passing into states characteristic of the second and third forms below. It seems to us that it is to be distinguished from the preceding by its less slender and less tapering branchlets.

Figure S8. Setchell (1903) report of *Corallina officinalis var. chilensis*.

Corallina LAMOUR.

Corallina chilensis DECN.[1]

Harv. Ner. austr. p. 103.

Young specimens have been found in the Beagle Channel infested with *Herposiphonia Sullivana* (H. et H.) FALKBG., and on the coast of the Falkland Islands (Berkeley Sound, Port Louis) infested with the following species: *Ceramium rubrum* f. *involutum* KG., *Ceramium circinnatum* KG., *Sphacelaria furcigera* KG., *Codium fragile* (SURING), and *Enteromorpha Hookeriana* KG. (SKOTTSBERG).

Figure S9. Foslie (1907) report of *Corallina chilensis*.

My botanical survey in 1907—09 covered only a part of the area I had visited during the Antaretic cruise five years earlier, but was extended much farther north, where very little algological work had been done before. From the region between the western entrance to the Magellan Strait and Valparaiso my material is, however, rather scanty. While the coast of Peru on one side, and Fuegia on the other, are tolerably well known in this respect, the enormous intermediate region has been neglected, a region most important from a geographical point of view. It is recommended to future explorers to collect as much as possible along the coast of Chile (in West Patagonia not only in the inner, but also in the outer channels), so that the connection between the marine floras of Peru and Fuegia will become firmly established.

In the present paper I have followed the arrangement of genera and species etc. in the memoir by KYLIN and the writer, with exception of the Delesseria family. Last time I had to leave the species of that family under current names, until a detailed study of all my new material and an examination of a number of AGARDH's and other authors' types could be undertaken. I have done my best to supply this need, and some of the results are laid down in this treatise. My thanks are due to Professor KYLIN for kind assistance on various occasions, and to the museums at Kew, Stockholm and Upsala for the loan of material.

Botanic Gardens, Gothenburg, February 1922.

Corallina (L.) LAMX

C. chilensis DCNE; HARVEY, Ner. Austr. p. 103; MONTAGNE ex GAY, Hist. de Chile, Bot. VIII p. 319.

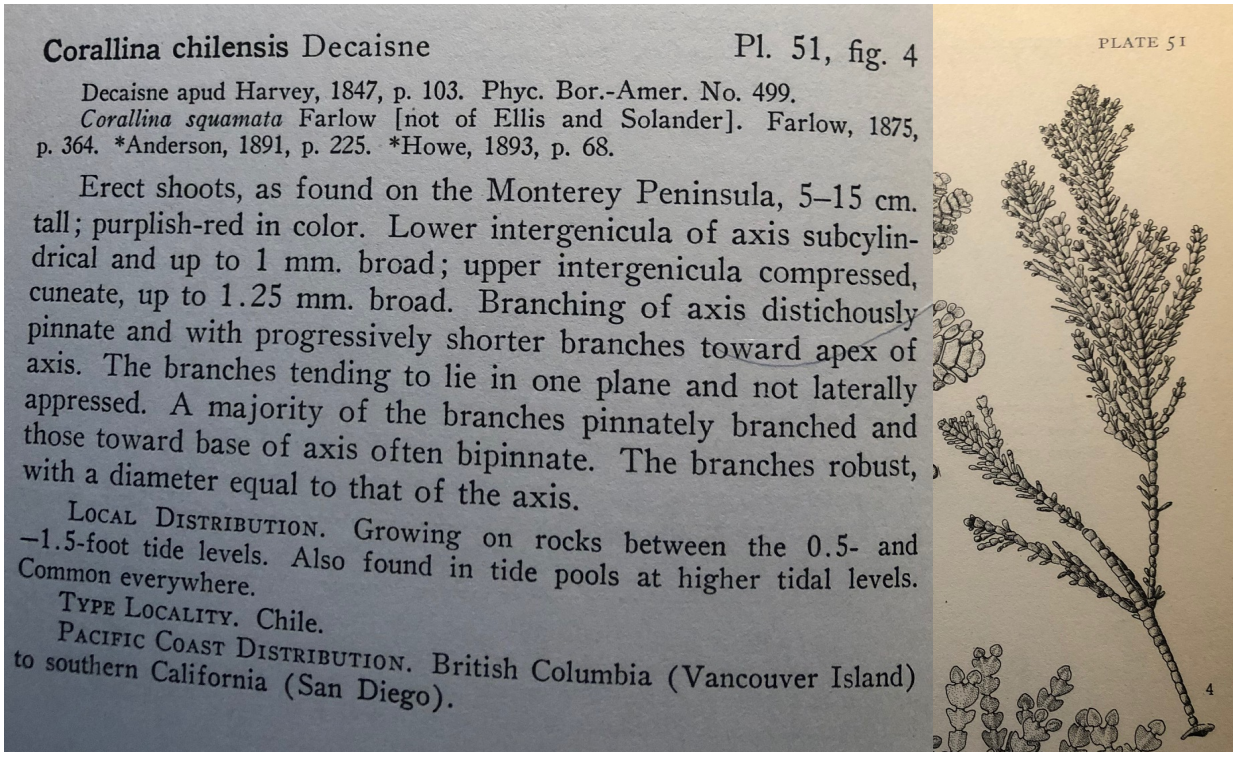
Syn. *C. officinalis* L. f. *chilensis*, KÜTZ., Tab. phyc. VIII t. 66; YENDO, Corall. verae of Port Renfr. p. 718, t. 54 f. 1, 56 f. 15.

Falkland Islands: Cape Pembroke, in tide-pools (St. 3 b, c, 7. 11. 07).

It is quite possible that this is only a form of *C. officinalis*, but further studies of the southern Corallines are necessary to settle this and other questions. The ramification is dense, almost every joint carrying lateral branches, all of about equal length and branched again in the same feather-like fashion.

Distribution: Japan, NW. Amer., Peru, Chile, Falkl. (first record).

Figure S10. Skottsberg (1923) report of *Corallina chilensis*.



Corallina chilensis Decaisne Pl. 51, fig. 4

Decaisne apud Harvey, 1847, p. 103. Phyc. Bor.-Amer. No. 499.
Corallina squamata Farlow [not of Ellis and Solander]. Farlow, 1875, p. 364. *Anderson, 1891, p. 225. *Howe, 1893, p. 68.

Erect shoots, as found on the Monterey Peninsula, 5–15 cm. tall; purplish-red in color. Lower intergenicula of axis subcylindrical and up to 1 mm. broad; upper intergenicula compressed, cuneate, up to 1.25 mm. broad. Branching of axis distichously pinnate and with progressively shorter branches toward apex of axis. The branches tending to lie in one plane and not laterally appressed. A majority of the branches pinnately branched and those toward base of axis often bipinnate. The branches robust, with a diameter equal to that of the axis.

Local Distribution. Growing on rocks between the 0.5- and −1.5-foot tide levels. Also found in tide pools at higher tidal levels. Common everywhere.

Type Locality. Chile.

Pacific Coast Distribution. British Columbia (Vancouver Island) to southern California (San Diego).

PLATE 51

4

Figure S11. Smith (1944) report of *Corallina chilensis.*

Appendix II: Sequences

Table S1. Table of all sequence data.

* Indicates that corresponding sequence(s) are not in tree, ABGD, or distance matrix analyses.

Name	Collector#	Accession#	Justification for designation	psbA	CO1	rbcL	Location	Collector/ References
Bossiella frondifera		A90727	Published sequence/authority	KT782243	KT782032	KT782137	Brady's Blow Hole, Bamfield, Vancouver Island, Canada	Hind et al. 2015
Calliarthron cheilosporioides	GWS010084		GenBank BLAST is 100% match with *C. cheilosporioides* JQ7410 published by van der Merwe et al. 2015.	JQ422199			British Columbia, Canada	Hind & Saunders 2013A (Note: referred to as "Corallina tuberculosum" in publication and GenBank)
Calliarthron cheilosporioides	GWS021537		Published sequence/criteria for name application unknown		KM254472		Point Lobos, California, United States	Saunders 2014
Calliarthron cheilosporioides		NCU585611	Published sequence/molecular & morpho comparison of topotype material			HQ322294	Catalina Island, California, United States	Gabrielson et al. 2011
Chiharaea bodegensis	GWS009079		Published sequence/morpholgical comparison to topotype material	JQ677009	JQ615596		Wizard I, Bamfield, British Columbia, Canada	Hind & Saunders 2013B
Chiharaea bodegensis	GWS010828		Published sequence/morphological comparison to topotype material			JQ677000	"Most collections were from the Canadian northeast Pacific" (Hind & Saunders 2013)	Hind & Saunders 2013B
Corallina aberrans	PTM 1445	A92983	Clustered with psbA JQ422201 & C01 JQ615597	x	x	x	Katsuura, Japan	Patrick T. Martone
Corallina aberrans	GWS013777		Published sequence morphological comparison to designated lectotype illustrations (Hind & Saunders 2013A)	JQ422201	JQ615597		Chibaken, Japan	Hind & Saunders 2013A
Corallina caespitosa holotype	BM000804549		Published sequence/holotype		DQ191343		Devon, England	Robba et al. 2006; Walker et al. 2009
Corallina chilensis	PTM 182	A88708	Formed clade with PTM 332	x	x		Botanical Beach, Vancouver Island, British Columbia, Canada	Patrick T. Martone
Corallina chilensis	PTM 332	A89284	Identical rbcL sequence with 263 bp Darwin type rbcL sequence	x	x	x	Hakai, Fifth Beach, Calvert Island, British Columbia, Canada	Patrick T. Martone
Corallina chilensis	PTM 738	A91487	Formed clade with PTM 332	x			Scott's Bay, Bamfield, Vancouver Island, British Columbia, Canada	Katherine R. Hind
Corallina chilensis	PTM 740	A91489	Formed clade with PTM 332	x			Scott's Bay, Bamfield, Vancouver Island, British Columbia, Canada	Katherine R. Hind
Corallina chilensis	PTM 742	A91491	Formed clade with PTM 332	x			Scott's Bay, Bamfield, Vancouver Island, British Columbia, Canada	Katherine R. Hind
Corallina chilensis	PTM 743	A91492	Formed clade with PTM 332	x			Scott's Bay, Bamfield, Vancouver Island, British Columbia, Canada	Katherine R. Hind
Corallina chilensis	PTM 788	A91531	Formed clade with PTM 332	x			Hakai, Fifth Beach, Calvert Island, British Columbia, Canada	Katherine R. Hind
Corallina chilensis	PTM 789	A91532	Formed clade with PTM 332	x			Hakai, Fifth Beach, Calvert Island, British Columbia, Canada	Katherine R. Hind
Corallina chilensis	PTM 1244	A92161	Formed clade with PTM 332	x	x	x	Yaquina Head, Oregon, United States	Katherine R. Hind
Corallina chilensis	Cor.chi.12ii09*	NCU 656905	99.88% similar to PTM 332 over 851 bp in psbA gene				Playa Cocholue, Concepcion, Chile (drift)	Paul W. Gabrielson
Corallina chilensis	Remnants from Paul Silva's collection	XXXXX	Identical to type specimen (over 263 bp length)			x	Unknown location, Chile	Alcide d'Orbigny
Corallina chilensis type		XXXXX	Type specimen sequence for *C. chilensis* (263 bp)			x	Valparaiso, Chile	Charles Darwin
Corallina chilensis	PTM 789*	A91532	Determined by Katherine R. Hind (using DNA)				Calvert Island, Fifth Beach, British Columbia, Canada	Katherine R. Hind
Corallina chilensis	PTM 487*	A89808	Determined by Katherine R. Hind (using DNA)				Calvert Island, Fifth Beach, British Columbia, Canada	Katherine R. Hind
Corallina chilensis	PTM 182*	A88708	Determined by Patrick T. Martone (using DNA)				Botanical Beach, Vancouver Island, British Columbia, Canada	Patrick T. Martone
Corallina chilensis	PTM 333*	A89285	Determined by Katherine R. Hind (using DNA)				Calvert Island, Fifth Beach, British Columbia, Canada	Patrick T. Martone
Corallina chilensis	PTM 629*	A89961	Determined by Katherine R. Hind and Patrick T. Martone (using DNA)				Vancouver Island, Botany Bay, British Columbia, Canada	Katherine R. Hind
Corallina chilensis	PTM 326*	A89279	Determined by Katherine R. Hind (using DNA)				Calvert Island, Fifth Beach, British Columbia, Canada	Patrick T. Martone
Corallina chilensis	PTM 1588*	A93226	Determined by Patrick T. Martone (using DNA)				Calvert Island, North Beach Bench, British Columbia, Canada	Patrick T. Martone
Corallina chilensis	PTM 1*	A88572	100% match to rbcL sequence for PTM 332 over 1107 bp				Pacific Grove, Hopkins Marine Station, California, USA	Patrick T. Martone
Corallina chilensis	PTM 10*	A88577	100% match to rbcL sequence for PTM 332 over 1107 bp				Laguna Beach, Crystal Cove, California USA (drift)	Patrick T. Martone
Corallina chilensis	PTM 11*	A88578	100% match to rbcL sequence for PTM 332 over 1107 bp				Laguna Beach, Crystal Cove, California USA (drift)	Patrick T. Martone

Name	Collector#	Accession#	Justification for designation	psbA	CO1	rbcL	Location	Collector/ References
Corallina crassissima	PTM 1447	A92985	Clustered with CO1 JQ615605		x	x	Katsuura, Japan	Patrick T. Martone
Corallina crassissima	PTM 1490	A93028	Clustered with psbA JQ422203	x	x	x	Chibaken, Japan	Patrick T. Martone
Corallina crassissima	GWS013776		Published sequence morphological comparison to designated lectotype illustrations (Hind & Saunders 2013A)	JQ422203	JQ615605		Chiba prefecture, Katsuura, Japan	Hind & Saunders 2013A
Corallina declinata	PTM 1488	A93026	Clustered with psbA JQ422204 & CO1 JQ615613	x	x		Chibaken, Japan	Patrick T. Martone
Corallina declinata	GWS013767		Published sequence morphological comparison to designated lectotype illustrations (Hind & Saunders 2013A)	JQ422204	JQ615613		Chibaken, Japan	Hind & Saunders 2013A
Corallina ferreyrae	PTM 819	A91593	Monophyletic with isotype in psbA tree, closest sequence to isotype in CO1 tree	x	x		Quintay, Chile	Katherine R. Hind
Corallina ferreyrae	PTM 821	A91595	Monophyletic with isotype in psbA tree	x			Quintay, Chile	Katherine R. Hind
Corallina ferreyrae	PTM 826	A91600	Formed clade with isotype in psbA and rbcL trees	x	x	x	Quintay, Chile	Katherine R. Hind
Corallina ferreyrae	PTM 827	A91601	In clade with isotype in psbA tree	x			Quintay, Chile	Katherine R. Hind
Corallina ferreyrae	PTM 830	A91604	In clade with isotype in psbA tree	x			Quintay, Chile	Katherine R. Hind
Corallina ferreyrae	PTM 832	A91606	In clade with isotype in psbA tree	x			Quintay, Chile	Katherine R. Hind
Corallina ferreyrae	PTM 833	A91607	In clade with isotype in psbA tree	x			Quintay, Chile	Katherine R. Hind
Corallina ferreyrae	PTM 847	A91617	In clade with isotype in psbA tree	x			Valparaiso, Chile	Katherine R. Hind
Corallina ferreyrae isotype		UC1404138	Published sequence/isotype	MK408748	MK408747	MK408748	Pucusana, Peru	Bustamante et al. 2019
Corallina ferreyrae-like	PTM 1408	A92946	Close sister to C. ferreyrae isotype clade in CO1 & rbcL trees		x	x	Oshoro Bay, Japan, Oshoro Bay Marine Station	Patrick T. Martone
Corallina ferreyrae-like	PTM 1416	A92954	Formed clade in psbA tree with PTM1440 & PTM 1417	x	x	x	Muroran, Japan	Patrick T. Martone
Corallina ferreyrae-like	PTM 1417	A92955	Formed clade in psbA tree with PTM1440 and PTM 1416	x			Muroran, Japan	Patrick T. Martone
Corallina ferreyrae-like	PTM 1440	A92978	Present in same clade as all other C. ferreyrae-like sequences across all three trees	x	x	x	Cape Tachimachi, Hakodate, Japan	Patrick T. Martone
Corallina maxima	GWS013782		Published sequence morphological comparison to designated lectotype illustrations (Hind & Saunders 2013A)	JQ422207	JQ615680		Chibaken, Japan	Hind & Saunders 2013A
Corallina melobesioides		UBCa62034	Published sequence/topotype			JN701477	Chibaken, Japan	Martone et al. 2012
Corallina officinalis		NCU588445	Published sequence/matched other sequences that matched epitype	KJ637651			Alaska, United States	Hind et al. 2014
Corallina officinalis	GWS006989		Published sequence/matched other sequences that matched epitype	JQ422209			Newfoundland & Labrador, Canada	Hind & Saunders 2013A
Corallina officinalis		NCU590595	Published sequence/matched other sequences that matched epitype	JQ637652			Foster Island, British Columbia, Canada	Hind et al. 2014
Corallina officinalis	BM001004107		Published sequence/matched other sequences that matched epitype	JQ917413			Somerset, England	Hind et al. 2014, van der Merwe et al. 2015
Corallina officinalis	GWS006989		Published sequence/matched with epitype		JQ615681		Cape Ray, Newfoundland & Labrador, Canada	Hind & Saunders 2013A
Corallina officinalis		NCU588445	Published sequence/in clade with epitype			KJ591672	Chichigof Harbor, Attu Island, Alaska, United States	Hind et al. 2014
Corallina officinalis		NCU590595	Published sequence/in clade with epitype			KJ591674	Foster Island, British Columbia, Canada	Hind et al. 2014
Corallina officinalis	BM001004107		Published sequence/matched epitype			JN701476	Lilstock, Somerset Co., England	Listed in GenBank as Martone et al. unpublished. Gabrielson must have gotten the sequence or specimen from Brodie, given collector number and location?)
Corallina officinalis	BM001062598		Published sequence/epitype		FM180073	JX315329	Devon, Sidmouth, England	Walker et al. 2009; Brodie et al. 2013

Name	Collector#	Accession#	Justification for designation	psbA	CO1	rbcL	Location	Collector/ References
Corallina pinnatifolia		UBCa88590	Published representative sequence			HQ322333	Laguna Beach, Orange County, California, United States	Gabrielson et al. 2011
Corallina sp. 1 california	PTM 363	A89705	Formed clade in psbA tree with JQ422238	x	x	x	Hakai, Calvert Island, British Columbia, Canada	Sandra Lindstrom
Corallina sp. 1 california	PTM 515	A89836	Formed clade in psbA tree with JQ422238	x		x	Hakai, Calvert Island, British Columbia, Canada	Sandra Lindstrom
Corallina sp. 1 california	PTM 1188	A92117	Formed clade with JQ615736 in CO1 tree	x	x	x	Hakai, Calvert Island, British Columbia, Canada	Patrick T. Martone
Corallina sp. 1 california	PTM 1247	A92164	Formed clade with JQ615736 in CO1 tree	x	x	x	California, United States	Patrick T. Martone
Corallina sp. 1 california	GWS021316		Published sequence/provisional name source	JQ422238	JQ615736		Pigeon Point Lighthouse, California, United States	Hind & Saunders 2013
Corallina sp. 1 chile	PTM 862	A91632	Name designated by Martone lab for group in psbA tree	x			Curinaco, Chile	Katherine R. Hind
Corallina sp. 1 chile	PTM 863	A91633	Name designated by Martone lab for group in psbA tree	x	x	x	Curinaco, Chile	Katherine R. Hind
Corallina sp. 1 chile	PTM 868	A91638	Name designated by Martone lab for group in psbA tree	x			Curinaco, Chile	Katherine R. Hind
Corallina sp. 1 chile	PTM 869	A91639	Name designated by Martone lab for group in psbA tree	x			Curinaco, Chile	Katherine R. Hind
Corallina sp. 1 chile	PTM 876	A91646	Name designated by Martone lab for group in psbA tree	x			Curinaco, Chile	Patrick T. Martone
Corallina sp. 1 chile	PTM 879	A91649	Name designated by Martone lab for group in psbA tree	x			Curinaco, Chile	Patrick T. Martone
Corallina sp. 1 chile	PTM 889	A91657	Name designated by Martone lab for group in psbA tree	x	x		Bonifacio, Chile	Patrick T. Martone
Corallina sp. 1 chile	PTM 891	A91659	Name designated by Martone lab for group in psbA tree	x			Bonifacio, Chile	Patrick T. Martone
Corallina sp. 1 chile	PTM 898	A91666	Name designated by Martone lab for group in psbA tree	x			Bonifacio, Chile	Patrick T. Martone
Corallina sp. 1 chile	PTM 899	A91667	Name designated by Martone lab for group in psbA tree	x		x	Bonifacio, Chile	Patrick T. Martone
Corallina sp. 1 chile	PTM 910	A91676	Name designated by Martone lab for group in psbA tree	x	x	x	Mar Brava, Chile	Patrick T. Martone
Corallina sp. 1 chile	PTM 926	A91927	Name designated by Martone lab for group in psbA tree	x	x		Cucao, Chile	Patrick T. Martone
Corallina sp. 1 chile	PTM 1325	NO VOUCHER	Clade with PTM 926 in CO1 & PTM 1337 in rbcL tree		x	x	Cucao, Chile	No record
Corallina sp. 1 chile	PTM 1337	NO VOUCHER	Clase with PTM1325 in rbcL tree			x	Pucatrihue, Chile	No record
Corallina sp. 1 gws	PTM 1457	A92995	Formed clade with JQ422217 in psbA tree& JQ615738 in the CO1 tree	x	x	x	Chiba University Marine Institute, Katsuura, Japan	Patrick T. Martone
Corallina sp. 1 gws	GWS013769		Published sequence/provisional name source	JQ422217	JQ615738		Chibaken, Katsuura, Japan	Hind & Saunders 2013
Corallina sp. 1 gws-like	PTM 1401	A92939	Formed clade/sister to C. sp. 1 gws sequences across all three trees	x	x	x	Oshoro Bay, Oshoro Marine Station, Japan	Patrick T. Martone
Corallina sp. 1 gws-like	PTM 1402	A92940	Corresponded with PTM 1401			x	Oshoro Bay, Oshoro Marine Station, Japan	Patrick T. Martone
Corallina sp. 1 gws-like	PTM 1409	A92947	Corresponded with PTM 1401			x	Oshoro Bay, Oshoro Marine Station, Japan	Patrick T. Martone
Corallina sp. 2 chile	PTM 867	A91637	Name designated by Martone lab for group in psbA tree	x			Curinaco, Chile	Katherine R. Hind
Corallina sp. 2 chile	PTM 870	A91640	Name designated by Martone lab for group in psbA tree	x	x	x	Curinaco, Chile	Katherine R. Hind
Corallina sp. 2 chile	PTM 873	A91643	Name designated by Martone lab for group in psbA tree	x			Curinaco, Chile	Patrick T. Martone
Corallina sp. 2 chile	PTM 880	A91650	Name designated by Martone lab for group in psbA tree	x			Curinaco, Chile	Patrick T. Martone
Corallina sp. 2 chile	PTM 895	A91663	Name designated by Martone lab for group in psbA tree	x	x		Bonifacio, Chile	Patrick T. Martone
Corallina sp. 2 chile	PTM 905	A91672	Name designated by Martone lab for group in psbA tree	x	x		Mar Brava, Chile	Patrick T. Martone
Corallina sp. 2 chile	PTM 1254	A92169	Name designated by Martone lab for group in psbA tree	x	x	x	Arrowhead point, Stillwater cove Pebble Beach CA	Patrick T. Martone
Corallina sp. 2 chile	PTM 1262	NO VOUCHER	Name designated by Martone lab for group in psbA tree	x			Arrowhead point, Stillwater cove Pebble Beach CA	No record
Corallina sp. 2 chile	PTM 1265	A92173	Name designated by Martone lab for group in psbA tree	x			Arrowhead point, Stillwater cove Pebble Beach CA	Patrick T. Martone
Corallina sp. 2 chile	PTM 1266	A92174	Name designated by Martone lab for group in psbA tree	x			Arrowhead point, Stillwater cove Pebble Beach CA	Patrick T. Martone
Corallina sp. 2 chile	PTM 1519	A93057	Name designated by Martone lab for group in psbA tree	x			East of Shimen Harbour, Keelung, Taiwan	Patrick T. Martone
Corallina sp. 2 chile	PTM 1985*	XXXXX	Field identified as "C. officinalis var. chilensis"	(x)			Biobio, Chile	Erasmo Macaya
Corallina sp. 2 chile		PC0028646, or PC0028647, or PC0040576	Clustered with C. sp. 2 chile specimens in rbcL type tree (263 bp)			x	"San Carlos De Chiloe" (Ancud), Chile, Collected year 1836.	Claudio Gay

Name	Collector#	Accession#	Justification for designation	psbA	CO1	rbcL	Location	Collector/ References
Corallina sp. 2 frondescens	PTM 489	A89810	Formed clade with PTM 1178 in psbA tree	x			Hakai, Calvert Island, British Columbia, Canada	Katherine R. Hind
Corallina sp. 2 frondescens	PTM 1178	A92108	Formed clade with JQ615748 in CO1 tree	x	x	x	Hakai, North Beach, Calvert Island, British Columbia, Canada	Patrick T. Martone
Corallina sp. 2 frondescens	GWS003062		Published sequence/provisional name source		JQ615748		Seapool Rock, Bamfield, Vancouver Island, British Columbia, Canada	Hind & Saunders 2013
Corallina sp. 2 vancouveriensis	GWS009913		Published sequence/provisional name source	JQ422229	JQ615760		Tahsis, Island #40 on Esperenza Inlet Chart, British Columbia, Canada	Hind & Saunders 2013
Corallina sp. 3 frondescens	PTM 1400	A92938	Formed clade with JQ2221 in psbA tree	x	x	x	Oshoro Bay, Japan, Oshoro Bay Marine Station	Patrick T. Martone
Corallina sp. 3 frondescens	PTM 1405	A92943	Formed clade with JQ2221 in psbA tree	x	x	x	Oshoro Bay, Japan, Oshoro Bay Marine Station	Patrick T. Martone
Corallina sp. 3 frondescens	PTM 1442	A92980	Formed clade with JQ2221 in psbA tree	x	x	x	Cape Tachimachi, Hakodate, Japan	Patrick T. Martone
Corallina sp. 3 frondescens	GWS006466		Published sequence/provisional name source	JQ22221	JQ615765		Stephenson Pt., British Columbia, Canada	Hind & Saunders 2013
Corallina sp. 3 frondescens	GWS011941		Published sequence/provisional name source		JQ615766		Hokkaido University Marine Station, Oshoro Bay, Japan	Hind & Saunders 2013
Corallina sp. 3 frondescens-like	PTM 1419	A92957	Formed clade sister to C. sp. 3 frondescens	x		x	Muoran, Japan	Patrick T. Martone
Corallina sp. 3 frondescens-like	PTM 1439	A92977	Formed clade sister to C. sp. 3 frondescens	x	x	x	Cap	Patrick T. Martone
Corallina sp. 4 frondescens	PTM 822	A91596	Formed clade with JQ422222 in psbA tree	x			Oshoro Bay, Japan, Oshoro Bay Marine Station	Katherine R. Hind
Corallina sp. 4 frondescens	PTM 842	A91612	Formed clade with JQ422222 in psbA tree	x			Valparaiso Torpederas Chile	Katherine R. Hind
Corallina sp. 4 frondescens	PTM 844	A91614	Formed clade with JQ422222 in psbA tree	x	x	x	Valparaiso Torpederas Chile	Katherine R. Hind
Corallina sp. 4 frondescens	PTM 846	A91616	Formed clade with JQ422222 in psbA tree	x	x		Valparaiso Torpederas Chile	Katherine R. Hind
Corallina sp. 4 frondescens	PTM 881	A91651	Formed clade with JQ422222 in psbA tree	x	x		Curinaco, Chile	Patrick T. Martone
Corallina sp. 4 frondescens	PTM 1235	NO ACCESSION	Formed clade with JQ422222 in psbA tree	x		x	Seal rocks state park beach, Oregon USA	No record
Corallina sp. 4 frondescens	GWS010351		Published sequence/provisional name source	JQ422222			British Columbia, Canada	Hind & Saunders 2013
Corallina sp. 4 frondescens	GWS021267		Published sequence/provisional name source		JQ615770		Pigeon Point Lighthouse, California, United States	Hind & Saunders 2013
Corallina sp. 4 frondescens	GWS010351		Published sequence/provisional name source		JQ615787		Point Holmes, Comox, British Columbia, Canada	Hind & Saunders 2013
Corallina sp. 5 frondescens	PTM 420	A89741	Fell into clad with other C. sp. 5 frondescens in psbA & CO1 trees	x	x	x	Hakai, Wolf Beach, Calvert Island, British Columbia, Canada	Katherine R. Hind
Corallina sp. 5 frondescens	GWS006561		Published sequence/provisional name source	JQ422226	JQ615794		Tahsis, Princesa Channel, British Columbia, Canada	Hind & Saunders 2013
Corallina sp. 5 frondescens	GWS012660		Published sequence/provisional name source	JQ422227	HM918986		Mazarredo Islands, NW of Masset, Haida Gwaii, British Columbia, Canada	Hind & Saunders 2013
Corallina sp. 5 Korea	GWS018201		Published sequence/provisional name source		JQ615795		Lighthouse Point, Piyangdo Island, South Korea	Hind & Saunders 2013
Corallina vancouveriensis	PTM 760	A91506	Compared to topotype specimens	x	x		Hakai, Calvert Island, British Columbia, Canada	Katherine R. Hind
Corallina vancouveriensis	PTM 767	A91513	Compred to topotype specimens	x	x	x	Hakai, Calvert Island, British Columbia, Canada	Katherine R. Hind
Corallina vancouveriensis	GWS010831		Published sequence/compared with topotype	JQ422228	JQ615834		Seppings I, Bamfield, British Columbia, Canada	Hind & Saunders 2013
Corallina vancouveriensis	PTM 179*	A88705	Determined by Patrick T. Martone/comparison to topotype				Botanical Beach, Vancouver Island, British Columbia, Canada	Patrick T. Martone
Crusticorallina muricata		UBCa89963	Published sequence/authority	KU983300			Botany Bay, Vancouver Island, British Columbia, Canada	Hind et al. 2016
Crusticorallina muricata		UBCa91387	Published sequence/authority		KU983192		Brady's Beach Blowhole, Bamfield, Vancouver Island, British Columbia, Canada	Hind et al. 2016
Crusticorallina muricata		UBCa89963	Published sequence/authority			KU983253	Botany Bay, Vancouver Island, British Columbia, Canada	Hind et al. 2016
Ellisolandia elongata	GWS001818		Published sequence/authority	JQ422231			Leitrim, Ireland	Hind & Saunders 2013
Ellisolandia elongata	GWS001818		Published sequence/authority		JQ615843		Leitrim, Ireland	Hind & Saunders 2013
Ellisolandia elongata	BM000806006		Published sequence/criteria for name application unknown			KP834400	Llanes, Asturias, Spain	Williamson et al. 2015
Lithothamnion glaciale	GWS007312		Published sequence/criteria for name application unknown	KP224290			Newfoundland & Labrador, Maerl bed, Canada	Hind et al. 2018
Lithothamnion glaciale	none given		Published sequence/criteria for name application unknown		HM918805		Newfoundland & Labrador, Canada	Hind et al. 2018; iBOL data release, 2018
Lithothamnion glaciale	GWS007312		Published sequence/criteria for name application unknown			KC134336	Newfoundland & Labrador, Maerl bed, Canada	Hind et al. 2018
Genus that has yet to be described	PTM 1984*	XXXXX	Field identified as "C. officinalis var. chilensis"	(x)			Biobio, Chile	Erasmo Macaya

Table S2. Concatenated outgroup sequences GenBank numbers for concatenated tree.

Name	psbA	CO1	rbcL
Lithothamnion glaciale	KP224290	HM918805	KC134336
Calliarthron cheilosporioides	JQ422199	KM254472	HQ322294
Chiharaea bodegensis	JQ677009	JQ615596	JQ677000
Bossiella frondifera	KT782243	KT782032	KT782137
Ellisolandia elongata	JQ422231	JQ615843	KP834400
Crusticorallina muricata	KU983300	KU983192	KU983253

Table S3. Table of (N=91) *Corallina chilensis* specimens collected by Hind & Saunders (2013A), corresponding GenBank sequence numbers, and locations.

GenBank#	BLAST Results	Species determination	Collection #	Province/State	Country	lat	long
HM918990	**Matched 100% with HQ545178 CO1**	*Corallina chilensis*	GWS012704	British Columbia	Canada	54.111	-132.37
HM919003	**Matched 100% with HQ544623 CO1**	*Corallina chilensis*	GWS012926	British Columbia	Canada	52.446	-131.23
HM919004	**CO1 99.39% match to PTM 332**	*Corallina chilensis*	GWS012933	British Columbia	Canada	52.446	-131.23
HQ544551	**CO1 99.39% match to PTM 332**	*Corallina chilensis*	GWS019642	British Columbia	Canada	48.838	-125.13
HQ544596	**CO1 99.70% match to PTM 332**	*Corallina chilensis*	GWS019732	British Columbia	Canada	52.442	-131.32
HQ544623	**CO1 100% match PTM 332**	*Corallina chilensis*	GWS019794	British Columbia	Canada	52.442	-131.32
HQ544655	**CO1 100% match with HQ544623**	*Corallina chilensis*	GWS019852	British Columbia	Canada	52.442	-131.32
HQ544681	**CO1 100% match with HQ544623**	*Corallina chilensis*	GWS019949	British Columbia	Canada	52.428	-131.38
HQ544693	**CO1 100% match with JQ615658**	*Corallina chilensis*	GWS019973	British Columbia	Canada	52.433	-131.37
HQ544765	**CO1 100% match with HQ545178**	*Corallina chilensis*	GWS020126	British Columbia	Canada	52.45	-131.29
HQ544777	**CO1 100% match with HQ545178**	*Corallina chilensis*	GWS020165	British Columbia	Canada	52.45	-131.29
HQ544839	**CO1 99.24% match to PTM 332**	*Corallina chilensis*	GWS020272	British Columbia	Canada	52.358	-131.17
HQ544903	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS020417	British Columbia	Canada	52.578	-131.44
HQ544917	**CO1 100% match with HQ545178**	*Corallina chilensis*	GWS020436	British Columbia	Canada	52.578	-131.44
HQ544937	**CO1 100% match with HQ544623**	*Corallina chilensis*	GWS020482	British Columbia	Canada	52.579	-131.44
HQ544993	**CO1 100% match with HQ545178**	*Corallina chilensis*	GWS020579	British Columbia	Canada	52.762	-131.61
HQ545000	**CO1 99.19% match to PTM 332**	*Corallina chilensis*	GWS020590	British Columbia	Canada	52.762	-131.61
HQ545012	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS020614	British Columbia	Canada	54.033	-132.05
HQ545022	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS020648	British Columbia	Canada	54.033	-132.05
HQ545055	**CO1 100% mach to HQ545178**	*Corallina chilensis*	GWS020744	British Columbia	Canada	53.217	-131.99
HQ545057	**CO1 100% match to HQ545174**	*Corallina chilensis*	GWS020746	British Columbia	Canada	53.217	-131.99
HQ545067	**CO1 100% match to HQ545174**	*Corallina chilensis*	GWS020761	British Columbia	Canada	53.217	-131.99
HQ545119	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS020834	British Columbia	Canada	53.242	-132.02
HQ545129	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS020848	British Columbia	Canada	53.248	-131.98
HQ545147	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS020874	British Columbia	Canada	53.248	-131.98
HQ545174	**CO1 99.54% match to PTM 332**	*Corallina chilensis*	GWS020910	British Columbia	Canada	53.248	-131.98
HQ545178	**CO1 99.24% match to PTM 332**	*Corallina chilensis*	GWS020917	British Columbia	Canada	53.248	-131.98
HQ545198	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS020952	British Columbia	Canada	54.107	-132.37
HQ545202	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS020957	British Columbia	Canada	54.107	-132.37
HQ545209	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS020965	British Columbia	Canada	54.107	-132.37
JQ615617	**CO1 99.24% match to PTM 332**	*Corallina chilensis*	GWS013274	British Columbia	Canada	52.604	-131.45
JQ615619	**CO1 99.54% match to PTM 332**	*Corallina chilensis*	GWS010231	British Columbia	Canada	49.821	-126.98
JQ615620	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS010230	British Columbia	Canada	49.821	-126.98
JQ615621	**CO1 100% match to HQ544551**	*Corallina chilensis*	GWS010119	British Columbia	Canada	49.609	-126.61
JQ615622	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS010026	British Columbia	Canada	49.813	-126.99
JQ615623	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS010020	British Columbia	Canada	49.813	-126.99
JQ615624	**CO1 100% match to HQ544551**	*Corallina chilensis*	GWS009931	British Columbia	Canada	49.813	-126.99
JQ615625	**CO1 99.24% match to PTM 332**	*Corallina chilensis*	GWS009929	British Columbia	Canada	49.813	-126.99
JQ615626	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS009923	British Columbia	Canada	49.813	-126.99
JQ615627	**CO1 99.21% match to PTM 332**	*Corallina chilensis*	GWS009920	British Columbia	Canada	49.813	-126.99
JQ615628	**CO1 100% match to JQ615625**	*Corallina chilensis*	GWS009911	British Columbia	Canada	49.813	-126.99

GenBank#	BLAST Results	Species determination	Collection #	Province/State	Country	lat	long
JQ615629	**CO1 99.39% match to PTM 332**	*Corallina chilensis*	GWS009910	British Columbia	Canada	49.813	-126.99
JQ615630	**CO1 99.39% match to PTM 332**	*Corallina chilensis*	GWS009698	British Columbia	Canada	49.813	-126.99
JQ615631	**CO1 99.09% match to PTM 332**	*Corallina chilensis*	GWS009695	British Columbia	Canada	49.813	-126.99
JQ615632	**CO1 100% match to JQ615647**	*Corallina chilensis*	GWS008233	British Columbia	Canada	48.824	-125.16
JQ615633	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS006658	British Columbia	Canada	49.813	-126.99
JQ615634	**CO1 99.24% match to PTM 332**	*Corallina chilensis*	GWS009442a	British Columbia	Canada	48.352	-123.73
JQ615635	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS010755	British Columbia	Canada	48.852	-125.12
JQ615636	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS010754	British Columbia	Canada	48.852	-125.12
JQ615637	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS010753	British Columbia	Canada	48.852	-125.12
JQ615638	**CO1 98.94% match to PTM 332**	*Corallina chilensis*	GWS010751	British Columbia	Canada	48.852	-125.12
JQ615639	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS010742	British Columbia	Canada	48.852	-125.12
JQ615640	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS010634	British Columbia	Canada	48.835	-125.15
JQ615641	**CO1 100% match to JQ615631**	*Corallina chilensis*	GWS004343	British Columbia	Canada	48.53	-124.45
JQ615642	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS002859	British Columbia	Canada	48.858	-125.16
JQ615643	**CO1 100% match to HQ544839**	*Corallina chilensis*	GWS002818	British Columbia	Canada	48.852	-125.12
JQ615644	**CO1 100% match to HQ544839**	*Corallina chilensis*	GWS001450	British Columbia	Canada	48.824	-125.16
JQ615645	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS008204	British Columbia	Canada	48.824	-125.16
JQ615646	**CO1 100% match to HQ544551**	*Corallina chilensis*	GWS004885	British Columbia	Canada	54.234	-130.8
JQ615647	**CO1 99.39% match to PTM 332**	*Corallina chilensis*	GWS010302	British Columbia	Canada	49.746	-126.64
JQ615648	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS010267	British Columbia	Canada	49.725	-126.64
JQ615649	**CO1 100% match to JQ615647**	*Corallina chilensis*	GWS010264	British Columbia	Canada	49.725	-126.64
JQ615650	**CO1 100% match to JQ615647**	*Corallina chilensis*	GWS010259	British Columbia	Canada	49.725	-126.64
JQ615651	**CO1 98.79% match to PTM 332**	*Corallina chilensis*	GWS022305	California	United	36.592	-121.96
JQ615652	**CO1 100% match to JQ615647**	*Corallina chilensis*	GWS021315	California	United	37.183	-122.39
JQ615653	**CO1 100% match to JQ615647**	*Corallina chilensis*	GWS021298	California	United	37.183	-122.39
JQ615654	**CO1 100% match to HQ544839**	*Corallina chilensis*	GWS021241	California	United	37.183	-122.39
JQ615655	**CO1 99.09% match to PTM 332**	*Corallina chilensis*	GWS011047	British Columbia	Canada	49.213	-123.94
JQ615656	**CO1 100% match to JQ615625**	*Corallina chilensis*	GWS011052	British Columbia	Canada	49.213	-123.94
JQ615657	**CO1 99.24% match to PTM 332**	*Corallina chilensis*	GWS013075	British Columbia	Canada	52.586	-131.37
JQ615658	**CO1 99.24% match to PTM 332**	*Corallina chilensis*	GWS013603	British Columbia	Canada	52.462	-131.45
JQ615659	**CO1 100% match with JQ615658**	*Corallina chilensis*	GWS013609	British Columbia	Canada	52.462	-131.45
JQ615660	**CO1 99.70% match to PTM 332**	*Corallina chilensis*	GWS013610	British Columbia	Canada	52.462	-131.45
JQ615661	**CO1 100% match with JQ615660**	*Corallina chilensis*	GWS013615	British Columbia	Canada	52.462	-131.45
JQ615662	**CO1 100% match with JQ615657**	*Corallina chilensis*	GWS013659	British Columbia	Canada	52.433	-131.37
JQ615663	**CO1 99.70% match to PTM 332**	*Corallina chilensis*	GWS013661	British Columbia	Canada	52.433	-131.37
JQ615664	**CO1 99.09% match to PTM 332**	*Corallina chilensis*	GWS012563	British Columbia	Canada	53.152	-132.59
JQ615665	**CO1 99.39% match to PTM 332**	*Corallina chilensis*	GWS012945	British Columbia	Canada	52.446	-131.23
JQ615666	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS013076	British Columbia	Canada	52.586	-131.37
JQ615667	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS013242	British Columbia	Canada	52.575	-131.44
JQ615668	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS013272	British Columbia	Canada	52.604	-131.45
JQ615669	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS013275	British Columbia	Canada	52.604	-131.45
JQ615670	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS013276	British Columbia	Canada	52.604	-131.45
JQ615671	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS013281	British Columbia	Canada	52.604	-131.45
JQ615672	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS013284	British Columbia	Canada	52.604	-131.45
JQ615673	**CO1 100% match to JQ615664**	*Corallina chilensis*	GWS013286	British Columbia	Canada	52.604	-131.45
JQ615674	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS013287	British Columbia	Canada	52.604	-131.45
JQ615675	**CO1 98.94% match to PTM 332**	*Corallina chilensis*	GWS002775	British Columbia	Canada	48.852	-125.12
JQ615676	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS013613	British Columbia	Canada	52.462	-131.45
JQ615677	**CO1 100% match to HQ545178**	*Corallina chilensis*	GWS013614	British Columbia	Canada	52.462	-131.45
JQ615678	**CO1 99.09% match to PTM 332**	*Corallina chilensis*	GWS013657	British Columbia	Canada	52.433	-131.37

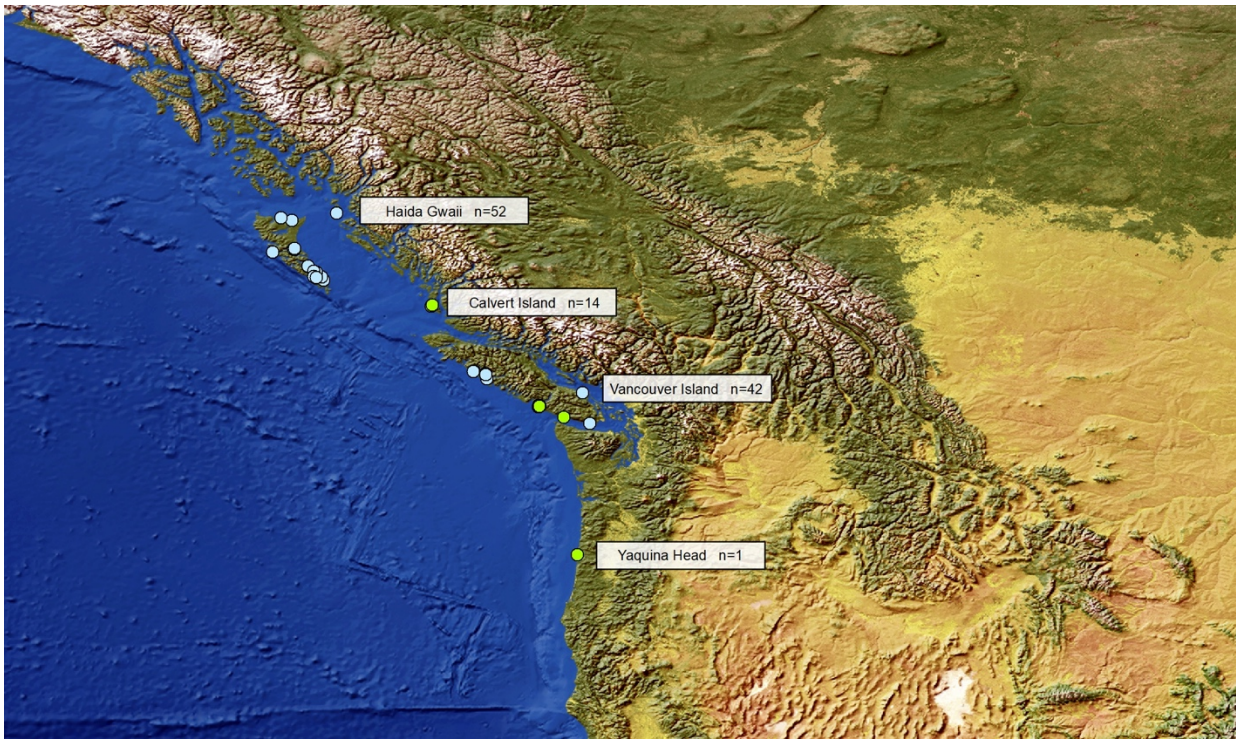


Figure S12. *Corallina chilensis* collected from the Northeast Pacific sites north of Oregon. Green dots indicate PTM collection sites for specimens included in morphometric analysis (Table S4), blue dots indicate Hind & Saunders collection sites (Hind & Saunders 2013A, see Table S3).

Appendix III: Morphological measurements

Table S4. *Corallina chilensis* measurements for morphological analysis.

*UC2050474 corresponds with NC_042901 and MK598845 in GenBank. The specimen is from Tomales, California. Publication: Alejo et al. 2019

Species	PTM	Herbarium#	grounds for name	Country	Lat	Long	Random frond width (mm)	Random frond length (mm)	Crown length (mm)	Stem length (mm)	Main axis mid intergen. max width (um)	Main axis mid intergen. min width (um)	Main axis mid intergen. length (um)	Basal intergen. width (um)	Basal intergen. length (um)	Branch intergen. max width (um)	Branch intergen. min width (um)	Branch intergen. length (um)	Full branch length (mm)	Concept. width (um)	Concept. length (um)	Tallest frond width (mm)	Tallest frond length (mm)
C. chilensis	182	A88708	Strong clade with PTM 332 & PTM 1244 in psbA & CO1 trees. PTM 332 & 1244 rbcL matches with Darwin 2151 in rbcL tree. (PTM 182 matches 99.58% in psbA to UC2050474 in NCBI BLASTn)	Canada	48.529253	-124.453704	40.09	67.68	67.68	0	1862	1440	1922	1367	2113	1331	771.5	1512	20.49	700.2	1339	34.87	115.31
C. chilensis	209	A89561	100% rbcL BLAST match with UC2050474, (UC2050474 matched PTM182)	Canada	51.651533	-128.146583	20.39	50.09	21.7	28.39	1620	785.9	1263	1060	691.8	890.5	606.6	1940	18.79			56.18	57.2
C. chilensis	306	A89266	99.84% rbcL BLAST match with UC2050474	Canada	51.66454	-128.1347	25.07	39.18	23.01	16.17	1859	816.7	1834	1517	2319	1127	474.6	1565	17.53			43.13	57.55
C. chilensis	311	A89662	99.52% rbcL BLAST match with UC2050474	Canada	51.651533	128.146583	15.83	40.42	25.73	14.69	1818	1092	1622	1084	1234	1428	779.2	2023	8.43			15.83	40.42
C. chilensis	326	A89279	99.71% rbcL BLAST match with UC2050474	Canada	51.64358056	-128.15815	46.96	66.24	45.52	20.72	2019	1048	1702			1139	740.3	1515	37.92			54.04	71.83
C. chilensis	332	A89284	Supported by psbA, CO1, & rbcL trees	Canada	51.64358056	-128.15815	37.09	43.95	38.85	5.1	1760	1170	1337	1460	1243	1235	468.9	1776	16.77			37.09	43.95
C. chilensis	333	A89285	99.17% rbcL BLAST match with UC2050474	Canada	51.64358056	-128.15815	29.01	35.17	35.17	0	2042	1162	1458	1830	1387	1949	1065	1585	29.2			17.76	47.15
C. chilensis	335	A89286	99.2% rbcL BLAST match with UC2050474	Canada	51.64358056	-128.15815	14.68	29.32	19.01	10.31	1580	891.1	1460	824.4	739.3	1330	576.1	1377	8.45			27.85	38.4
C. chilensis	337	A89288	99.76% rbcL BLAST match with UC2050474	Canada	51.64358056	-128.15815	30.24	54.86	54.86	0	1509	895.1	1459	1348	1468	1387	705.6	1722	11.65			30.24	54.86
C. chilensis	362	A89704	99.54% rbcL BLAST match with UC2050474	Canada	51.66545	-128.136033	21.42	32.76	20.49	12.27	1490	872.3	1321	1604	1054	1447	630.5	1408	8.79			22.09	42.83
C. chilensis	487	A89808	99.57% psbA BLAST match with UC2050474	Canada	51.64358	-128.1581667	21.21	48.99	22.91	26.08	1922	1302	2409	1191	848.3	2488	1026	1965	12.7			51.52	54.16
C. chilensis	629	A89961	99.67% psbA BLAST match with UC2050474	Canada	48.52959	-124.45472	51.17	95.2	87.2	8	1493	732.2	1503	1112	872.2	1244	702.7	1366	33.97	664.3	958.8	60.1	93.91
C. chilensis	726	A91477	98.47% psbA BLAST match with UC2050474	Canada	48.82432778	-125.1610139	17.22	18.23	18.23	0	1380	781.9	1254	1183	1084	1096	408.6	1165	7.13			17.3	26.33
C. chilensis	738	A91487	Supported by psbA tree	Canada	48.8341	-125.1456194	15.85	14.24	11.08	3.16	1627	921.8	1424	1239	1574	997.2	602.5	1579	6.49	615.7	1289	11.82	15.98
C. chilensis	740	A91489	Supported by psbA tree	Canada	48.8341	-125.1456194	15.57	34.87	34.87	0	1676	854.7	1548	1167	1057	1585	622.4	1613	11.63	627.9	1557	12.14	36.72
C. chilensis	742	A91491	Supported by psbA tree	Canada	48.8341	-125.1456194	23.55	31.15	31.15	0	2049	1288	1697	1666	1564	1417	677.6	1629	19.27	705.2	1723	25.25	30.85
C. chilensis	743	A91492	Supported by psbA tree	Canada	48.8341	-125.1456194	15.13	17.13	17.13	0	1677	1009	1262	1619	1203	1304	592.4	1358	6.62	615	1216	7.19	24.2
C. chilensis	763	A91509	98.86% psbA BLAST match with UC2050474	Canada	51.66453889	-128.1347	21	42.98	32.34	10.64	1096	614.6	1644	887	1653	1446	645.6	1868	8.4			29	54.13
C. chilensis	788	A91531	Supported by psbA tree	Canada	51.64358056	-128.15815	31.56	54.86	31.1	23.76	1365	910.7	1229	752.2	871.5	1221	529.6	1552	16.34	604.8	2544	30.34	79.03
C. chilensis	789	A91532	Supported by psbA tree	Canada	51.64358056	-128.15815	14.24	48.35	23.73	24.62	1338	888.8	1507	689.9	1211	979.2	671.2	1804	10.8			31.79	63.28
C. chilensis	975	A91962	99.57% psbA BLAST match with UC2050474	Canada	51.66454	-128.1347	8.97	25.65	17.79	7.86	1075	841.3	1825	1066	1142	2159	993.4	1814	7.39			17.21	41.95
C. chilensis	1244	A92161	Supported psbA, CO1, & rbcL trees	USA	44.67526	-124.07826	10.15	17.79	12.79	5	1330	1015	1676	1515	1642	1218	661.8	1936	9.32			7.3	22.83

Table S5. *Corallina vancouveriensis* measurements for morphological analysis.

*Intergeniculum located middle of main axis

PTM#	Herbarium#	Species	Reason for determination	Country	Lat	Long	Intergeniculum* max width (um)	Intergeniculum* min width (um)	Intergeniculum* length (um)	Conceptacle width (um)	Random frond length (mm)
5	**A88574**	*vancouveriensis*	Determined genetically or morphologically by PTM & KRH	Canada	36.6217444	-121.9057944	881.7	506.9	618.1	425.9	44.73
12	**A88579**	*vancouveriensis*	Determined genetically or morphologically by PTM & KRH	Canada	33.574153	-117.843647	650.4	366.3	660	445.8	44.4
96	**A88624**	*vancouveriensis*	Determined genetically or morphologically by PTM & KRH	Canada	48.529253	-122.770666	824.3	551.7	942.9	493.7	47.44
156	**A88682**	*vancouveriensis*	Determined genetically or morphologically by PTM & KRH	Canada	48.529253	-124.453704	791.8	503.9	958.2	579.5	61.66
158	**A88684**	*vancouveriensis*	In Genbank: KJ637656 Hind et al. 2014	Canada	48.529253	-124.453704	964.1	542.9	943.9	581.6	72.62
161	**A88687**	*vancouveriensis*	Determined genetically or morphologically by PTM & KRH	Canada	48.529253	-124.453704	776.6	498.2	821.9	471.2	70.42
163	**A88689**	*vancouveriensis*	Determined genetically or morphologically by PTM & KRH	Canada	48.529253	-124.453704	1214	674.2	966.8	590.4	53
173	**A88699**	*vancouveriensis*	Determined genetically or morphologically by PTM & KRH	Canada	48.529253	-124.453704	998.3	404.1	841.6	556	48.96
177	**A88703**	*vancouveriensis*	Determined genetically or morphologically by PTM & KRH	Canada	48.529253	-124.453704	888.2	454	675.4	496	52.69
179	**A88705**	*vancouveriensis*	Determined genetically or morphologically by PTM & KRH	Canada	48.529253	-124.453704	1102	565.8	1095		56.4
212	**A89564**	*vancouveriensis*	Determined genetically or morphologically by PTM & KRH	Canada	51.651533	-128.1347	862.8	533.5	821.6	586.7	64.2
313	**A89448**	*vancouveriensis*	99.10% rbcL BLAST match with NCU588197/HQ322334 Gabrielson et al. 2011	Canada			766.4	456.5	936.6	377.5	71.55
320	**A89274**	*vancouveriensis*	100% rbcL BLAST match NCU588197/HQ322334 Gabrielson et al. 2011	Canada			627.5	433.2	807.8	462.4	60.36
328	**A89280**	*vancouveriensis*	99.71% rbcL BLAST match HQ3223341/NCU588197 Gabrielson et al. 2011	Canada			708.1	442.9	889.1	461.6	61.01
714	**A91468**	*vancouveriensis*	98.63% BLAST psbA match to UBCA88684/KJ637656 Hind et al. 2014	Canada	48.8243278	-125.1610139	848.3	492.7	657.1	442.9	34.61
715	**A91469**	*vancouveriensis*	98.01% psbA BLAST match to A88684/KJ637656 Hind et al. 2014	Canada	48.8243278	-125.1610139	768.8	410.2	715.5	433.1	39.88
758	**A91504**	*vancouveriensis*	100% psbA BLAST match to JQ422228 & KJ637656 Hind & Saunders 2013A/UBC A88684 Hind	Canada	51.6645389	-128.1347	604.8	380.2	834.1	472.3	46.23
760	**A91506**	*vancouveriensis*	psbA tree in clade ith JQ422228	Canada	51.6645389	-128.1347	806.8	678.7	384	695	30.72
767	**A91513**	*vancouveriensis*	psbA tree in clade ith JQ422228, CO1 tree with JQ615834, in rbcL tree	Canada	51.6645389	-128.1347	851.4	538.6	801.1	538.9	45.36

Appendix IV: Methods tables

Table S6. Thermal cycler settings.

psbA 20 ul	Temp (C)	Time	Step
Initial denaturation	94	4:00	1
Denaturation	94	1:00	2
Annealing	50	0:30	3
Extension	72	1:00	4
Go to step 2 30x			5
Final extension	72	7:00	6

psbA 25 ul	Temp (C)	Time	Cycle
Initial denaturation	94	5:00	1
Denaturation	94	0:30	2
Annealing	50	0:30	3
Extension	72	0:42	4
Go to step 2 30x			5
Final extension	72	7:00	6

CO1 20 ul	Temp (C)	Time	Step
Initial denaturation	94	2:00	1
Denaturation	94	0:30	2
Annealing	45	0:30	3
Extension	72	1:00	4
Go to step 2 5x			5
Denaturation	94	0:30	6
Annealing	46.5	0:30	7
Extension	72	1:00	8
Go to step 6 35x			9
Final extension	72	7:00	10

CO1 25 ul	Temp (C)	Time	Cycle
Initial denaturation	94	5:00	1
Denaturation	94	0:10	2
Annealing	46.5	0:20	3
Extension	72	0:30	4
Go to step 2 40x			5
Final extension		7:00	6

rbcL 20 ul	Temp (C)	Time	Step
Initial denaturation	95	2:00	1
Denaturation	93	1:00	2
Annealing	47	1:00	3
Extension	72	2:00	4
Go to step 2 35x			5
Final extension	72	2:00	6

rbcL 25 ul	Temp (C)	Time	Cycle
Initial denaturation	95	5:00	1
Denaturation	93	0:10	2
Annealing	47	0:20	3
Extension	72	0:30	4
Go to step 2 40x			5
Final extension	72	7:00	6

Table S7. Rates of evolution and models for tree analyses.

Alignment	Gene	Codon	Iqtree MAC aLRT/I	MrBayes "set models"	Partition mymodels on PC MrBayes
psbA	psbA	1	TNe+I	nst=6 rates=propinv; statefreqpr=fixed(equal)	SYM+I
	psbA	2	F81+F+I	nst=1 rates=propinv	F81+F+I
	psbA	3	HKY+F+G4	nst=2 rates=gamma	HKY+F+G4
CO1	CO1	1	TN+F+G4	nst=6 rates=gamma	GTR+F+G4
	CO1	2	F81+F+I	nst=1 rates=propinv	F81+F+I
	CO1	3	K3Pu+F+I+G4	nst=1 rates=propinv	GTR+F+I+G4
rbcL	rbcL	1	TIM+F+I	nst=6 rates=propinv	GTR+F+I
	rbcL	2	F81+F+I	nst=1 rates=propinv	F81+F+I
	rbcL	3	TIM3+F+G4	nst=6 rates=gamma	GTR+F+G4
rbcL type	rbcL	1	TIM+F+I	nst=6 rates=propinv	GTR+F+I
	rbcL	2	F81+F+I	nst=1 rates=propinv	F81+F+I
	rbcL	3	TIM3+F+G4	nst=6 rates=gamma	GTR+F+G4

Appendix V: Distance matrices

Table S8. Percent difference matrix of *psb*A sequences.

	C. sp. 1 chile	C. sp. 1 gws	C. sp. 2 chile	C. aberrans	C. sp. 1 california	C. officinalis	C. crassissima	C. vancouveriensis	C. declinata	C. ferreyrae	Lithothamnion glaciale	C. ferreyrae-like	C. gws-like	C. sp. 3 frondescens-like	C. maxima	Bossiella frondifera	Calliarthron cheilosporioides	Chiharaea bodegensis	Crusticorallina muricata	Ellisolandia elongata	C. chilensis	C. sp. 2 frondescens	C. sp. 2 vancouveriensis	C. sp. 3 frondescens	C. sp. 4 frondecens	C. sp. 5 frondescens
C. sp. 1 chile	0.12 0.00	1.18 1.06	1.29 1.06	1.41 1.18	0.82 0.71	0.94 0.82	1.53 1.41	1.65 1.53	1.29 1.06	1.18 0.94	10.34 10.22	1.06 0.94	1.29 1.18	1.76 1.65	1.41 1.29	6.70 6.58	6.51 6.39	6.68 6.56	4.94 4.82	6.00 5.88	1.29 1.06	1.06 0.94	2.00 1.88	1.65 1.41	1.41 1.29	1.06 0.82
C. sp. 1 gws		0.00 0.00	1.76 1.65	1.65 1.53	1.06 1.06	1.18 1.18	1.76 1.76	1.41 1.41	1.53 1.41	1.41 1.30	9.99 9.99	1.53 1.53	0.24 0.24	2.00 2.00	1.06 1.06	6.23 6.23	6.63 6.63	6.43 6.43	4.70 4.70	6.13 6.13	1.41 1.29	1.18 1.18	2.12 2.12	1.88 1.76	1.53 1.53	1.18 1.06
C. sp. 2 chile			0.12 0.00	1.53 1.29	0.94 0.82	1.06 0.94	1.65 1.53	1.53 1.41	1.41 1.29	0.82 0.59	9.64 9.52	1.06 0.94	2.00 1.88	1.88 1.76	1.76 1.65	6.58 6.46	6.88 6.76	6.68 6.56	5.05 4.94	6.00 5.88	1.41 1.18	1.18 1.06	1.88 1.76	1.76 1.53	1.18 1.06	1.18 0.94
C. aberrans				0.12 0.12	0.82 0.71	0.94 0.82	0.59 0.47	1.41 1.29	0.12 0.00	1.18 0.94	9.64 9.52	1.53 1.41	1.88 1.76	1.29 1.18	1.41 1.29	5.41 5.41	6.27 6.14	5.53 5.40	4.47 4.35	5.88 5.88	1.29 1.06	0.82 0.71	1.76 1.65	1.18 0.94	1.18 1.06	1.06 0.82
C. sp. 1 california					0.00 0.00	0.59 0.59	0.94 0.94	1.06 1.06	0.71 0.71	0.59 0.47	9.99 9.99	1.18 1.18	1.29 1.29	1.18 1.18	1.06 1.06	6.11 6.11	6.39 6.39	6.17 6.17	4.58 4.58	5.88 5.88	0.47 0.35	0.47 0.47	1.41 1.41	1.06 0.94	0.59 0.59	0.24 0.12
C. officinalis						0.00 0.00	0.82 0.82	1.18 1.18	0.82 0.82	0.94 0.82	9.64 9.64	1.06 1.06	1.41 1.41	1.29 1.29	1.18 1.18	5.99 5.99	6.27 6.27	6.04 6.04	4.70 4.70	5.51 5.51	1.06 0.94	0.35 0.35	1.53 1.53	1.18 1.06	0.94 0.94	0.82 0.71
C. crassissima							0.00 0.00	1.53 1.53	0.47 0.47	1.30 1.18	9.52 9.52	1.65 1.65	2.00 2.00	1.65 1.65	1.53 1.53	5.76 5.76	6.51 6.51	5.66 5.66	4.47 4.47	5.88 5.88	1.41 1.29	0.71 0.71	1.88 1.88	1.53 1.41	1.29 1.29	1.18 1.06
C. vancouveriensis								0.00 0.00	1.29 1.29	1.18 1.06	9.99 9.99	1.53 1.53	1.65 1.65	1.76 1.76	1.29 1.29	6.23 6.23	7.00 7.00	6.68 6.68	4.94 4.94	6.25 6.25	1.18 1.06	0.94 0.94	0.71 0.71	1.65 1.41	1.29 1.29	1.18 1.06
C. declinata									0.00 0.00	1.06 0.94	9.52 9.52	1.41 1.41	1.76 1.76	1.18 1.18	1.29 1.29	5.41 5.41	6.14 6.14	5.40 5.40	4.35 4.35	5.88 5.88	1.18 1.06	0.71 0.71	1.65 1.65	1.06 0.94	1.05 1.06	0.94 0.82
C. ferreyrae										0.12 0.00	9.89 9.78	0.82 0.71	1.65 1.53	1.53 1.41	1.30 1.18	6.36 6.24	6.88 6.76	6.43 6.30	5.18 5.06	5.89 5.76	1.06 0.82	0.82 0.71	1.53 1.41	1.41 1.30	0.94 0.82	0.82 0.59
Lithothamnion glaciale												9.87 9.87	10.11 10.11	10.11 10.11	9.64 9.64	9.52 9.52	9.83 9.83	10.15 10.15	10.81 10.81	10.81 10.81	9.87 9.64	9.75 9.75	10.34 10.34	10.34 10.22	9.87 9.87	9.99 9.87
C. ferreyrae-like												0.00 0.00	1.76 1.76	1.88 1.88	1.53 1.53	6.58 6.58	6.88 6.88	6.43 6.43	5.05 5.05	6.13 6.13	1.65 1.53	1.18 1.18	1.88 1.88	1.76 1.65	1.53 1.53	1.41 1.29
C. sp. 1 GWS-like													2.23 2.23	1.29 1.29	6.46 6.46	6.88 6.88	6.68 6.68	4.94 4.94	6.37 6.37	1.65 1.53	1.41 1.41	2.35 2.35	2.12 2.00	1.76 1.76	1.41 1.29
C. sp. 3 frondescens-like													0.00 0.00	1.76 1.76	5.99 5.99	7.00 7.00	6.30 6.30	4.82 4.82	5.88 5.88	1.65 1.53	1.18 1.18	2.12 2.12	0.59 0.41	1.53 1.53	1.41 1.29
C. maxima															6.23 6.23	6.88 6.88	6.43 6.43	5.29 5.29	6.13 6.13	1.18 0.94	0.82 0.82	1.76 1.76	1.65 1.53	1.18 1.18	1.06 0.94
Bossiella frondifera																4.05 4.05	2.96 2.96	7.52 7.52	7.60 7.60	6.23 6.11	6.11 6.11	6.58 6.58	6.23 6.11	6.23 6.23	6.35 6.23
Calliarthron cheilosporioides																	4.37 4.37	7.49 7.49	7.97 7.97	6.39 6.27	6.63 6.63	7.13 7.13	7.00 6.88	6.88 6.88	6.51 6.51
Chiharaea bodegensis																		7.33 7.33	7.13 7.13	6.30 6.30	6.17 6.17	16.68 6.68	6.17 6.04	6.43 6.43	6.30 6.30
Crusticorallina muricata																			8.47 8.47	4.70 4.58	4.70 4.70	5.41 5.41	4.94 4.82	4.94 4.94	4.70 4.58
Ellisolandia elongata																				6.00 6.00	5.76 5.76	6.50 6.50	5.63 5.51	5.88 5.88	5.88 5.88
C. chilensis																				0.24 0.00	0.82 0.59	1.53 1.29	1.53 1.29	0.94 0.71	0.59 0.24
C. sp. 2 frondescens																					0.00 0.00	1.18 1.18	1.06 0.94	0.59 0.59	0.47 0.35
C. sp. 2 vancouveriensis																							2.00 1.76	1.53 1.53	1.29 1.18
C. sp. 3 frondescens																							0.24 0.00	1.41 1.29	1.29 1.06
C. Sp. 4 frondescens																								0.00 0.00	0.59 0.47
C. Sp. 5 frondescens																									0.12 0.00

Table S9. Percent difference matrix of CO1 sequences.

	C. aberrans	*C. caespitosa*	*C. crassissima*	*C. declinata*	*C. ferreyrae*	*C. maxima*	*C. officinalis*	C. sp. 1 california	C. sp. 1 chile	*C. chilensis*	C. sp. 1 gws	C. sp. 2 chile	C. sp. 2 frondescens	C. sp. 2 vancouveriensis	C. sp. 3 frondescens	C. sp. 4 frondescens	C. sp. 5 frondescens	C. sp. 5 korea	C. ferreyrae-like	C. sp. 1 gws-like	C. sp. 3 frondescens-like	*C. vancouveriensis*	*Bossiella frondifera*	*Calliarthron cheilosporioides*	*Chiharea bodegensis*	*Crusticorallina muricata*	*Ellisolandia elongata*	*Lithothamnion glaciale*
C. aberrans	0.00	20.72	4.54	5.06	8.50	9.53	7.11	8.93	9.08	7.87	6.51	8.70	7.56	7.56	9.00	7.11	7.11	4.69	9.83	8.17	9.23	7.56	13.98	14.67	14.52	12.41	13.16	18.91
	0.00	20.72	4.54	4.54	8.18	9.53	6.68	8.77	8.77	6.96	6.51	7.87	7.56	7.56	7.18	6.35	7.11	4.69	9.83	8.17	9.23	7.26	13.98	14.67	14.52	12.41	13.16	18.91
C. caespitosa			19.96	19.96	2.97	21.63	20.57	22.08	18.30	20.42	21.02	14.36	20.72	21.48	22.46	20.27	20.87	20.57	16.33	21.03	21.63	20.87	25.53	26.17	25.57	23.75	25.57	28.14
			19.96	19.81	2.60	21.63	20.57	22.08	17.84	19.96	21.02	14.20	20.57	21.48	21.93	92.11	20.87	20.57	16.33	21.93	21.63	20.57	25.53	26.17	25.57	23.75	25.57	28.14
C. crassissima			0.00	5.22	8.04	8.62	7.41	7.41	8.62	6.66	7.56	8.07	6.81	8.17	9.08	7.72	6.81	4.08	9.38	8.62	9.38	8.47	14.76	15.73	14.52	12.56	13.62	19.36
			0.00	4.84	7.73	8.62	6.12	7.26	8.32	6.20	7.56	7.72	6.51	8.17	7.66	7.41	6.81	4.08	9.38	8.62	9.38	8.17	14.76	15.73	14.52	12.56	13.52	19.36
C. declinata				0.47	8.23	9.49	7.44	7.44	9.34	7.75	8.07	8.82	7.59	8.54	8.86	7.28	6.96	5.38	9.18	9.49	8.86	8.54	15.78	16.30	16.46	13.61	15.03	18.91
				0.47	7.28	8.93	6.68	6.66	8.32	6.81	7.41	7.26	7.11	7.87	7.18	6.51	6.51	4.99	8.62	8.77	8.62	8.02	15.38	16.04	15.89	12.86	14.07	18.67
C. ferreyrae					0.30	10.02	8.80	10.02	6.83	9.10	8.50	3.33	8.04	9.86	10.47	8.04	8.65	8.50	3.03	9.56	10.32	9.56	13.82	15.78	15.78	13.35	14.57	18.51
					0.30	10.00	8.35	9.55	6.07	8.33	8.18	2.88	7.59	9.55	9.71	7.73	8.33	8.18	3.03	9.26	10.02	9.24	13.51	15.48	15.48	13.05	14.26	18.21
C. maxima							8.72	9.38	8.47	9.83	8.02	9.48	7.11	9.53	10.14	9.08	7.41	9.23	10.44	9.23	9.38	9.83	15.85	15.28	15.58	11.65	15.13	20.12
							8.47	9.23	8.02	9.38	8.02	8.62	6.96	9.53	9.53	8.32	7.41	9.23	10.44	9.23	9.38	9.53	15.85	15.28	15.58	11.65	15.13	20.12
C. officinalis							0.00	6.81	8.32	7.26	8.16	8.85	7.11	4.24	8.32	7.11	5.60	7.26	9.46	9.46	8.02	4.08	15.38	15.73	15.73	13.46	14.83	19.82
							0.00	6.66	7.61	6.31	8.02	7.79	6.49	3.53	8.42	6.68	5.57	7.05	9.38	9.23	7.42	3.90	14.98	15.40	15.58	12.99	14.66	18.37
C. sp. 1 california								0.15	9.23	8.02	8.93	10.26	6.66	8.17	9.23	6.96	5.14	7.72	10.44	8.47	8.77	7.87	16.47	16.94	17.40	13.16	14.98	18.46
								0.00	8.62	7.41	8.93	9.53	6.51	8.02	8.13	6.51	4.99	7.72	10.29	8.47	8.62	7.56	16.32	16.79	17.25	13.16	14.98	18.31
C. sp. 1 chile									0.45	8.32	8.47	5.89	8.02	9.53	10.59	8.17	8.17	8.93	7.41	9.38	9.68	9.53	15.69	16.79	15.58	13.31	14.98	19.36
									0.15	7.26	8.17	4.39	7.41	9.23	9.89	7.41	7.72	8.62	6.96	9.08	9.23	9.08	15.38	16.49	15.13	13.01	14.83	19.06
C. chilensis										0.91	8.47	8.47	7.26	8.02	9.53	6.66	6.05	6.35	9.83	9.53	8.93	8.62	15.54	16.49	16.49	13.01	14.98	19.52
										0.45	7.56	7.56	6.96	7.72	8.45	5.90	5.90	6.05	9.38	8.62	8.32	7.72	15.23	15.58	15.89	12.41	14.22	18.61
C. sp. 1 gws											0.00	8.85	7.41	8.62	9.76	8.02	7.56	8.17	9.83	3.18	9.23	9.53	14.76	14.98	16.04	12.71	13.77	19.36
											0.00	8.17	7.26	8.62	9.08	7.41	7.56	8.17	9.83	3.18	9.23	9.23	14.76	14.98	16.04	12.71	13.77	19.36
C. sp. 2 chile												1.37	8.23	10.26	11.35	8.23	8.54	8.54	3.48	9.63	10.73	9.79	14.60	15.28	14.98	13.53	14.47	18.91
												0.00	7.72	9.23	9.53	7.72	8.17	8.32	3.03	9.08	9.83	9.08	14.14	14.78	14.83	13.16	13.92	18.53
C. sp. 2 frondescens													0.30	8.17	9.61	6.20	5.14	7.87	8.77	8.32	8.47	9.08	5.07	15.13	15.43	12.56	13.92	18.76
													0.30	7.87	8.77	5.90	5.14	7.72	8.62	8.17	8.47	8.47	14.91	14.98	15.28	12.41	13.77	18.61
C. sp. 2 vancouveriensis															8.55	7.56	6.35	8.62	10.89	9.83	8.77	4.39	15.23	16.49	16.19	13.62	14.52	19.67
															7.34	7.26	6.35	8.62	10.89	9.83	8.77	4.08	15.23	16.49	16.19	13.62	14.52	19.67
C. sp. 3 frondescens															2.80	9.30	8.93	8.93	11.80	10.82	5.30	8.85	17.02	17.93	17.40	13.16	17.02	20.65
															0.16	8.77	8.17	8.13	11.32	10.05	3.03	7.66	15.58	16.34	16.43	12.44	15.73	19.46
C. sp. 4 frondescens																1.82	4.69	6.96	9.23	9.08	9.23	7.87	14.29	14.67	14.67	12.86	13.92	19.67
																0.00	4.54	6.66	8.93	8.17	8.77	7.26	13.82	14.22	14.07	11.95	13.62	18.76
C. sp. 5 frondescens																	0.00	6.35	9.23	8.02	7.87	7.26	14.76	16.19	15.89	12.56	14.07	19.36
																	0.00	6.35	9.23	8.02	7.87	7.26	14.76	16.19	15.89	12.56	14.07	19.36
C. sp. 5 korea																			9.23	9.23	8.62	8.32	15.07	15.43	14.83	12.25	13.31	19.06
																			9.23	9.23	8.62	8.02	15.07	15.43	14.83	12.25	13.31	19.06
C. ferreyrae-like																			0.00	10.14	11.04	10.14	14.76	16.64	15.73	13.46	14.67	19.06
																			0.00	10.14	11.04	9.83	14.76	16.64	15.73	13.46	14.67	19.06
C. sp. 1 gws-like																					9.53	9.68	15.07	16.19	16.79	13.77	14.22	19.67
																					9.53	9.53	15.07	16.19	16.79	13.77	14.22	19.67
C. sp. 3 frondescens-like																						8.77	15.85	17.7	16.49	13.16	15.73	19.97
																						8.47	15.85	17.7	16.49	13.16	15.73	19.97
C. vancouveriensis																						0.45	15.54	16.49	16.49	13.92	16.04	20.42
																						0.00	15.23	16.19	16.19	13.92	15.73	20.42
Bossiella frondifera																								11.02	9.31	15.38	13.82	17.56
																								11.02	9.31	15.38	13.82	17.56
Calliarthron cheilosporioides																									10.69	16.64	15.13	18.52
																									10.69	16.64	15.13	18.52
Chiharea bodegensis																										15.58	15.73	19.43
																										15.58	15.73	19.43
Crusticorallina muricata																											13.92	18.46
																											13.92	18.46
Ellisolandia elongata																												16.94
																												16.94
Lithothamnion glaciale																												

Table S10. Percent difference matrix of *rbc*L sequences.

	C. crassissima	*C. aberrans*	*C. ferreyrae*	*C. melobesioides*	*C. officinalis*	*C. pinnatifolia*	*C. sp. 1 california*	*C. sp. 1 chile*	*C. chilensis*	*C. sp. 1 gws*	*C. sp. 2 chile*	*C. sp. 2 frondescens*	*C. sp. 3 frondescens*	*C. sp. 4 frondescens*	*C. sp. 5 frondescens*	*C. ferreyrae-like*	*C. sp. 1 gws-like*	*C. sp. 3 frondescens-like*	*C. vancouveriensis*	*Bossiella frondifera*	*Calliarthron cheilosporioides*	*Chiharaea bodegensis*	*Crusticorallina muricata*	*Ellisolandia elongata*	*Lithothamnion glaciale*
C. crassissima	0.00 0.00	0.82 0.82	2.32 2.32	2.62 2.62	3.83 2.62	1.80 1.80	1.72 1.65	2.32 2.32	2.08 1.95	2.32 2.32	2.48 2.17	2.02 2.02	3.54 3.45	2.15 2.13	1.77 1.77	2.47 2.47	2.55 2.55	3.37 3.30	2.82 2.82	10.57 10.57	10.12 10.12	10.33 10.33	5.55 5.55	9.30 9.30	16.12 16.12
C. aberrans			2.55 2.55	2.85 2.85	3.98 3.00	2.77 2.77	1.95 1.80	2.55 2.55	2.26 2.10	2.62 2.62	2.70 2.40	2.25 2.25	3.85 3.75	2.32 2.28	1.93 1.93	2.70 2.70	2.85 2.85	3.67 2.82	2.82 2.82	10.79 10.79	10.04 10.04	10.33 10.33	5.40 5.40	9.30 9.30	15.90 15.90
C. ferreyrae				0.67 0.67	3.10 2.25	0.60 0.60	1.80 1.65	0.97 0.97	1.90 1.72	1.95 1.95	0.68 0.37	2.03 2.03	3.78 3.67	2.28 2.23	1.69 1.69	0.82 0.82	2.10 2.10	3.52 3.45	2.74 2.74	10.57 10.57	10.12 10.12	10.25 10.25	5.40 5.40	9.00 9.00	15.97 15.97
C. melobesioides					3.10 2.55	0.37 0.37	2.10 1.95	1.05 1.05	2.02 1.99	2.25 2.25	0.45 0.45	2.32 2.32	3.95 3.82	2.66 2.59	2.01 2.01	0.90 0.90	2.40 2.40	3.67 3.60	2.90 2.90	10.34 10.34	10.04 10.04	10.02 10.02	5.40 5.40	9.15 9.15	15.82 15.82
C. officinalis					0.59 0.15	3.24 2.47	1.83 1.42	3.24 2.47	2.36 1.80	3.10 2.62	3.41 2.25	2.51 1.95	3.24 2.62	3.10 2.41	2.36 1.77	3.24 2.70	3.24 2.85	3.39 2.77	3.10 2.74	10.79 10.62	10.57 10.18	10.25 9.52	6.19 5.32	9.22 8.41	16.20 15.04
C. pinnatifolia							2.02 1.87	0.97 0.97	1.99 1.95	2.17 2.17	0.37 0.08	2.25 2.25	4.03 3.90	2.58 2.51	1.93 1.93	0.82 0.82	2.32 2.32	3.75 3.67	2.98 2.98	10.49 10.49	10.19 10.19	10.18 10.18	5.47 5.47	9.22 9.22	15.90 15.90
C. sp. 1 california							0.15 0.00	1.92 1.72	1.08 0.90	1.80 1.65	2.00 1.50	1.27 1.12	2.83 2.62	1.55 1.33	0.73 0.64	2.10 1.95	2.10 1.83	2.92 2.67	1.77 1.67	10.42 10.27	10.25 10.12	10.00 9.87	5.33 5.17	9.30 9.22	16.17 16.05
C. sp. 1 chile								0.00 0.00	2.08 1.87	1.80 1.80	0.90 0.60	2.17 2.17	3.62 3.45	2.49 2.44	1.85 1.85	1.20 1.20	2.10 2.10	3.37 3.30	2.74 2.74	10.19 10.19	10.04 10.04	9.95 9.95	5.25 5.25	8.85 8.85	15.59 15.59
C. chilensis									0.09 0.09	1.80 1.63	1.99 1.57	1.17 1.12	3.43 2.98	1.63 1.55	0.81 0.81	2.08 1.87	2.10 21.99	3.16 2.92	2.18 2.08	10.75 10.64	10.57 10.49	10.25 10.21	6.05 5.55	9.58 9.37	16.08 15.97
C. sp. 1 gws											2.10 1.80	1.95 1.95	3.62 3.38	1.98 1.90	1.61 1.61	2.17 2.17	0.52 0.52	3.15 3.07	2.66 2.66	9.90 9.90	9.90 9.90	9.71 9.71	5.40 5.40	8.70 8.70	15.44 15.44
C. sp. 2 chile											0.30 0.30	2.18 1.87	3.95 3.52	2.58 2.13	1.86 1.53	0.75 0.60	2.25 1.95	3.68 3.30	2.91 2.58	10.44 10.42	10.29 10.27	10.18 10.18	5.41 5.25	9.32 9.00	15.90 15.90
C. sp. 2 frondescens													3.00 2.82	1.75 1.63	1.05 1.05	2.32 2.32	2.32 2.32	2.62 2.55	2.10 2.10	10.79 10.79	10.87 10.87	10.49 10.49	5.32 5.32	9.82 9.82	16.43 16.43
C. sp. 3 frondescens													0.48 0.15	3.39 2.90	3.06 2.74	4.03 3.97	3.85 3.62	1.20 0.72	3.71 3.55	10.06 9.85	9.82 9.55	9.63 9.43	5.64 5.55	9.42 9.16	16.03 15.75
C. sp. 4 frondescens														0.17 0.17	1.18 1.13	2.66 2.59	2.15 2.05	3.12 2.92	2.36 2.34	11.00 10.58	10.22 10.20	10.14 9.95	5.63 5.41	9.67 9.54	16.15 15.99
C. sp. 5 frondescens																1.93 1.93	1.93 1.93	2.74 2.66	1.85 1.85	10.64 10.64	10.64 10.64	10.22 10.22	5.56 5.56	9.59 9.59	16.53 16.53
C. ferreyrae-like																0.00 0.00	2.32 2.32	3.82 3.75	2.82 2.82	10.42 10.42	10.42 10.42	10.18 10.18	5.40 5.40	9.15 9.15	15.59 15.59
C. sp. 1 gws-like																	0.00 0.00	3.37 2.30	2.90 2.90	9.97 9.97	9.90 9.90	9.79 9.79	5.47 5.47	8.70 8.70	15.59 15.59
C. sp. 3 frondescens-like																		0.07 0.07	3.55 3.46	9.82 9.75	9.67 9.60	9.48 9.41	5.25 5.17	8.85 8.77	15.59 15.52
C. vancouveriensis																				10.48 10.48	10.31 10.31	10.05 10.05	5.72 5.72	9.67 9.67	16.45 16.45
Bossiella frondifera																					5.10 5.10	4.39 4.39	10.12 10.12	10.79 10.79	14.69 14.69
Calliarthron cheilosporioides																						4.78 4.78	9.45 9.45	10.64 10.64	14.16 14.16
Chiharaea bodegensis																							9.79 9.79	10.79 10.79	15.57 15.57
Crusticorallina muricata																								9.45 9.45	15.44 15.44
Ellisolandia elongata																									15.59 15.59
Lithothamnion glaciale																									