RESEARCH ARTICLE

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Resolving cryptic species of *Bossiella* (Corallinales, Rhodophyta) using contemporary and historical DNA[1]

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PREMISE OF THE STUDY: Phenotypic plasticity and convergent evolution have long complicated traditional morphological taxonomy. Fortunately, DNA sequences provide an additional basis for comparison, independent of morphology. Most importantly, by obtaining DNA sequences from historical type specimens, we are now able to unequivocally match species names to genetic groups, often with surprising results.

METHODS: We used an integrative taxonomic approach to identify and describe Northeast Pacific pinnately branched species in the red algal coralline genus *Bossiella*, for which traditional taxonomy recognized only one species, the generitype, *Bossiella plumosa*. We analyzed DNA sequences from historical type specimens and modern topotype specimens to assign species names and to identify genetic groups that were different and that required new names. Our molecular taxonomic assessment was followed by a detailed morphometric analysis of each species.

KEY RESULTS: Our study of *B. plumosa* revealed seven pinnately branched *Bossiella* species. Three species, *B. frondescens*, *B. frondifera*, and *B. plumosa*, were assigned names based on sequences from type specimens. The remaining four species, *B. hakaiensis*, *B. manzae*, *B. reptans*, and *B. montereyensis*, were described as new to science. In most cases, there was significant overlap of morphological characteristics among species.

CONCLUSIONS: This study underscores the pitfalls of relying upon morpho-anatomy alone to distinguish species and highlights our likely underestimation of species worldwide. Our integrative taxonomic approach can serve as a model for resolving the taxonomy of other plant and algal genera.

KEY WORDS *Bossiella*; COI-5P; coralline algae; *Corallina frondescens*; cryptic species; *psbA*; *rbcL*; integrative taxonomy

The process of species identification and description has changed dramatically over the last 50 years. Traditionally, taxonomists relied solely on morpho-anatomical criteria to delineate species. However, we now know that this approach can be problematic due to high levels of intraspecific variation in morphological characters resulting from phenotypic plasticity (Stewart, 2006; Koehl et al., 2008; Monro and Poore, 2009) and convergent morphologies between distantly related species due to analogous environmental pressures (Johansen, 1981; Steneck, 1986). For example, morphologically distinct specimens have turned out to reflect morphological variants of a single species (e.g., Gabrielson et al., 2011; Hind et al., 2014a), and genetically distinct species sometimes exhibit nearly identical morphologies (e.g., van der Merwe et al., 2015). More recently, the incorporation of molecular sequence data, particularly DNA barcodes (standardized regions of DNA), into taxonomic research has achieved more accurate estimates of species diversity and at less cost than traditional morphological methods (Hebert et al., 2003, 2004; Thompson and Newmaster, 2014). A growing trend in diversity studies is to use integrative approaches (Padial et al., 2010), such as molecular-assisted alpha taxonomy (MAAT) (Saunders, 2005, 2008; Cianciola et al., 2010), that combine molecular and classic morpho-anatomical data to identify and describe organisms.

After species have been delineated, taxonomists are challenged with applying names to these species. Naming botanical species is governed by the International Code of Nomenclature for Algae, Fungi and Plants (McNeill et al., 2012) and is based on a "type" system of nomenclature. This task involves an analysis of the type specimen, the specimen to which the name is permanently attached (McNeill et al., 2012, Article 7.2), and an exploration of all available

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species names that might be applied to a genetic group or clade. Sometimes type specimens are inaccessible, missing, or unavailable for anatomical investigation and/or DNA extraction. Consequently, in place of the type specimen, topotype material (contemporary specimens from the collection site of the type specimen) is often used as a surrogate, even though type locality information can be vague and environmental conditions may have changed dramatically since the original collection was made. For example, anthropogenic changes in the environment such as fish-farming or coastal development may increase algal abundance (Sanderson et al., 2012) or decrease algal diversity (Bates et al., 2009). In addition, herbarium records from the last 70 years documented significant poleward shifts of seaweed species in Australia (Wernberg et al., 2011). Thus, returning to the original type locality to collect specimens does not guarantee the presence of the target species.

Another technique to apply species names to contemporary collections is the use of historical DNA sequencing. Historical DNA approaches have been used extensively across many taxa including woolly mammoths (Hagelberg et al., 1994) and algal fossils (Hughey et al., 2008). Adoption of these techniques has come with some apprehension as to the reliability of these data due to contamination and proper experimental controls (Saunders and McDevit, 2012). However, these issues have been addressed (Hughey and Gabrielson, 2012), and sequences from type specimens have been essential to apply names to contemporary collections of morphologically variable genera (Hughey et al., 2001; Gabrielson, 2008; Lindstrom et al., 2011, 2015; Hind et al., 2014b).

In this paper, we used both topotype collections and historical DNA approaches to apply names to contemporary specimens in a group of red algae with known taxonomic uncertainties. The Corallinales is a large order (over 700 species) of calcified red algae that are notoriously difficult to identify due to convergent and simple morphologies (Hind et al., 2014a; Pardo et al., 2014). They are important components of marine ecosystems, acting as ecosystem engineers (Asnaghi et al., 2015), providing structural support to reefs (Nelson, 2009) and emitting chemical settlement cues to marine invertebrate species (Whalan et al., 2012), yet we know little about their specific identities. The order comprises species with upright, usually jointed fronds (geniculate species) and prostrate crusts (nongeniculate species). Over the past 20 years, molecular phylogenetic studies have revealed that some nongeniculate species are more closely related to geniculate species than they are to other nongeniculate species (Bailey and Chapman, 1998; Hind and Saunders, 2013b), raising interesting questions about morphological evolution and speciation of coralline algae.

Recently, *Bossiella* P.C.Silva, a common eastern Pacific geniculate genus, was revised to include 17 species (Hind et al., 2014b) although only four were recognized previously in taxonomic keys (Gabrielson et al., 2012) and in the literature (Johansen, 1971). Gabrielson et al. (2011) showed that *Bossiella* was monophyletic within the subfamily Corallinoideae by including the generitype, *Bossiella plumosa* (Manza) P.C.Silva in their phylogenetic analysis, but the precise application of this name was not adequately documented.

In this study, we compared DNA from type specimens of *Bossiella plumosa* and its synonym, *Bossea frondifera* Manza, with contemporary topotype specimens and additional collections from Alaska, United States to Baja California, Mexico to demonstrate that six other species (with pinnate branching), including four new to science, have been passing under this name. Analysis of the type specimen of *Corallina frondescens* Postels & Ruprecht showed that this species also belongs in *Bossiella*.

Previously, Hind et al. (2014b) reassessed those *Bossiella* species with dichotomous branching passing under the names *B. californica* (Decaisne) P.C.Silva and *B. orbigniana* (Decaisne) P.C.Silva. Future studies will assess those species with irregular branching, falling under the name *B. chiloensis* (Decaisne) H.W.Johansen.

MATERIALS AND METHODS

***Sample collection*—**This work represents a collaborative effort among many laboratories whose goal was to conduct floristic surveys of algae along the Northeast (NE) Pacific coast from Alaska, United States through Baja California, Mexico using an integrative taxonomic approach. Together, we analyzed morphological and sequence data for 331 *Bossiella* specimens, including both contemporary and historic collections. Specific collection localities, GPS coordinates, and collector information are listed in Appendix S1 (see Supplemental Data with the online version of this article). Efforts were made to obtain topotype material from type localities (Appendix S1). Multiple specimens of each currently recognized *Bossiella* species in the NE Pacific (Gabrielson et al., 2012; Hind et al., 2014b), i.e., *B. californica, B. chiloensis, B. dichotoma* (Manza) P.C.Silva, *B. heteroforma* K.R.Hind, P.W.Gabrielson & G.W.Saunders, *B. orbigniana*, *B. plumosa* (Manza) P.C.Silva, and *B. schmittii* (Manza) H.W.Johansen were made at each collection locality. Specimens were collected from the high, mid, and low intertidal zones and subtidally to depths of 20 m (Appendix S1). A portion (3 × 3 cm) of each collection was silica dried for DNA analyses, and the remaining material was dried as a voucher and accessioned at UBC, UNB, NCU or UC (Appendix S1; see Thiers (2015) for herbarium acronyms).

Type collections were observed and photographed, and tissue samples (intergenicula) were excised for genetic analyses (Appendix S1). Specimens from the following herbaria were examined: TRH, S, UBC, and UC.

***DNA extraction and PCR*—**All contemporary samples were ground and total DNA was extracted following the method of Saunders (2008). Historical specimens were extracted following the protocol of Hughey et al. (2001) as modified by Gabrielson et al. (2011) and for some (Appendix S1), following the recommendations of Saunders and McDevit (2012) and Hughey and Gabrielson (2012).

PCR was used to amplify the mitochondrial cytochrome *c* oxidase subunit 1 (664 bp, COI-5P) marker, the plastid-encoded large subunit of RuBisCO (*rbcL*, 1387 bp) and the photosystem II reaction center protein D1 gene (*psbA*, 863 bp). All regions were amplified and sequenced as described in Hind and Saunders (2013b). In addition, 135 (*rbcL*-135) or 296 (*rbcL*-296) bp of the *rbcL* were amplified from type collections for comparison with contemporary specimens as in Hind et al. (2014a). PCR products were sequenced using the PE Applied Biosystems Big Dye kit (version 3.0) following the manufacturer's protocol (Applied Biosystems, Foster City, California, USA). All forward and reverse fragments were edited and aligned using the program Sequencher 4.8 (Gene Codes Corp., Ann Arbor, Michigan, USA), and a multiple sequence alignment was constructed by eye using the program MacClade 4 (version 4.06) for OSX (Maddison and Maddison, 2003). Corrected evolutionary distances and neighbor-joining trees were calculated in the

program Geneious v6.1 (Biomatters, Newark, New Jersey, USA) using the default Tamura–Nei model of sequence evolution.

***Phylogenetic analyses*—**A concatenated data set of three gene regions, COI-5P, *psbA*, and *rbcL* (2848 bp), for a single representative of each genetic species group resolved through barcode analyses was used. In addition, each gene region was analyzed independently to assess congruence. Outgroup taxa were chosen from the order Hapalidiales (Broom et al., 2008). Maximum likelihood analysis was conducted using the program RAxMLGUI v1.3 (Silvestro and Michalak, 2012) with a general-time-reversible (GTR+I+G) substitution model, determined by the program jModelTest2 version 2.1.1 (Darriba et al., 2012). The concatenated data set was partitioned by both gene and codon. Bootstrap resampling (1000 replicates) was conducted to estimate branch support. Bayesian analysis was performed using the program MrBayes version 3.1 (Huelsenbeck and Ronquist, 2001) under the GTR+I+G model. For each of the three gene regions, the analysis was partitioned by codon, and sampling was performed every 1000 generations. Analyses were run for 6 million generations and the burn-in was determined after convergence of the tree samples was obtained (5000 in our case).

***Morphological assessment*—**We measured characters following Baba et al. (1988) and Johansen (1971) that were found to be informative for species segregation using morphology alone. Specimens were decalcified in 1 N HCl for no longer than 12 h (usually 2 h) and sectioned on a Leica CM1850 cryostat microtome (Leica Microsystems GmbH, Wetzlar, Germany). Measurements were made using the imaging software ImageJ (Rasband, 1997–2014), on an Olympus SZ61 dissecting microscope (Olympus, Tokyo, Japan) and an Olympus BX51WI compound microscope. Measurements were taken from the main axis on the 3rd to 10th intergenicula from the plant apex. Morphological data were plotted in the program R v3.0.1 (R Core Team, 2014) as either boxplots or pie charts, for continuous and categorical data, respectively. For the boxplots, default parameters were used for whisker lengths and outlier detection.

RESULTS

Of the predominantly pinnately branched *Bossiella* species, nine genetic clusters were resolved using the COI-5P barcode (Fig. 1). The same genetic clusters were resolved for both the *psbA* and *rbcL* genes (data not shown). Using *rbcL*-296, three clusters matched the type specimen sequences of *Corallina frondescens*, *Bossea frondifera*, and *Bossiella plumosa*, respectively. Four clusters were distinct and warranted designation as new species: *B. manzae* sp. nov., *B. reptans* sp. nov., *B. hakaiensis* sp. nov., and *B. montereyensis* sp. nov. Two clusters, *B. plumosa* Haida Gwaii and *B. plumosa* Vancouver Island, had sufficient genetic divergence values for all three genes indicative of population structuring (Hind and Saunders, 2013a) and were considered genetically distinct populations of *Bossiella plumosa* (Fig. 1). A distance matrix with percent divergence values for CO1-5P is included in Appendix S2 (see online Supplemental Data).

A phylogram was generated from combined COI-5P, *psbA*, and *rbcL* data for *Bossiella* species along with confirmed coralline taxa that were previously published (Fig. 2). Both Bayesian and ML analyses resulted in identical tree topologies (Fig. 2). The genus *Bossiella* was monophyletic, and the generitype (confirmed by sequencing holotype specimen DNA) resolved in the lineage containing all other *Bossiella* species with strong support (Fig. 2). The genus *Johansenia* resolved as sister to the *Bossiella* lineage with weak support (Fig. 2).

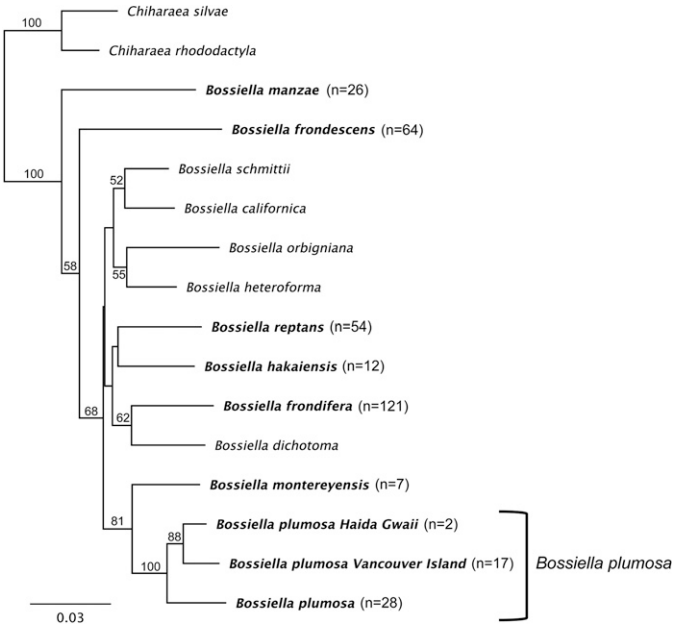


FIGURE 1 Phylogram (neighbor-joining) generated from CO1-5P data for specimens included in this study. Bootstrap support values are provided for nodes with support values >50. Species in boldface were reviewed in this study with current names applied following taxonomic assessment. Scale bar refers to substitutions per site.

From our extensive floristic surveys in the NE Pacific, the distributions of seven pinnately branched *Bossiella* species were assessed (Fig. 3). *Bossiella frondescens* (Postels & Ruprecht) E.Y.Dawson had a nearly continuous distribution from the Commander Islands, Russia through the Aleutian Islands and southeast Alaska to Duxbury Reef, Marin County, California (CA), United States. Six of the seven species occurred in British Columbia (BC), Canada with *B. frondescens*, *B. frondifera* (Manza) comb. nov., *B. reptans*, and *B. manzae* occurring as far north as Alaska (Fig. 3). Four species were present in CA and had overlapping ranges: *B. plumosa*, *B. frondescens*, *B. frondifera*, and the CA endemic *B. montereyensis* (Fig. 3), but none were reported south of Point Conception, CA.

Before pooling morphological data from the entire range of a species, we tested whether there were significant differences between morphological parameters of southern and northern populations. We did not find significant differences between any morphological characters measured for southern and northern populations (data not shown) and therefore have analyzed these data as one large data set.

We found significant differences between at least one species pair in 14 of the continuous characters measured (Fig. 4; online Appendix S3). For example, intergenicular length in the main axis differed significantly between *B. hakaiensis*, *B. reptans*, and all other *Bossiella* species in this study (Fig. 4; Appendix S3). However, intergenicular length was not an informative character to differentiate between *B. frondescens*, *B. frondifera*, *B. manzae*, *B. montereyensis*,

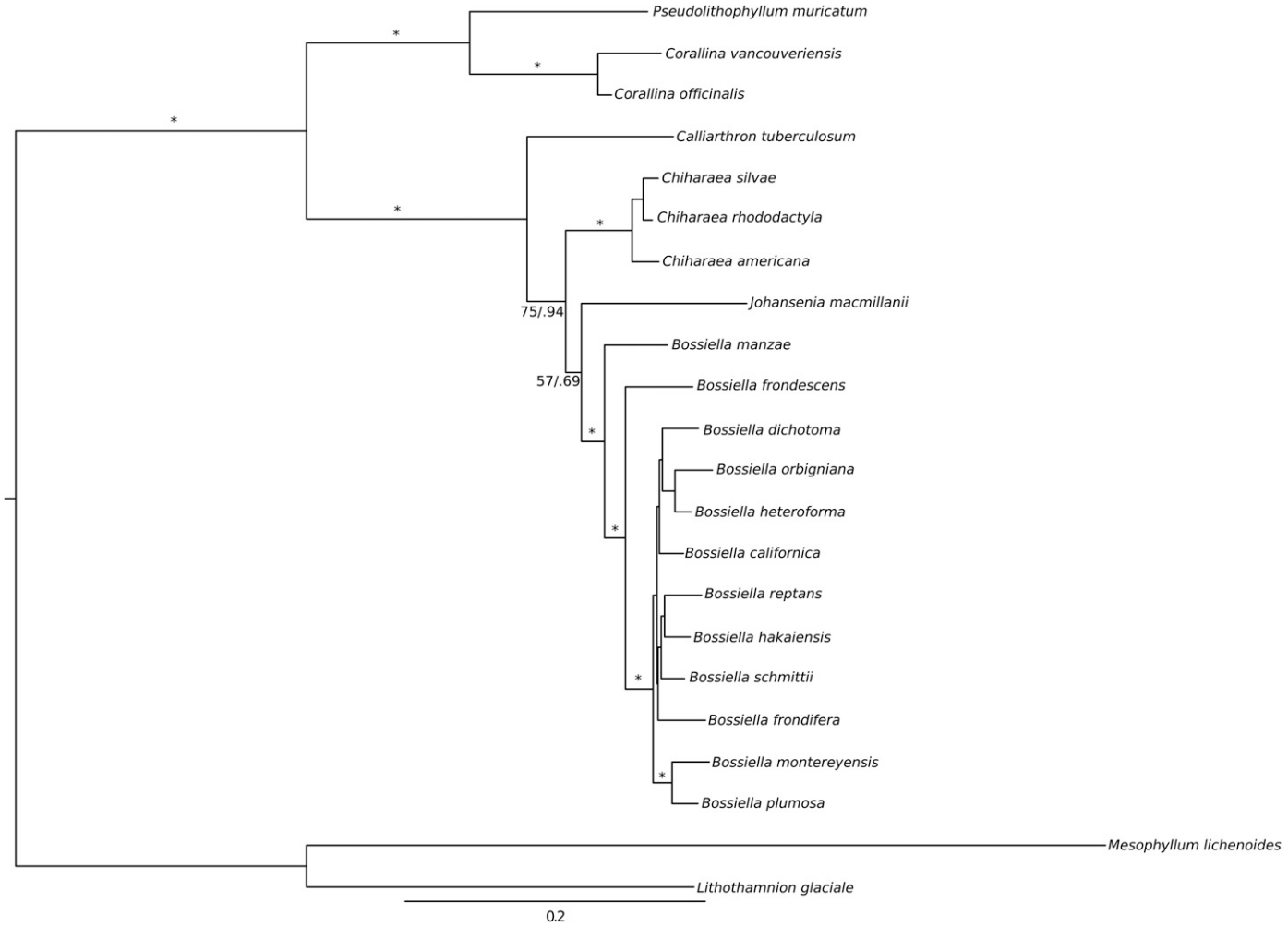


FIGURE 2 Phylogram inferred by maximum likelihood (ML) analysis of concatenated CO1-5P, *psbA*, and *rbcL* sequence data (2848 bp). Support values are listed as bootstrap for ML analyses and Bayesian posterior probabilities respectively. Asterisks denote nodes that are strongly supported (bootstrap values ≥ 98, posterior probabilities = 1.0) in all analyses. Support values are not indicated for all nodes (i.e., bootstrap values ≤ 50, posterior probabilities ≤ 0.65, or between closely related species). Scale bar refers to substitutions per site.

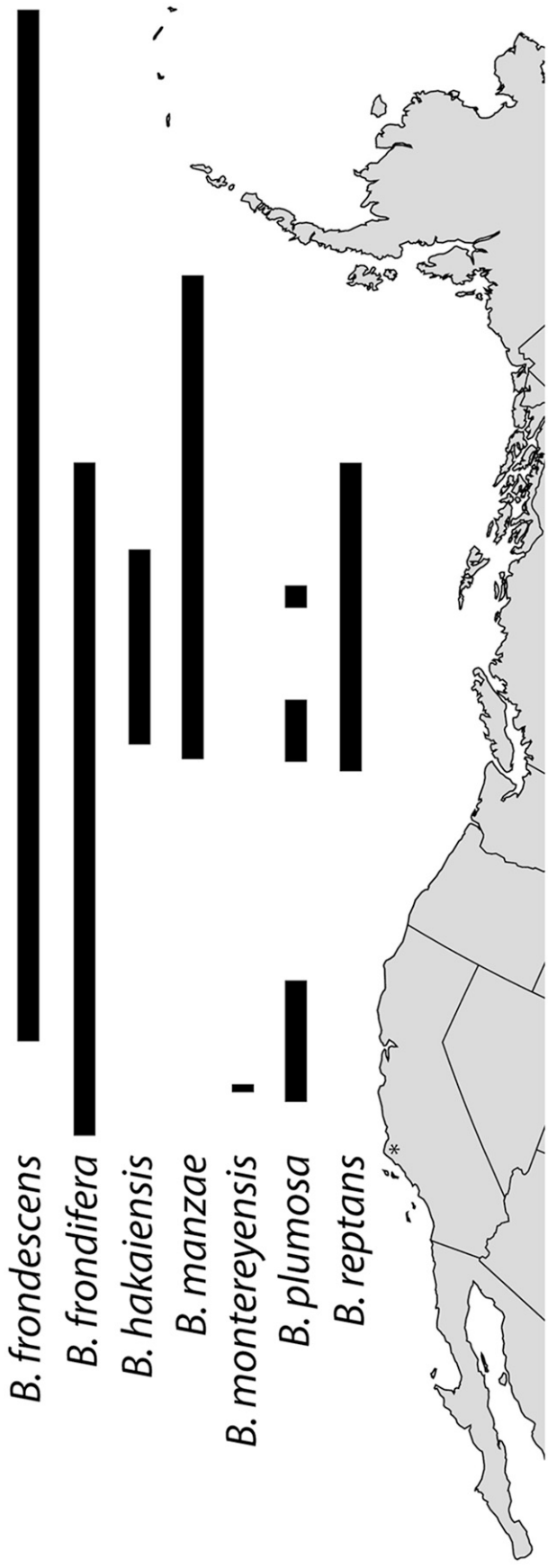
or *B. plumosa*. Other characters that showed this same trend were the maximum width of intergenicula, number of medullary tiers in the intergenicula, and the length of intergenicula in branches (Fig. 4; Appendix S3). Although there were significant differences between means for 14 continuous characters it is important to note that there was overlap in the ranges for almost all characters measured (online Appendix S4), and there was no single character that could be used to differentiate all species.

For the categorical characters (Fig. 5), we found very few that were able to differentiate species. Only the presence of axial conceptacles in *B. frondescens* and acentric conceptacles (when present) in *B. manzae* and *B. frondifera* were diagnostic characters for these species (see Taxonomic Results and Discussion). These data are interesting nonetheless in that they show that some features that were once considered to be diagnostic of a species are noninformative. For example, the presence of lateral branches consisting of only one intergeniculum (i.e., pinnae), which was thought to be a diagnostic character in *B. plumosa* (Johansen, 1971), was found in every species examined, except *B. reptans* (Fig. 5).

DISCUSSION

Four *Bossiella* species have been accepted as valid since the 1970s based on a morphological species concept (Johansen, 1971). Using a MAAT approach, Hind et al. (2014b) found at least 17 species in this genus, more than four times the previously recognized number of species. Estimates of the number of species on earth vary from three to over 100 million (Mora et al., 2011; Appeltans et al., 2012); however, only 1.58 million species are currently catalogued in the 2014 Species 2000 & ITIS Catalogue of Life database (Roskov et al., 2014), suggesting that between 52 and 98% of species on earth remain undescribed and in need of proper classification. For the genus *Bossiella*, we have found evidence that supports this claim, thus adding to the growing number of rhodophyte lineages that are significantly more speciose than previously thought (Guiry, 2012). This information provides insight into the extent of macroalgal species diversity in our oceans, an area of research that is largely understudied.

Our study used a combination of DNA sequence data and extensive morphometric analyses to establish that there are at least seven pinnately branched *Bossiella* species in the NE Pacific where



previously only one (*B. plumosa*) was recognized (Gabrielson et al., 2012). Using a combination of type specimen sequencing and topotype collections, we were able to assign previously recorded species names to three species (*B. frondescens*, *B. frondifera* and *B. plumosa*). The remaining four species (*B. manzae*, *B. hakaiensis*, *B. reptans*, and *B. montereyensis*) were determined to be new to science.

We were able to collect each of the three previously described species at their type localities (Appendix S1) suggesting that, in this case, the ranges of these species still include their type localities (see Fig. 3). Poleward shifts in species distributions are difficult to estimate since early records of species distributions relied on morphological species concepts that are known to be problematic. Moving forward, now that we have documented species ranges and confirmed species identifications using molecular sequence data, we will be able monitor poleward shifts or local extinctions as a result of climate change or other anthropogenic effects.

In our study, two very similar species, *B. frondifera* and *B. plumosa*, shared the same type locality: Moss Beach, San Mateo County, CA. Collection of topotype material (without DNA sequencing) in this case often resulted in the application of an incorrect species name. Since these two morphologically similar species shared the same type locality, one could not be confident that one had collected either species without DNA sequence-based identification. Reliance on collections from type localities should be made with caution since morphologically similar (in this case, often indistinguishable) species may share type localities.

Our morphometric analyses were able to detect distinguishing characteristics that were not apparent without first understanding the species boundaries delineated by DNA sequence data. For example, without a priori knowledge of genetic species boundaries between *B. hakaiensis* and *B. reptans*, these two species may have been lumped into one group based on a morphological species concept. The subtle differences in conceptacle chamber diameter, length and width of intergenicula in the main axis and branches, and the number of tiers of intergenicular medullary cells found in *B. hakaiensis* and *B. reptans* (Fig. 4; Appendices S3, S4) would not have been observed without a combination of DNA and morphometric analyses. The differences observed in morpho-anatomy likely would have been considered part of the natural range of intraspecific morphological variation. Furthermore, the MAAT approach provided insight into the prevalence of phenotypically variable traits within a species. For example, morphological variation and/or constraint was detected by assessing phenotypic variation for the same species in different habitats. Such morphological variation was particularly evident for the widespread species *B. frondifera* (Fig. 3). On a larger scale, we were able to test the hypothesis that there were morphological differences between northern and southern populations of one species, a trend that has been reported in the literature (Johansen, 1971). For example, Johansen (1971) suggested that warm-water populations (across multiple species)

FIGURE 3 Map showing distributions confirmed by DNA sequencing of pinnately branched *Bossiella* species. *Bossiella plumosa* populations were represented as having a disjunct distribution, since the three populations were genetically distinct and collections were absent from Oregon, Washington and the central coast of BC. Asterisk indicates Point Conception, California.

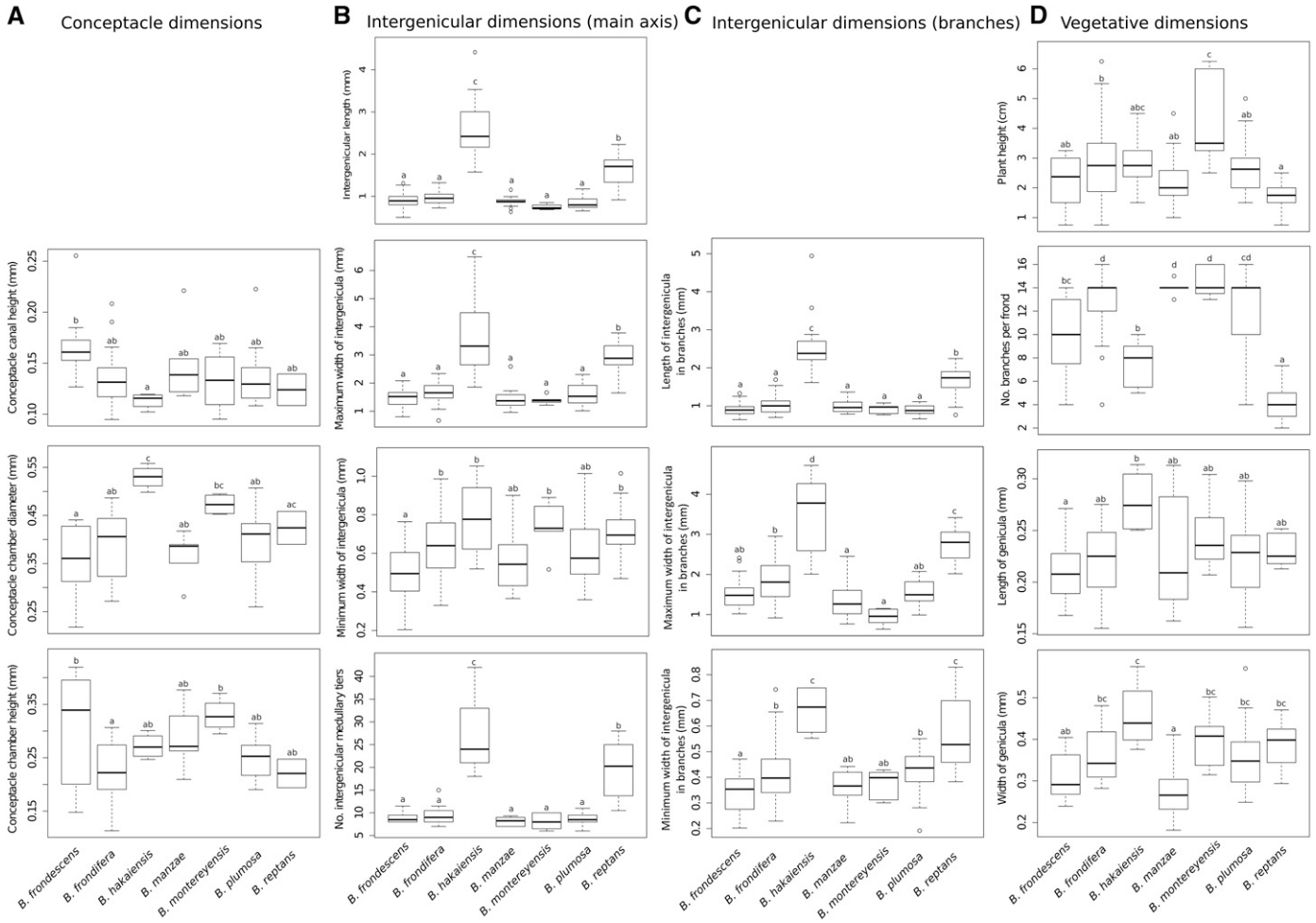


FIGURE 4 Observed morphological characteristics for pinnately branched *Bossiella* species (*B. frondescens*, *B. frondifera*, *B. hakaiensis*, *B. manzae*, *B. montereyensis*, *B. plumosa*, and *B. reptans*) with significant differences for at least one species pair. (A) Conceptacle dimensions. (B) Intergenicular dimensions (main axis). (C) Intergenicular dimensions (branches). (D) Vegetative dimensions. Box plots show median values (solid horizontal line), 50th percentile values (box), upper and lower quartiles (whiskers), and outlier values (open circles). Letters represent results of Tukey's HSD posthoc comparisons. ANOVA results provided in Appendix S3.

had longer fronds, smaller intergenicula, and fewer conceptacles, a trend that we did not observe using our integrative approach.

Our study demonstrates that the process of delineating species, including considerations of habitat, distribution, morphological variation, and possible species names, requires sequencing both contemporary and type specimens. The MAAT approach facilitates rapid assessment of species numbers, but without sequencing type specimens, applying names is problematic. We hope that the incorporation of DNA sequences from type specimens into an integrative taxonomic framework will facilitate timely and accurate naming of species.

TAXONOMIC RESULTS AND DISCUSSION

Our morpho-anatomical measurements and observations, as well as our review of heterotypic synonyms, habitat and distributional data, are based on specimens whose identities were confirmed by DNA sequence(s). We treat first the generitype species, *B. plumosa*, then previously named species, and finally the proposed new species.

Bossiella plumosa* (Manza) P.C.Silva 1957: 47—Basionym—Bossea plumosa* Manza, 1937a: 46.

Holotype—UC 545710, Moss Beach, San Mateo Co., CA, 5.v.1931, no habitat data, leg. A. V. Manza.

Representative DNA barcodes—UC 545710 (holotype), *rbcL* 135 sequence, GenBank KT819580; UC 1966627 (topotype) *rbcL*-1387 GenBank HQ 322280.

Homotypic synonyms—Pachyarthron plumosum (Manza) C.W.Schneider & M.J.Wynne, 2007: 231.

DNA sequences—The sequence *rbcL*-135 from the holotype (Manza, 1940, pl. 12) and from three isotypes were identical to each other and to the same portion of the *rbcL*-1387 from four topotype specimens

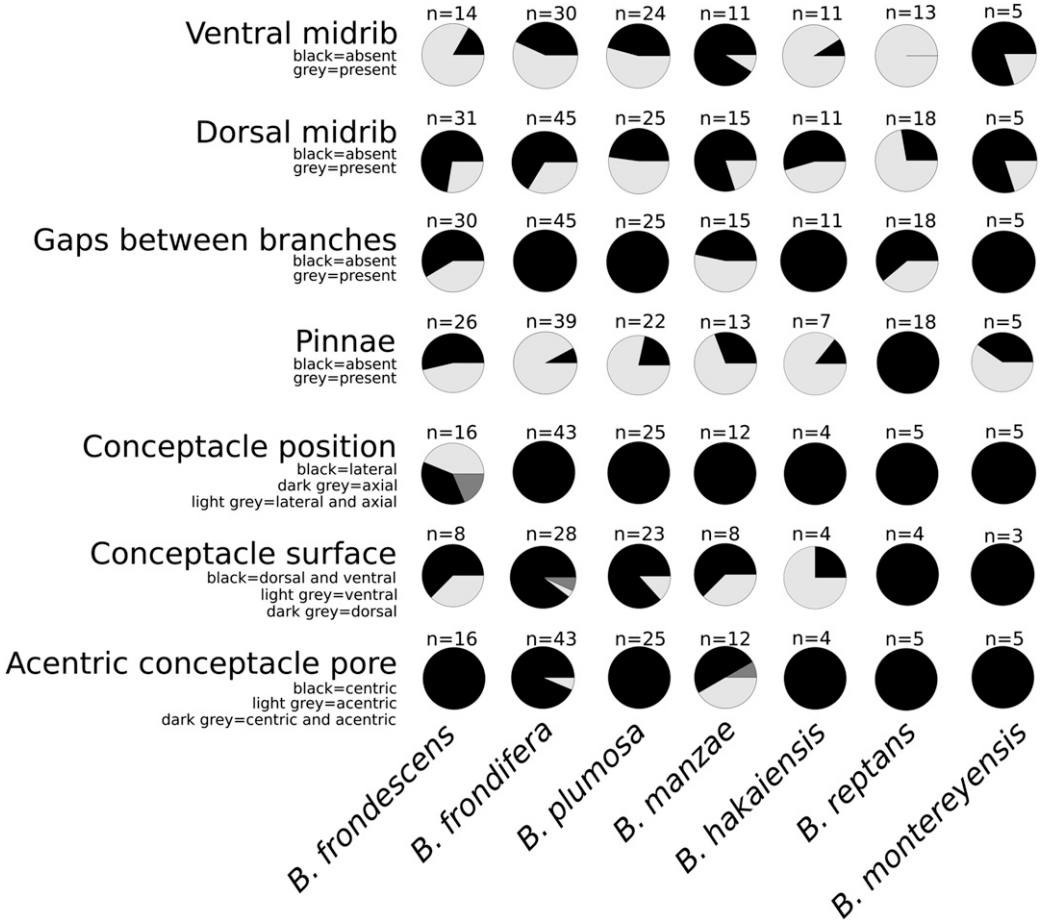


FIGURE 5 Observed categorical measurements for pinnately branched *Bossiella* species. *n* = number of individuals. Black pie slices represent absence; light gray represents presence. For conceptacle position, black is lateral, dark gray is axial, and light gray is both lateral and axial. For conceptacle surface, black is dorsal and ventral, dark gray is dorsal, and light gray is ventral. For acentric conceptacle, pore black is centric, light gray is acentric, and dark gray is both centric and acentric.

(Appendix S1). Recently collected specimens from southern BC and one collection from Bodega Head, Sonoma County, CA differed from topotype material by six single nucleotide polymorphisms (SNPs) in the rbcL-1387; *psbA* sequences also had six SNPs. Another specimen from Bodega Head and two collections from Mussel Point Monterey County, CA differed by only 1 SNP from the topotype material.

Morphology—Figure 6. Thalli solitary or in dense mats, erect to 5.0 cm tall from an inconspicuous crustose base; branching irregularly pinnate; indeterminate branches often densely layered or sometimes flattened with a prominent main axis; determinate branches consisting of single intergeniculum (pinna) common in some populations; basal intergenicula subterete to flattened, branched or unbranched; main axial intergenicula rectangular to hexagonal; intergenicula on secondary branches hexagonal to sagittate; intergenicula 0.6–1.2 mm long, 0.8–2.5 mm broad; 6–11 tiers of medullary cells per intergeniculum; genicula length ranges from 150.4–316.1 µm long and 195.7–570.4 µm wide; conceptacles lateral, typically 1–2/intergeniculum, often paired or solitary, pairs of conceptacles along main axis often separated by large gaps. Tetrasporangial conceptacle chamber diameter, chamber height, and canal height ranged from 248.9–517 µm, 174.6–370.3 µm, and 100.9–222.6 µm, respectively. Female and male conceptacles were not observed in this study.

Habitat and distribution—*Bossiella plumosa* was found disjunctly from Haida Gwaii and Vancouver Island, Canada to Pacific Grove, CA, USA (Fig. 3). It was not collected along the central coast of BC, despite significant sampling effort. It was collected from the low to high intertidal zones, in and out of pools.

Comments—DNA sequences generated from our collections of *Bossiella plumosa* displayed marked population structure with distinct populations in Haida Gwaii, Vancouver Island, and California (Fig. 3). Although there was substantial genetic differentiation among these populations (Appendix S2), we consider this to be a single species with a disjunct biogeographic range. The COI-5P, *rbcL*, and *psbA* barcodes revealed distinct northern and southern haplotypes. Interestingly, collections from Bodega Head, CA showed a mixed population of haplotypes.

***Bossiella frondifera* (Manza) P.W.Gabrielson, K.A.Miller, Martone, & K.R. Hind comb. nov.**—*Basionym*—*Bossea frondifera* Manza, 1937b: 562.

Holotype—UC 545757, Moss Beach, San Mateo Co., CA, 5.v.1931, no habitat data, leg. A. V. Manza.

DNA sequences—The sequence *rbcL*-135 from the holotype of *Bossea frondifera* (Manza, 1940, pl. 14) differed by 2.22% from the *Bossiella plumosa* holotype. The longer *rbcL*-1387 (1387 vs. 135 bp) had a sequence divergence of 1.9% between topotype specimens of *B. frondifera* and *B. plumosa*; *psbA* sequences of topotype specimens had 1.64% DNA sequence divergence indicating that these are distinct species.

All *rbcL* and *psbA* sequences from *B. frondifera* examined during this study (Appendix S1) were invariant. The COI-5P barcode was more variable within this species (0–0.8% intraspecific sequence divergence, Appendix S2).

Representative DNA barcodes—UC 545757 (holotype) *rbcL*-135, GenBank KT819581; UC 1966619, UC 1966624, UC 1966628, (topotype) *rbcL*-1387 GenBank KT782040, KT782124, KT782149, respectively.

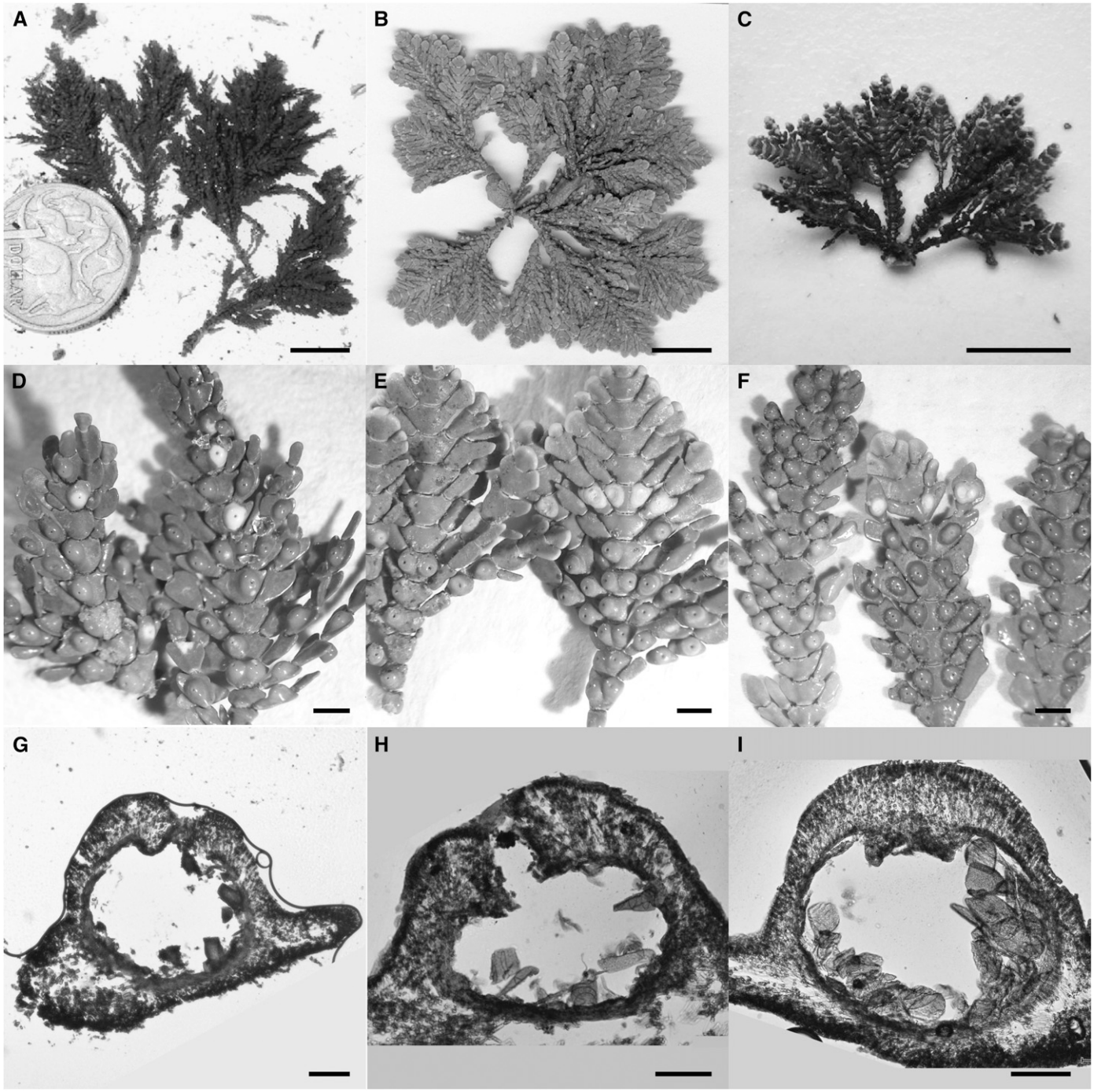


FIGURE 6 Morphology of *Bossiella plumosa*. (A) Specimen (GWS031422) from Haida Gwaii deposited at UNB. Scale bar = 1 cm. (B) Specimen (PTM178) from Vancouver Island deposited at UBC. Scale bar = 1 cm. (C) Specimen (GWS021435) from California deposited at UNB. Scale bar = 1 cm. (D–F) Close-up views of specimens (GWS031422, GWS010824 (Vancouver Island), PTM036 (California), respectively) displaying conceptacle placement, branching pattern and lack of pinnae. Scale bars = 1 mm. (G–I) Specimens (GWS031422, GWS010824, PTM036, respectively) showing tetrasporangial conceptacle morphology. Scale bars = 100 μm.

Morphology—Figure 7. Thalli often solitary, erect to 6.25 cm tall from an inconspicuous crustose base; branching irregularly pinnate; indeterminate branches often densely layered (Fig. 7B) or sometimes flattened with a prominent main axis (Fig. 7A); determinate branches consisting of a single intergeniculum common; secondary branches rarely terminated with unbranched, spindly intergenicula; basal intergenicula frequently flattened, branched or unbranched, rarely with rhizoidal projections; main axial intergenicula hexagonal to triangular; intergenicula on secondary branches hexagonal to irregular, rarely sagittate; intergenicula 0.6–1.5 mm

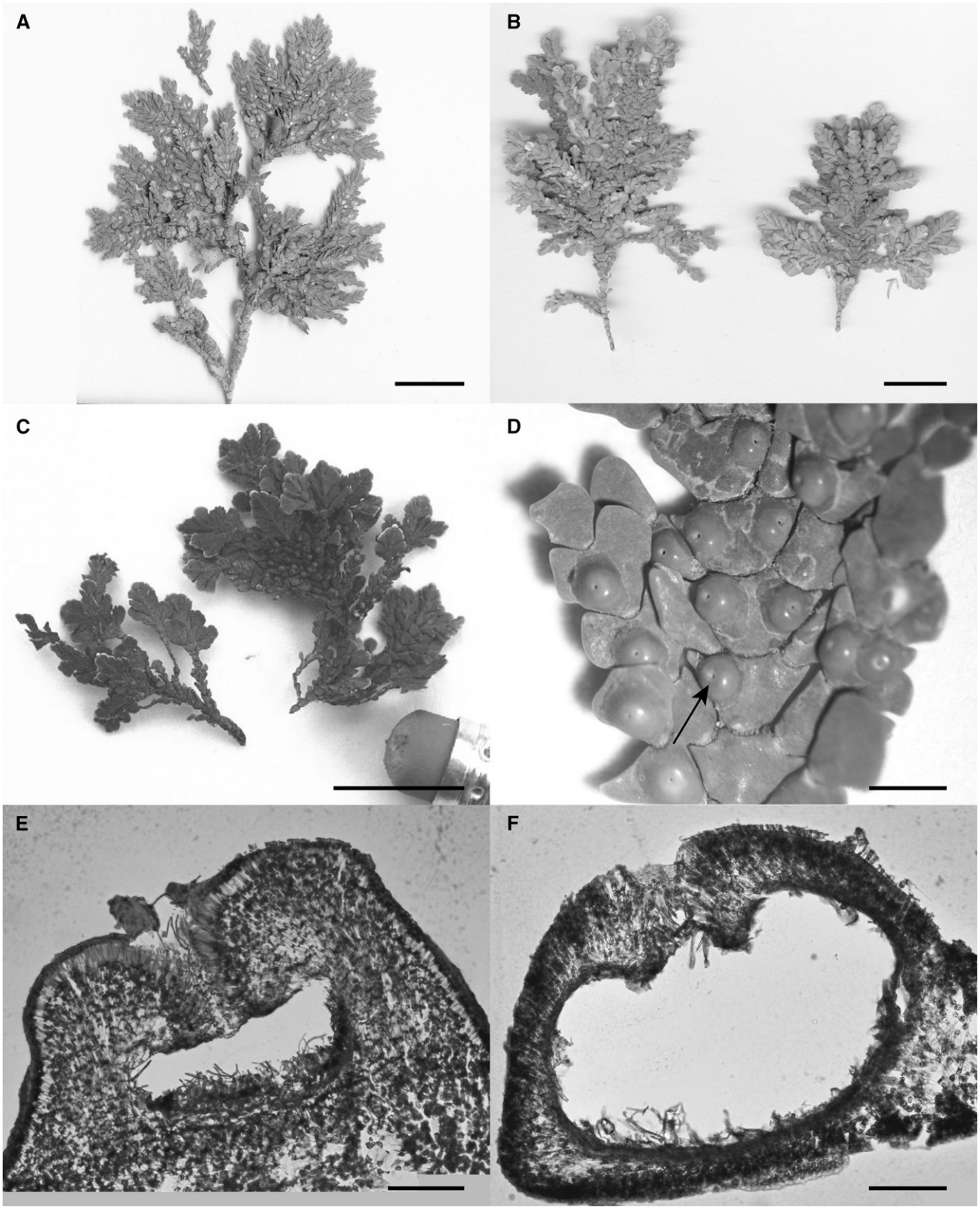


FIGURE 7 Morphology of *Bossiella frondifera*. (A) Specimen (PTM323) displaying a unique branching pattern in which flattened determinate fronds project from a prominent main axis. Scale bar = 1 cm. (B) Specimen (PTM094) showing densely layered determinate laterals. Scale bar = 1 cm. (C) Specimen (GWS010838) displays young, crowded, ovate branches as described by Manza (1937a). Scale bar = 1 cm. (D) Specimen (GWS021245) displays a mixture of centric and acentric conceptacular pores. Arrow indicates acentric conceptacle pore placement. Scale bar = 1 mm. (E) Male conceptacle (GWS012914) showing slight acentric pore placement. Scale bar = 100 µm. (F) Tetrasporangial conceptacle (GWS020255). Scale bar = 100 µm.

long 0.7–2.6 mm wide; 7–15 tiers of medullary cells per intergeniculum; genicula 141.1–314.6 μm long and 269.8–499.1 μm wide; conceptacles lateral, dorsal or ventral, typically 1 or 2/intergeniculum, solitary to densely clustered, often irregularly paired. Conceptacle pores rarely acentric (markedly off the central axis) and only documented in northern populations (Fig. 7D). Tetrasporangial conceptacle chamber diameter, chamber height, and canal height were 253.4–499.5 μm, 100.8–316.1 μm, and 81.9–216.4 μm respectively. One male conceptacle measured 292.2–352.3 μm, 169.1–181.1 μm, and 198.5–278.3 μm for chamber diameter, chamber height, and canal height, respectively. Female conceptacles were not observed.

Habitat and distribution—Bossiella frondifera was primarily found in the mid to low intertidal zone on rocks and rarely in tide pools. It was collected occasionally from the shallow subtidal zone, but not below 3 m depth. This species occurs commonly from Sitka, Alaska (AK), USA to San Luis Obispo County, CA (north of Point Conception).

Comments—Silva (1957) merged *Bossea frondifera* with *B. plumosa* when he proposed the substitute name *Bossiella* for *Bossea*. Manza's 27 isotype specimens of *B. plumosa* appear very similar to the holotype (Manza, 1940), with relatively few indeterminate axes per individual and determinate laterals either consisting of single intergenicula in two ranks along either side of the indeterminate axes or of progressively shorter laterals, resulting in a corymbose thallus. In *B. frondifera*, there are numerous, short, overlapping indeterminate axes produced along main indeterminate axes (Fig. 7A), but these do not develop into a corymbose thallus.

As Dawson and Silva in Dawson (1953, p. 161) stated, "The types of *B. plumosa* and *B. frondifera* seem to exemplify two peaks of variability, while other examples intermediate between them, but from the same collection, were not named by Manza." Indeed, our measurements of thallus height and various intergenicular characters overlapped for the two species; there were no significant differences between any of the continuous characters measured for these two species (Fig. 4, Appendix S3), and we could not reliably assign our topotype specimens to one species or the other without DNA sequences.

***Bossiella frondescens* (Postels & Ruprecht) E.Y.Dawson 1964: 540.**—*Basionym*—*Corallina frondescens* Postels & Ruprecht 1840: 20, pl. 40, fig. 103.

Lectotype—LE not numbered, Unalaska Island, AK, USA, 1827, leg. G. Kastalsky (by H. W. Johansen, 17 Oct 1967, unpublished).

Isolectotypes—FH 258854, S A2603.

Representative DNA barcode—S A2603 (isolectotype); *rbcL*-296 sequence, GenBank KT782066.

Homotypic synonyms—Cheilosporum frondescens (Postels & Ruprecht) Yendo 1902: 715.

Arthrocardia frondescens (Posels & Ruprecht) Areschoug in J. Agardh, 1852: 549.

Amphiroa tuberculosa f. *frondescens* (Postels & Ruprecht) Setchell & N.L.Gardner 1903: 362.

Pachyarthron frondescens (Postels & Ruprecht) C.W.Schneider & M.J.Wynne, 2007: 231.

Heterotypic synonyms—Joculator delicatulus Doty, 1947: 167–168.

Corallina delicatula ('*delicatulus*') (Doty) E.Y.Dawson, 1961: 418 nom. illeg.

DNA sequences—The *rbcL*-296 sequences from isolectotype specimens in S (Fig. 8B) were identical to two topotype specimens, one a 1932 collection (S A2698) and the other a contemporary collection (NCU 593283), and to all other field-collected specimens.

Morphology—Figure 8. Thalli frequently in dense mats on rock surfaces; individual fronds erect to 3.25 cm long from an inconspicuous crustose base; branching irregularly pinnate, sometimes sparse; branch length decreased toward apex (Fig. 8A); secondary branches infrequently terminated by up to 8 unbranched, spindly intergenicula; basal intergenicula most often flattened and branched, can form rhizoidal projections; main axial intergenicula trapezoidal to deeply sagittate, generally flattened, sometimes highly irregular and spathulate; intergenicular margins often irregular to frilled; intergenicula 0.5–1.3 mm long, 0.8–2.3 mm wide and may have dorsal and ventral midribs; 8–12 tiers of medullary cells per intergeniculum; genicula 150.8–295.7 μm long and 175.8–427.7 μm wide; conceptacles 1 or 2/intergeniculum, rarely paired, may be axial and/or lateral (Fig. 8C). Tetrasporangial conceptacle chamber diameter, chamber height and canal height ranged from 217.8–512.9 μm, 147.9–429 μm and 108.5–255.3 μm, respectively. One male conceptacle measured 262.6 μm for chamber diameter, 198.1 μm for chamber height and 299.8 μm for canal height. Female conceptacles were not observed in this study.

Habitat and distribution—Bossiella frondescens was found primarily in the mid to high intertidal zone; it was infrequent in the low intertidal zone and rare in tidal pools. It was confirmed from Bering Island, Commander Islands, Russia westward through the Aleutian Islands to the Gulf of Alaska and south to Marin County, CA, a range of over 6000 km. Reports of this species from the warm temperate eastern Mediterranean Sea (Taşkın et al., 2008) and from tropical seas (Guiry and Guiry, 2015), particularly those based on the purported heterotypic synonym *Corallina pinnatifolia* var. *digitata* E.Y.Dawson (see below) based only on morphological similarity, need to be confirmed with DNA sequence data.

Comments—Bossiella frondescens (as *Corallina frondescens*) was one of five species of geniculate corallines described and illustrated from the North Pacific by Postels and Ruprecht (1840). This species was collected by G. Kastalsky (Fig. 8B) on the von Lütke expedition (1826–1829) at Unalaska Island, AK, growing with *C. arbuscula* Postels & Ruprecht.

Yendo (1902) transferred *Corallina frondescens* to *Cheilosporum* and recognized four forms, *Ch. frondescens* f. *typica*, f. *maxima*, f. *intermedia* and f. *polymorpha*, the last three typified by specimens collected in 1901 at the Minnesota Seaside Station (now Botanical Beach Provincial Park) on southern Vancouver Island, BC, Canada. To our knowledge, the geniculate coralline specimens that Yendo collected at that locality are missing. Type material of the three forms were photographed by Yendo (1902), but cannot be identified confidently and therefore have not been included as heterotypic synonyms. Setchell and Gardner (1903) concluded that NE Pacific geniculate corallines with flattened intergenicula were forms of a single species that they placed in *Amphiroa*. They made the combination *A. tuberculosa* f. *frondescens*, but did not recognize the forms of Yendo.

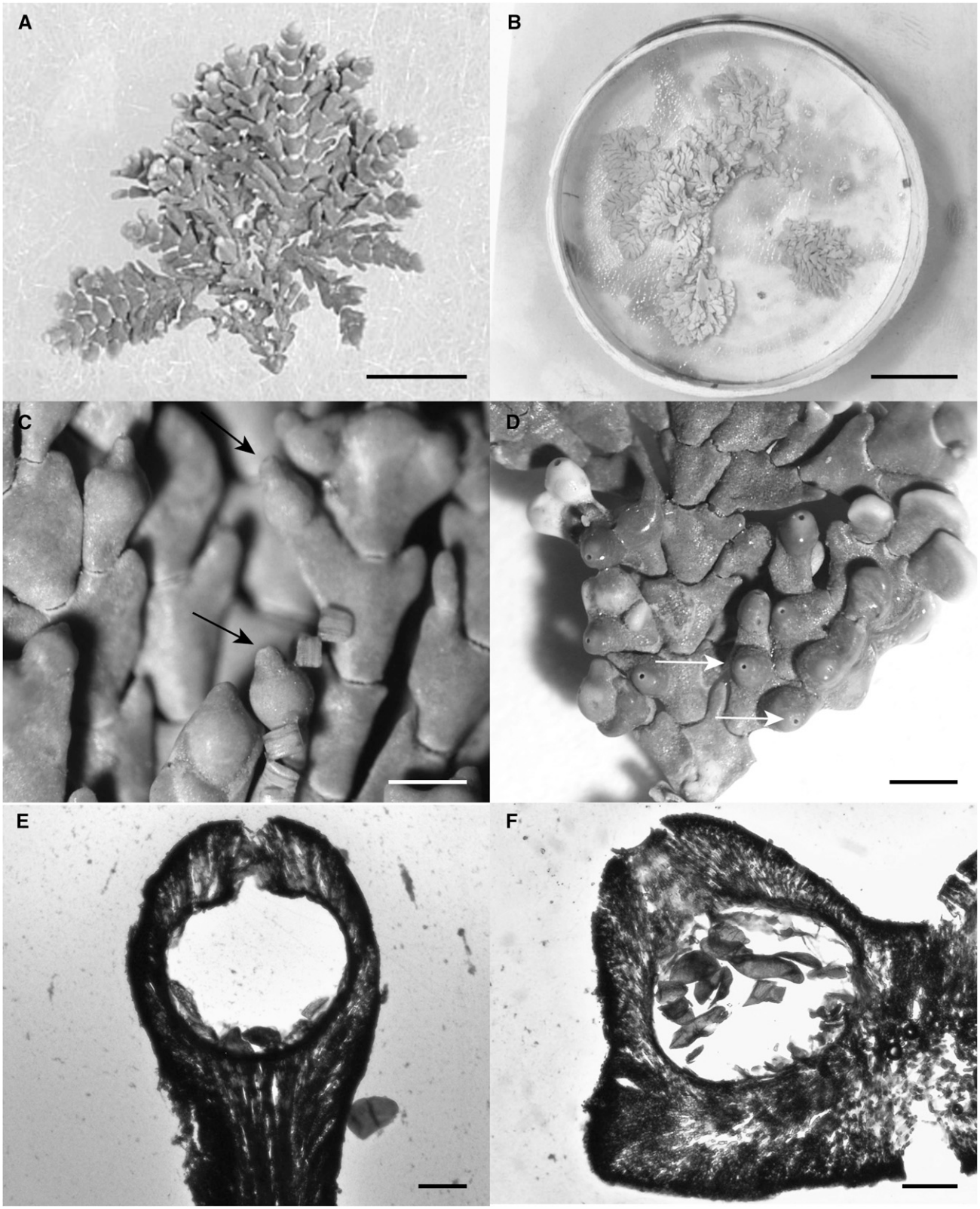


FIGURE 8 Morphology of *Bossiella frondescens*. (A) Specimen (NCU 585605) showing irregular pinnate branching and branch length decreasing toward the apex. Scale bar = 0.5 cm. (B) Image of isolectotype specimen of *Corallina frondescens* (FH258854) in the Swedish Museum of Natural History (S). Scale bar = 1 cm. (C) Specimen (PTM150) displaying axial conceptacle position (arrows). Scale bar = 1 mm. (D) Specimen (PTM376) displaying lateral conceptacle position (arrows). Scale bar = 1 mm. (E) Longitudinal section of axial conceptacle (PTM147). Scale bar = 100 μm. (F) Longitudinal section of lateral conceptacle (PTM376). Scale bar =100 μm.

Dawson (1964) transferred *C. frondescens* to *Bossiella*. Johansen (1971) stated that the type specimen of *Corallina frondescens* belonged in *Corallina*, not *Bossiella*, probably in recognition of both terminal and lateral conceptacles. Subsequently, Johansen in Abbott and Hollenberg (1976: 403) listed *Joculator delicatulus* Doty and *Corallina pinnatifolia* var. *digitata* E.Y.Dawson as synonyms of *C. frondescens*. We have confirmed by *rbcL*-296 sequence that the holotype of *J. delicatulus* (type locality: Cape Arago, OR) is indeed *B. frondescens*. We have not sequenced the type of *C. pinnatifolia* var. *digitata*, but its type locality in the northern Gulf of California, Guaymas, Sonora, Mexico (Dawson, 1953) makes it unlikely that this taxon is *B. frondescens*.

Historically, one of the distinguishing characters of *Bossiella* has been the presence of exclusively lateral conceptacles. *Bossiella frondescens* was unique in having both axial and lateral conceptacles (Fig. 8C and 8D), a diagnostic feature for this species that, when present, was an excellent character for field identification.

Bossiella hakaiensis K.R.Hind & Martone sp. nov.—*Holotype*—UBC A89922 (Fig. 9A), Exposed Point, Fifth Beach (51.63825°N, −128.1571°W), Calvert Island, Hakai Luxvbalis Conservancy, BC, Canada, *K.R. Hind*, 27 May 2013, epilithic, subtidal 7 m depth.

Representative DNA barcode—UBC A89922 (holotype), *rbcL*-1401, GenBank KT782118.

Etymology—Named for the Hakai Luxvbalis Conservancy Region where the most robust examples of this species have been found to date.

Morphology—Figure 9. Thallus prostrate (Fig. 9B) to erect to 4.5 cm long from an inconspicuous crustose base; branching irregularly pinnate, basal intergenicula may be terete and unbranched, but most often flattened and branched; intergenicula generally flattened, spatulate and often large (up to 5 mm in length), thin and compressed (0.24–0.53 mm thick), often chipped resulting in an irregular margin (Fig. 9C); intergenicula bear ventral and dorsal midribs; terminal intergenicula fan-shaped and broad, some axial intergenicula prominently digitate (often producing up to 8 digits each bearing a central midrib); intergenicula 1.3–5.3 mm long 1.9–7.0 mm wide; 18–42 tiers of medullary cells per intergeniculum (Fig. 9E); genicula range from 223.1–362.4 μm long and 338.8–913.1 μm wide; conceptacles 1–6 per intergeniculum, scattered on the dorsal surface and markedly depressed (low profile) (Fig. 9C). Tetrasporangial conceptacle chamber diameter, chamber height, and canal height are 434–672.4 μm, 209–382.2 μm, 92–132 μm, respectively. Female and male conceptacles were not observed in this study.

Habitat and distribution—Bossiella hakaiensis was epilithic from the low intertidal zone to 8 m depth; infrequently collected from mid intertidal pools and most often collected from surge channels or exposed coastlines. This species occurred from Wiah Point and Cape Edensaw northwest of Masset, Haida Gwaii to Bamfield, Vancouver Island, BC, Canada.

Comments—Bossiella hakaiensis most closely resembled *B. reptans*. Both species were found in the low intertidal and shallow subtidal zones of exposed coastlines. All continuous characters measured in this study overlapped in both species (Fig. 4; Appendix S4). Tetrasporangial conceptacle chamber diameter had little overlap but was still not significantly different between species (Fig. 4; Appendix S3). Both species had low profile or recessed conceptacles (Fig. 9C). More measurements of reproductive specimens are required to verify the utility of these characters. In general, *B. hakaiensis* had longer intergenicula and a greater number of medullary tiers than *B. reptans* (Fig. 9E). There were never gaps between successive branches of *B. hakaiensis* (Fig. 9A), but there were gaps between branches in one third of the *B. reptans* samples. Pinnae (branches composed of a single intergeniculum) were absent from *B. reptans*, but were reliably present in *B. hakaiensis*.

Bossiella manzae P.W.Gabrielson, K.R.Hind & Martone, sp. nov.—*Holotype*—UBC A88693, (Fig. 10A). Botanical Beach (48.52925°N, −124.453704°W), Juan de Fuca Provincial Park, Vancouver Island, BC, Canada, 11 July 2010, epilithic, mid to low intertidal under *Saccharina sessilis* and *Egregia menziesii*, leg. P.T.Martone PTM167.

Representative DNA barcode—UBC A88693 (holotype), *rbc*L-1387, GenBank KT782129.

Etymology—Named for A. V. Manza, a pioneer in coralline algal taxonomy in the NE Pacific and author of the genus *Bossea*.

Morphology—Figure 10. Thalli often solitary, forming dense corymbose clusters of fronds from an inconspicuous crustose base (Fig. 10B), erect to 4.5 cm tall; branching irregularly pinnate, determinate branches of one intergeniculum frequent, indeterminate branches sometimes densely layered, secondary branches sometimes terminated with a series of up to 10 unbranched, spindly intergenicula (Fig. 10A) or frilled with many new segments appearing at the margin simultaneously (Fig. 10C); basal intergenicula terete to subterete, sometimes devoid of branches; main axis prominent, axial intergenicula trapezoidal to hexagonal, rarely sagittate, upper axial intergenicula measure 0.6–1.3 mm long, 0.8–3.3 mm broad, rarely with midribs; intergenicula on secondary branches triangular, sometimes sagittate; 6–14 tiers of medullary cells per intergeniculum; genicula 149.5–350.3 μm long and 179.1–411 μm wide; conceptacles 1–2/intergeniculum, paired or unpaired, solitary conceptacles common. Acentric conceptacular pore common in tetrasporophytes (Fig. 10D). Tetrasporangial conceptacle chamber diameter, chamber height and canal height are 213.7–470.5 μm, 178.1–400.9 μm, 85.1–239.5 μm, respectively. Female and male conceptacles were not observed in this study.

Habitat and distribution—Bossiella manzae was epilithic primarily in the mid-low intertidal zone, typically in highly wave-exposed environments, rarely in tide pools; one specimen (GWS020953) was found at 2.5 m depth. This species occurred from Sitka, AK, USA to Botanical Beach, Port Renfrew, BC, Canada.

Comments—Johansen (1971) observed the presence of acentric (markedly off the central axis) conceptacular pores in the genus *Bossiella*, specifically in some forms of *B. chiloensis*. Most likely these forms of *B. chiloensis* were collections of *B. manzae* and/or *B. frondifera* both of which may have acentric conceptacular pores. They were recorded rarely (4.7%) in *B. frondifera* (sample size $n = 43$) and more commonly (50%) in *B. manzae* ($n = 12$)

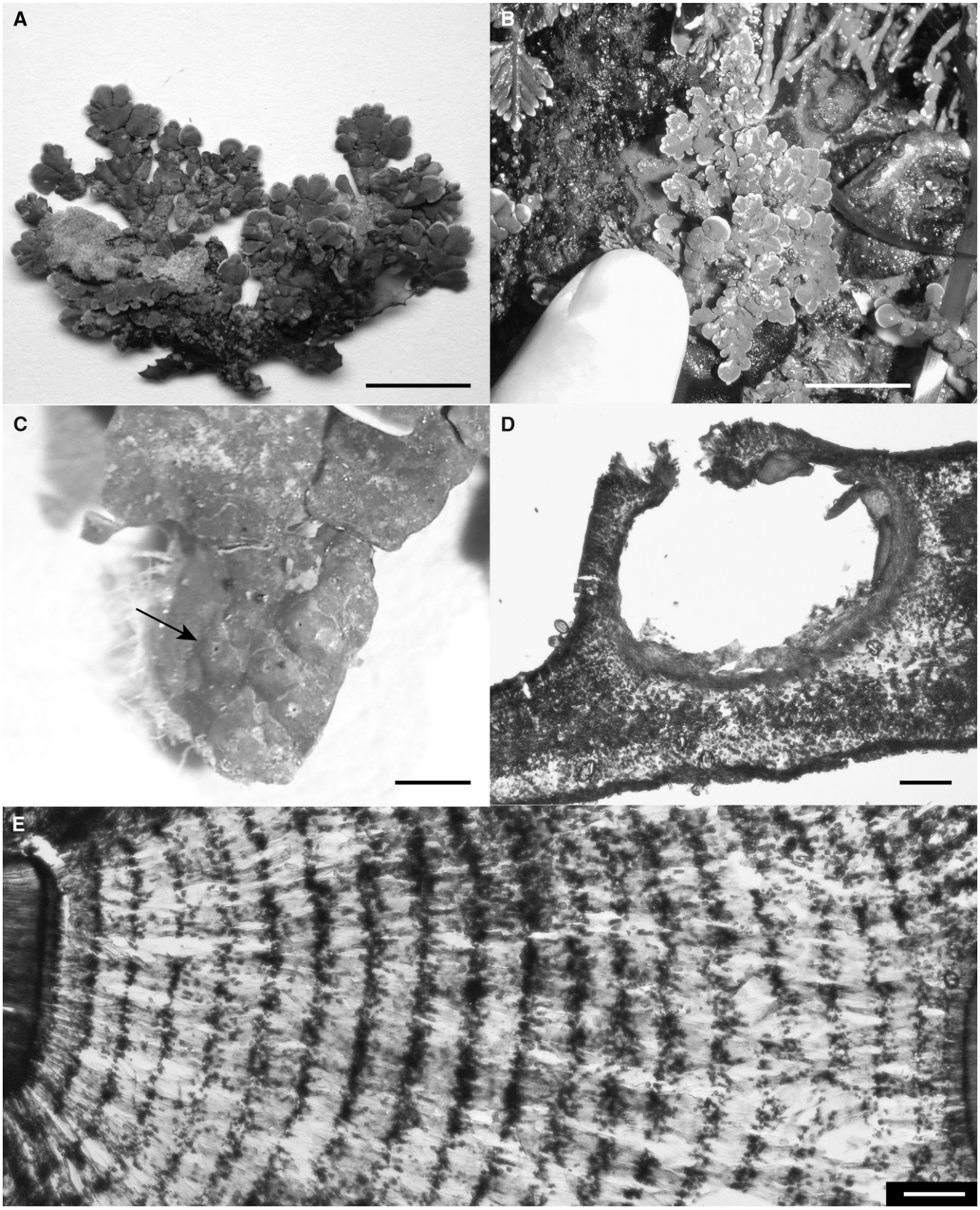


FIGURE 9 Morphology of *Bossiella hakaiensis*. (A) Holotype specimen (UBCA89922/PTM601) deposited at UBC. Scale bar = 1 cm. (B) Specimen (GWS030336) showing prostrate habit and comparatively large intergenicula. Scale bar = 1 cm. (C) Specimen (PTM488) showing distinguishable low profile (arrow) conceptacles. Scale bar = 1 mm. (D) Tetrasporangial conceptacle (PTM601). Scale bar = 100 µm. (E) *Bossiella hakaiensis* specimen (PTM568) displaying >20 tiers of intergenicular medullary cells. Scale bar = 100 µm.

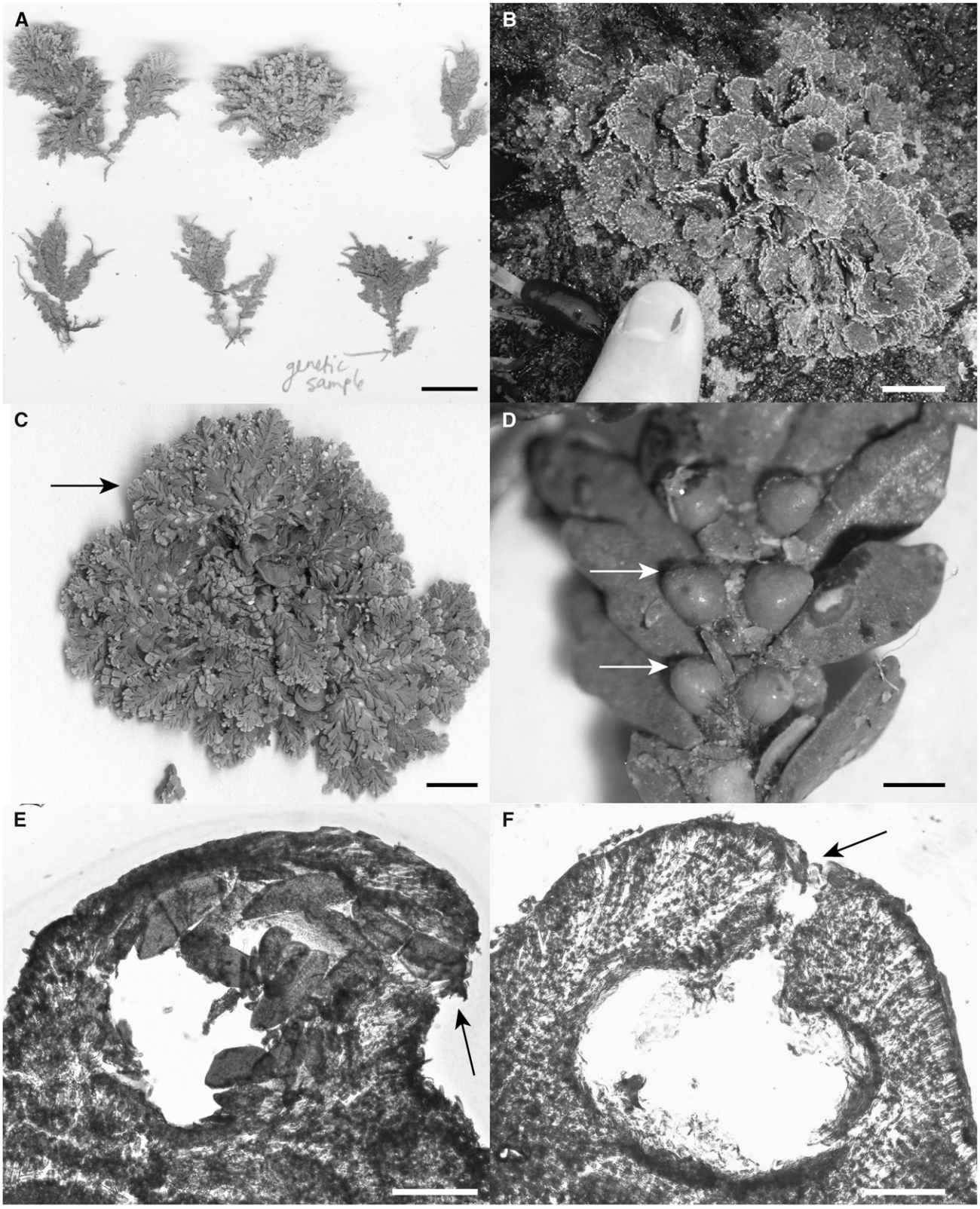


FIGURE 10 Morphology of *Bossiella manzae*. (A) Holotype specimen (UBC A88693/PTM167) deposited at UBC. Scale bar = 1 cm. (B) Specimen (GWS019296) showing densely layered, corymbose branches and solitary growth habit. Scale bar = 1 cm. (C) Specimen (PTM329) demonstrating frilled margins (arrow). Scale bar = 5 mm. (D) Specimen (PTM450) with acentric conceptacle pore placement (arrow). Scale bar = 0.5 mm. (E) Bisporangial conceptacle (PTM547) with an acentric conceptacular pore (arrow). Scale bar = 100 µm. (F) Biosporangial conceptacle (PTM329) with a centric conceptacular pore (arrow). Scale bar = 100 µm.

(Fig. 10D and 10E). This feature was not found in any other *Bossiella* taxon. *Bossiella manzae* and *B. frondifera* were morphologically similar, but sometimes were differentiated by the presence of gaps between successive branches in *B. manzae*. *Bossiella manzae* often lacked an apparent main axis, whereas *Bossiella frondifera* often had a prominent, flattened indeterminate main axis. They had significantly different values for measurements of width of intergenicula in branches and width of genicula (Fig. 4; Appendix S3), but the ranges for each of these characters overlapped (Appendix S4). Despite substantial genetic differences between these two species, they were phenotypically very similar and difficult to distinguish.

Bossiella montereyensis K.R.Hind, P.W.Gabrielson & Martone sp. nov.—*Holotype*—UBC A89692, (Fig. 11A). Mussel Beach, Hopkins Marine Station, (36.62125°, −121.90364°), Pacific Grove, CA, 6 June 2013, epizoic on mussel shell, mid-intertidal, leg. P. T. Martone PTM388.

Representative DNA barcode—UBC A89692 (holotype), *rbcL*-1401, GenBank KT782065.

Etymology—Named for Monterey County, an area of high macroalgal diversity where this species is endemic.

Morphology—Figure 11. Thalli erect, to 6.25 cm tall, from a small crustose base; branching irregularly pinnate, determinate branches frequently of one intergeniculum; indeterminate branches sometimes densely layered (Fig. 11A), secondary branches sometimes terminated with triangular shaped tips and sometimes with up to 15 intergenicula per branch (Fig. 11A); basal intergenicula subterete to flattened, often devoid of branches; main axis sometimes visible or hidden by dense lateral branches; axial intergenicula trapezoidal to hexagonal, rarely sagittate; upper axial intergenicula measure 0.6–0.9 mm long, 1.1–1.9 mm wide; dorsal and ventral midribs were rarely observed; 6–10 tiers of medullary cells per intergeniculum; genicula 204.2–308 μm long and 285.6–511.0 μm wide; conceptacles 1 or 2/intergeniculum, paired or unpaired, solitary conceptacles common. Tetrasporangial conceptacle chamber diameter, chamber height and canal height ranged from 438.2–551.4 μm, 282.6–370.6 μm, 88.7–187 μm, respectively. Male specimens had conceptacles that were 345.1–489.5 μm, 191.0–253.0 μm, 262.8–519.8 μm for conceptacle chamber diameter, chamber height and canal height, respectively. Female conceptacles were not observed.

Habitat and distribution—Bossiella montereyensis was found in the mid-low intertidal zone, occasionally in tidal pools, epilithic or epizoic on mussel shells. This species apparently has a limited distribution in Monterey County, CA.

Comments—Bossiella montereyensis was morphologically similar to *B. plumosa,* but had DNA sequence divergence values indicative of species level differentiation (at least 5.4% in COI-5P, 1.1% in *psbA* and 1.0% in *rbcL*). *Bossiella montereyensis* was difficult to differentiate from *B. plumosa* in the field. Since the morphological ranges of these two species overlap (Appendix S4), sequence data are recommended to identify this species with confidence. Plant height was the only character that differed significantly between these two species however there was still overlap in the ranges measured (Fig. 4; Appendices S3, S4).

Bossiella reptans K.R.Hind, P.W.Gabrielson & Martone sp. nov.—*Holotype*—UBC A89738, (Fig. 12A). Wolf Beach, East Rocks (51.6697°N, −128.117°W), Calvert Island, Hakai Luxvbalis Conservancy, BC, Canada, 22 May 2013, subtidal (5 m) on large (~1 m diam.) boulders, leg. K. Hind, (PTM417).

Representative DNA barcode—UBC A89738 (holotype), CO1-5P, GenBank KT782021.

Etymology—From the Latin *reptare*, meaning to creep or crawl. *Bossiella reptans* produces small, prostrate fronds.

Morphology—Figure 12. Prostrate fronds (Fig. 12B) from an inconspicuous crustose base to 2.5 cm long; branching irregular to irregularly pinnate throughout, primary overlapping branches proliferous (Fig. 12A), secondary branching infrequent, basal intergenicula often branched and flattened (Fig. 12A); intergenicula sagittate, thin and compressed (0.23–0.46 mm), often chipped resulting in an irregular margin; upper axial intergenicula extremely thin, flat, delicate, and brittle (Fig. 12C), 0.9–2.2 mm long 1.0–4.4 mm wide; 9–29 tiers of medullary cells per intergeniculum; intergenicula with midrib on ventral and/or dorsal surface; genicula 183.9–275 μm long and 251–472.3 μm wide; conceptacles emergent but depressed (low profile) (Fig. 12D), paired, infrequent and sometimes irregular. Tetrasporangial conceptacle chamber diameter, chamber height and canal height 355.4–475.7 μm, 151.3–257.1 μm, 103.1–166.1 μm, respectively. Female and male conceptacles were not observed in this study.

Habitat and distribution—Bossiella reptans was infrequent in the mid intertidal zone, but common in the low intertidal zone, usually under surfgrass (*Phyllospadix* spp.), and subtidally to 9 m depth. While most often epilithic, it was also found growing on benthic invertebrates. This species distribution ranged from Wiah Point and Cape Edensaw northwest of Masset, Haida Gwaii to Whiffen Spit, Vancouver Island, BC, Canada. It was also recorded from Hornby Island in the Salish Sea, BC, Canada.

Comments—Bossiella reptans resembled *B. hakaiensis*. The differences between these two species were discussed under *B. hakaiensis. Bossiella reptans,* named for its creeping growth habit, had two distinct morphological forms. The type (Fig. 12A) represents the form with robust intergenicula. The more delicate form bore irregular, thin intergenicula often separated by relatively long and thin genicula, making the intergenicula appear to float (Fig. 12C). The creeping growth habit is the easiest field character for identification (Fig. 12B).

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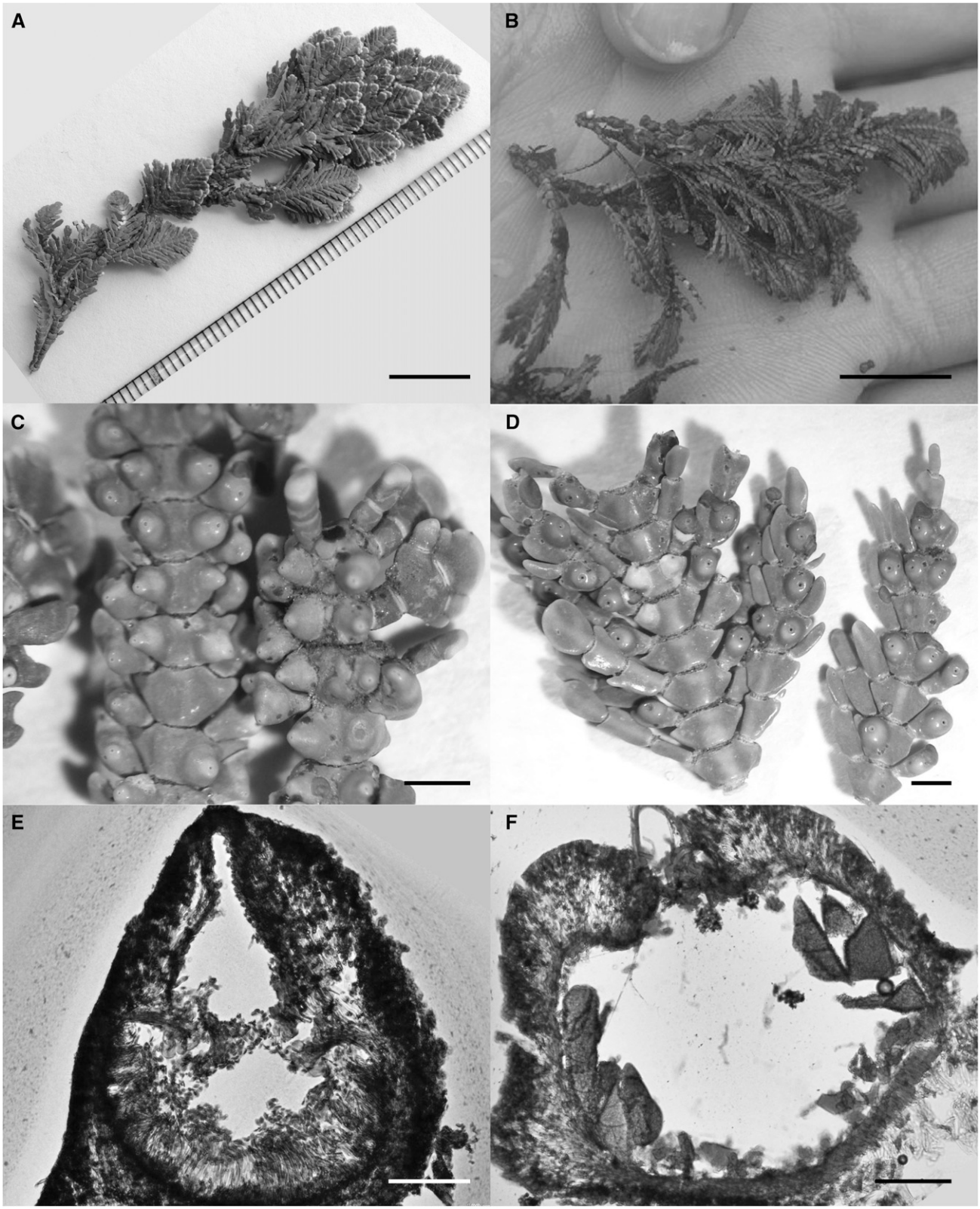

FIGURE 11 Morphology of *Bossiella montereyensis.* (A) Holotype specimen (UBC A89692/PTM388). Scale bar = 1 cm. (B) Specimen (GWS021674) showing dense branching and absence of pinnae. Scale bar = 1 cm. (C) Urceolate male conceptacles (GWS021619). Scale bar = 1 mm. (D) Tetrasporangial conceptacles with central pore (GWS021711). Scale bar = 1 mm. (E) Longitudinal section of male conceptacle (GWS021619). Scale bar = 100 µm. (F) Longitudinal section of tetrasporangial conceptacle (GWS021711). Scale bar = 100 µm.

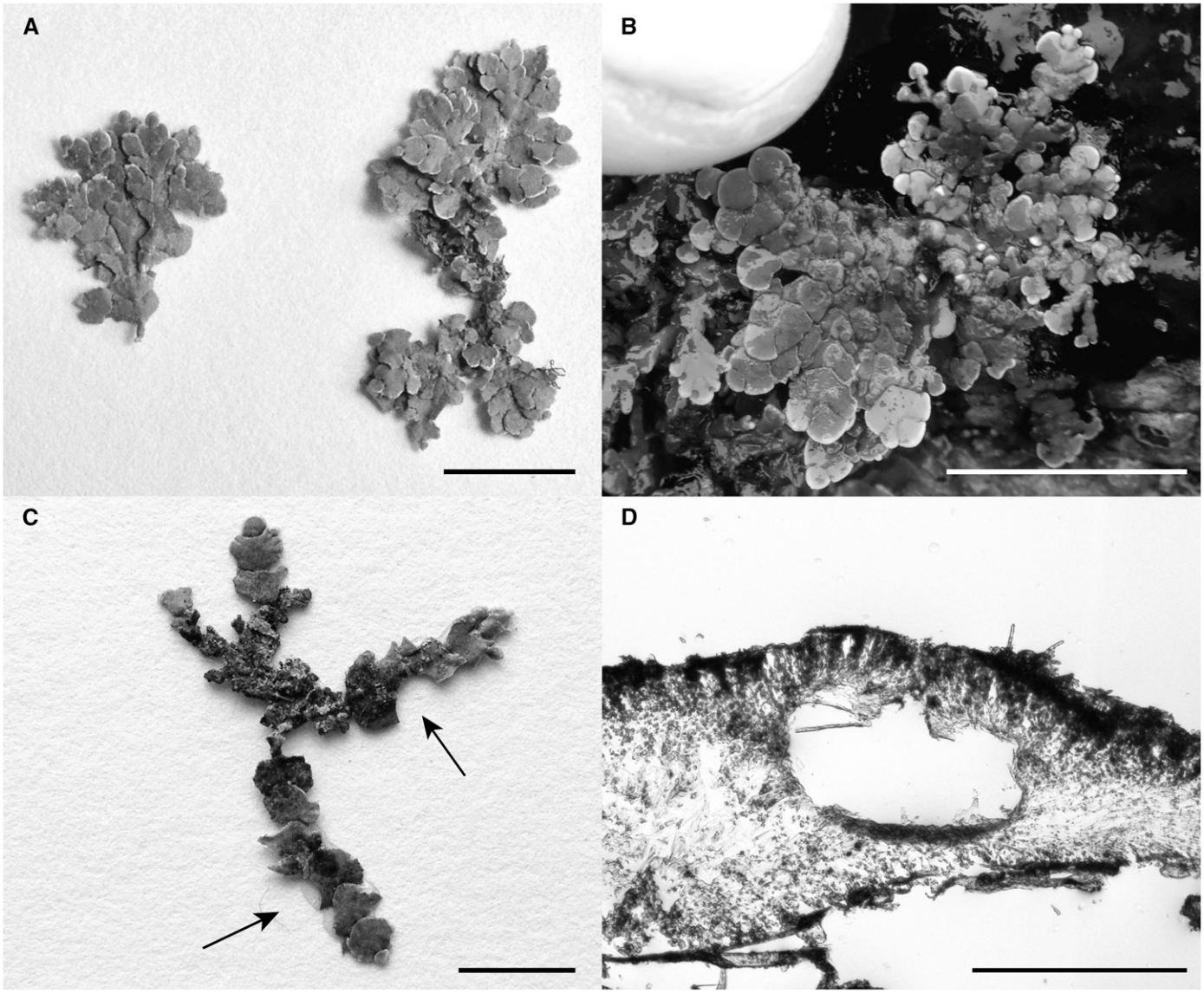

FIGURE 12 Morphology of *Bossiella reptans*. (A) Holotype specimen (UBC A89738/PTM417) deposited at UBC. Scale bar = 1 cm. (B) Specimen (PTM1174) showing prostrate growth habit and petite morphology. Scale bar = 1 cm. (C) Specimen (PTM413) demonstrating the "chipped" intergenicula (arrow) and the thin delicate nature of this species. Scale bar = 0.5 cm. (D) Specimen (GWS010227) displaying partially sunken, low profile conceptacle. Scale bar = 400 µm.

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