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Molecular-assisted alpha taxonomy reveals pseudocryptic diversity among species of *Bossiella* (Corallinales, Rhodophyta) in the eastern Pacific Ocean

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Abstract: A floristic survey of the red algal genus *Bossiella* was conducted using molecular-assisted alpha taxonomy (MAAT). The MAAT approach used DNA sequence data as a first pass to assess species diversity followed by additional study including detailed morphological observations to delimit species. In addition, type specimen sequencing was conducted to apply existing species names to genetic groups. Four *Bossiella* species were recognised in the eastern Pacific Ocean based on morphology, but a genetic screen using a DNA barcode marker, mitochondrial cytochrome *c* oxidase subunit 1 (COI-5P), showed 17 genetic species groups. Due to the large number of species requiring taxonomic assessment, we focused this study on species with predominantly dichotomous branching, that is, the recognised morphospecies *B. californica* and *B. orbigniana*. DNA sequences from three loci, *psb*A, *rbc*L and COI-5P, resolved five species: *B. californica*, *B. dichotoma*, *B. schmittii*, *Bossiella heteroforma sp. nov.* and *B. orbigniana* (the only species with a type locality not in the northeast Pacific). Morphology alone was an inadequate discriminator of these species, but incorporating distribution and habitat data facilitated identification of some species without DNA sequencing. All of these species were widely distributed in the northeast Pacific Ocean, from at least northern British Columbia, Canada, to Monterey Bay, California, USA, with two reaching Baja California Norte, Mexico.

Key Words: COI-5P, Coralline algae, DNA barcode, *psb*A, *rbc*L

INTRODUCTION

Alpha taxonomy is the science of identifying, describing and naming species and is usually partnered with phylogenetic inference (Wheeler 2004). Over the last 30 yr the use of DNA-based identifications has become an established taxonomic practice for biologists and has facilitated the creation of DNA reference libraries such as the Barcode of Life Data System (BOLD; Ratnasingham & Hebert 2007) and GenBank (Benson *et al.* 2005). Molecular-assisted alpha taxonomy (MAAT) is a technique that uses molecular markers to assign collections to genetic groups followed by detailed morphological observations (Saunders 2008; Cianciola *et al.* 2010). This process has been used to uncover pseudocryptic species complexes and identify potentially informative morphological characters that are diagnostic of a species group (Saunders 2008; Cianciola *et al.* 2010). It is particularly useful for algal studies due to their predominantly simple morphologies, convergent morphological characters and phenotypic plasticity (Saunders 2005).

Coralline algae (Corallinales, Rhodophyta) are an excellent candidate group for this type of study because they are often overlooked by phycologists and have demonstrated a high degree of phenotypic plasticity and morphological convergence in the marine environment (Johansen & Colthart 1975; Gabrielson *et al.* 2011; Hind & Saunders 2013b). Coralline algae exist in a diversity of forms, from flat, smooth crusts on rocks to elaborate calcified fronds. The erect, geniculate species are composed of alternating calcified (intergenicula) and uncalcified (genicula) segments giving them a jointed morphology that grants them flexibility during wave action. Johansen & Colthart (1975) examined phenotypic plasticity in geniculate coralline algae and showed that morphological dimensions can differ under varying laboratory conditions. Specifically, they demonstrated that intergenicular size, length of medullary cells, distance between successive branches and the thickness of cuticles and cortices were influenced by the environment.

Using the MAAT approach, combined with sequencing type specimens to apply species names (Hughey & Gabrielson 2012), we examined the taxonomic diversity of algal species in the northeast Pacific Ocean. The objective of this study was to examine the diversity of species in the geniculate coralline algal genus *Bossiella* P.C. Silva. The last taxonomic assessment of northeast Pacific *Bossiella* species occurred before the use of DNA sequencing techniques and suggested that *Bossiella* species displayed substantial phenotypic variation due to responses to physical parameters such as depth and wave exposure (Johansen 1971).

In the current study we used the mitochondrial cytochrome *c* oxidase subunit 1 (664 bp, COI-5P) DNA barcode, the photosystem II reaction center protein D1 gene (863 bp, *psb*A) and the internal transcribed spacer (998 bp, ITS) of the nuclear ribosomal cistron to assess the number of *Bossiella* species present in the northeast Pacific region. Furthermore, comparisons were made between contemporary plastid-encoded large subunit of RuBisCO (*rbc*L) sequences and those obtained from type specimens. Our

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analyses revealed 17 *Bossiella* species in the northeast Pacific region, where only four were recognised. In addition, we constructed a multigene phylogeny using the COI-5P and the *psb*A gene regions to assess the monophyly of *Bossiella*.

We focused this study on *Bossiella* species with predominantly dichotomous branching [i.e. *Bossiella californica* (Decaisne) P.C. Silva and *B. orbigniana* (Decaisne) P.C. Silva]. Species with predominantly pinnate or irregular branching will receive taxonomic assessment in future publications.

MATERIAL AND METHODS

Specimens in this study ($n = 409$) and their collection information are included in Table S1. Specimens were collected from subtidal and intertidal habitats at both sheltered and exposed sites. We collected multiple individuals of all *Bossiella* species listed as current in the regional taxonomic keys (Gabrielson *et al*. 2012). Efforts were made to collect from type localities when possible. Fresh material was dried in silica and stored as vouchers in the Connell Memorial Herbarium (UNB) at the University of New Brunswick, Canada (Table S1).

DNA extraction, PCR, sequencing and barcode analyses

Total DNA was extracted from contemporary specimens using an automated protocol for dried red algal samples (Hind & Saunders 2013b). The COI-5P region of the mitochondrion was PCR amplified using a low-volume protocol (12.5µl reaction; Hamsher *et al*. 2011) with the actual primer combination used for each collection listed with each accession at GenBank (Table S1). Cycling consisted of one cycle at 94°C for 2 min, five cycles of 30 s at 94°C, 30 s at 45°C and 1 min at 72°C, followed by 35 cycles of 30 s at 94°C, 30 s at 46.5°C and 1 min at 72°C with a final cycle at 72°C for 7 min.

In addition, the *psb*A gene of the chloroplast genome and the nuclear ITS region of the rDNA were amplified and cleaned following Hind & Saunders (2013b). These molecular markers have been useful at assessing species boundaries for other coralline algal groups in the past (Broom *et al*. 2008; Hind & Saunders 2013b) and were used here in order to assess congruence between COI-5P and molecular markers from the plastid and nucleus. The ITS was sequenced only for specimens of genetic species that showed less than 3.3% sequence divergence in the COI-5P marker.

Following the manufacturer's protocol, PCR products were sequenced using the PE Applied Biosystems Big Dye kit (v3.0, ABI, Foster City, California USA), and forward and reverse fragments were edited using Sequencher™ 4.8 (Gene Codes Corp., Ann Arbor, Michigan USA). Multiple sequence alignments were constructed using MacClade 4 (v 4.06) for OSX (Maddison & Maddison 2003). For all sequence data, corrected evolutionary distances and neighbour-joining (NJ) trees were calculated in Geneious v6.1 (Biomatters, available from http://www.geneious.com) using a Tamura–Nei model of sequence evolution for the purpose of assigning collections to genetic groups.

Extraction of DNA from type specimens followed the protocol in Gabrielson *et al*. (2011) that was modified for coralline algae from the protocol of Hughey *et al*. (2001) and further explained in Hughey & Gabrielson (2012). The primer pair F-1152Cor/R-1308Cor (Gabrielson *et al*. 2011) amplified a 135bp variable region of *rbc*L, herein called *rbc*L 135. In addition, F-1152Cor was paired with R-*rbc*S (Freshwater & Rueness 1994) to yield a 296bp variable region of *rbc*L, herein called *rbc*L 296. Amplification and sequencing protocols were those of Hughey *et al*. (2001). Mock DNA extraction controls as outlined in Saunders & McDevit (2012) were not included at the time of this study. Sequences were obtained from an ABI 3100 Genetic Analyzer (DNA Analysis Core Facility, Center for Marine Sciences, University of North Carolina,-Wilmington, Wilmington, North Carolina USA), and were manually aligned and compiled using Sequencher (Gene Codes Corp.) and Sequence Alignment Editor available at http://evolve.zoo.ox.ac.uk/Se-Al/Se-Al.html.

A multigene phylogeny was constructed using the COI-5P and *psb*A sequence data (1527 bp) for a single representative of each genetic species group resolved through the barcode analyses. Outgroup taxa were chosen from within the family Corallinaceae (Hind & Saunders 2013b). Maximum likelihood (ML) analysis was conducted using PhyML 3.0 (Guindon & Gascuel 2003) with a general-time-reversible (GTR+I+Γ) substitution model [determined by jModelTest v0.1.1 (Posada 2008)], with PhyML-estimated proportion of invariable sites and a rate heterogeneity parameter based on a gamma distribution. Bootstrap resampling (1000 replicates) was conducted to estimate branch support. In addition, each gene region was analysed independently to assess marker congruence. Bayesian analysis was performed using MrBayes v3.1 (Huelsenbeck & Ronquist 2001) under the GTR+I+Γ model. The concatenated data set (COI-5P and *psb*A) was partitioned by gene and codon, and sampling was performed every 1000 generations. Two separate analyses consisting of four chains each, starting from random starting trees, were run (i.e. MrBayes default settings). Analyses were run for six million generations, and the burn-in was determined after convergence of the tree samples was obtained. Convergence of tree samples was determined by plotting log-likelihood vs generation time.

Morphology

Vegetative features were measured on two to three intergenicula for four to 20 plants from each genetic species group. Measurements were made on the third to fifth intergeniculum from the apex of the main axial branch. We measured intergenicular length and thickness using digital calipers accurate to 0.02 mm. We also assessed midrib presence, branching pattern and the number and distribution of conceptacles per intergeniculum. These specific morphological characters were assessed since they were presumed to be effective at discriminating species of *Bossiella* in the past (Johansen 1971).

Specimen photographs were taken using a Canon EOS 60D camera with a Sigma 70-mm f/2.8 macro lens (Canon USA, Melville, New York USA). Several photographs were taken of each specimen and merged into one image using Helicon Focus v4.2.7 Pro from HeliconSoft (http://mac.brothersoft.com/helicon-focus-pro-x64.html). This allowed the entire depth of field to be in focus.

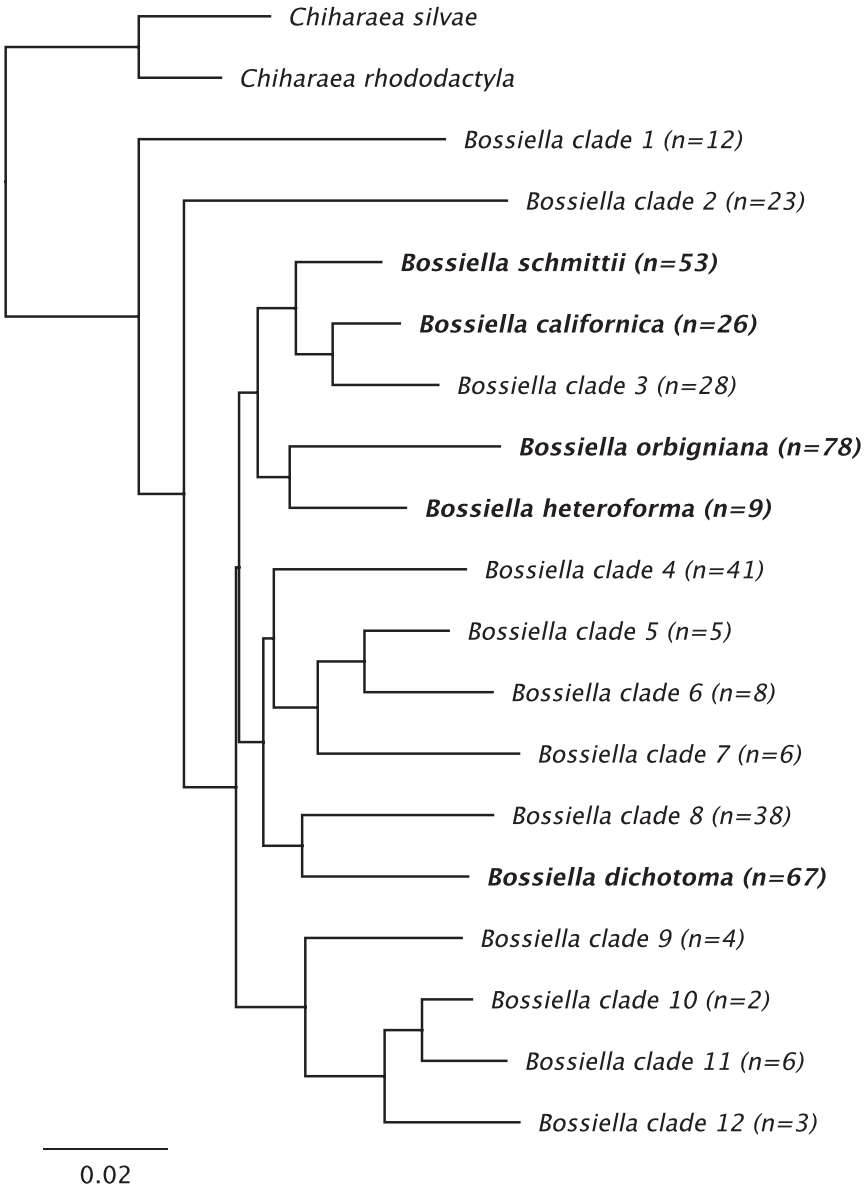


Fig. 1. Unrooted phylogram (neighbour-joining) generated from CO1-5P data for specimens included in this study. Species in bold type were reviewed in this study with current names applied following taxonomic assessment. Scale bar refers to substitutions per site.

RESULTS

A total of 409 specimens were collected along the Pacific coast of North America and sequenced for the COI-5P DNA barcode region (Table S1). Using NJ analysis 17 genetic species groups were resolved for the genus *Bossiella* with intraspecific variation ranging between 0% and 1.4% and interspecific variation of at least 2.0% (Fig. 1). All genetic species groups were given provisional names (e.g. *Bossiella* clade 1) based on their clustering in the COI-5P analysis. The ITS region was sequenced ($n = 19$) for two to four specimens from each COI-5P group that showed less than 3.3% sequence divergence from its respective nearest neighbour. We were unable to obtain clean ITS sequences from *Bossiella* clade 10 or *Bossiella* clade 11. Genetic species groups from the ITS analyses were completely congruent with the COI-5P results with 0.1% to 3.8% sequence divergence within and at least 5.3% sequence divergence between genetic species groups (data not shown). One exception was between specimens of *Bossiella* clade 3 and *B. californica* from California, where the minimum interspecific ITS divergence was 3.7%.

The *psb*A gene region was also sequenced for representatives ($n = 96$) of each genetic group designated by the COI-5P NJ analysis. All genetic species groups from the *psb*A analysis were consistent with the COI-5P groups except for one case. Using both the COI-5P and the ITS markers, *Bossiella* clade 3 specimens resolved as a single lineage; however, using the *psb*A marker, California collections (GWS021434, GWS021750, and GWS021309, GenBank accession nos. KJ637842, KJ637841, and KJ637843, respectively) resolved with *B. californica*; whereas, samples from

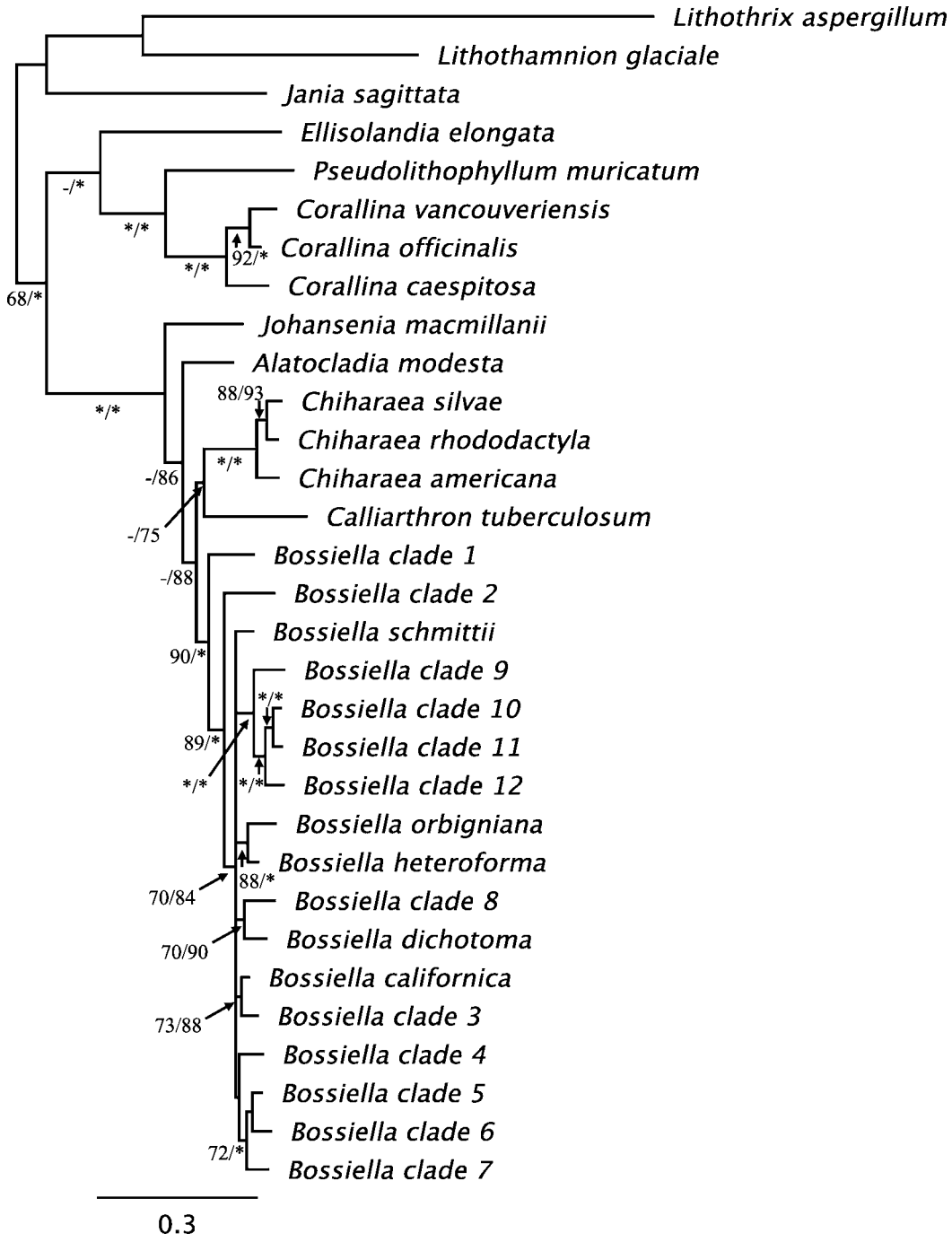
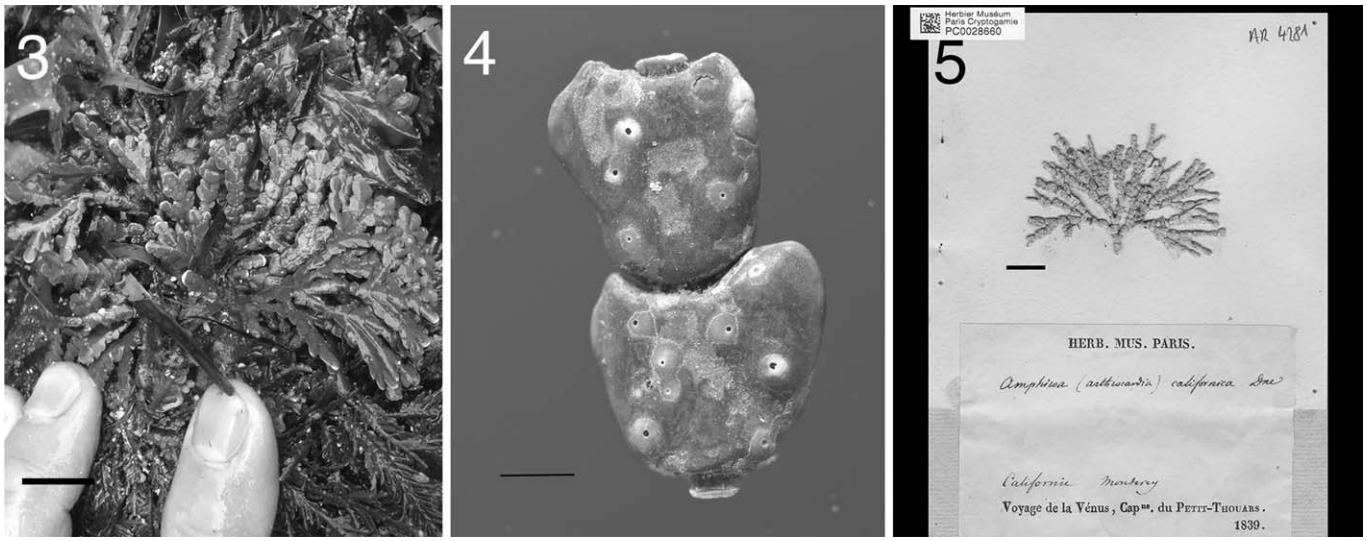


Fig. 2. Phylogram inferred by Bayesian analysis of concatenated CO1-5P and *psb*A sequence data. Support values are listed as bootstrap for maximum likelihood analyses and Bayesian posterior probabilities respectively. Asterisks denote nodes that are strongly supported (bootstrap values $\geq$ 96, posterior probabilities $\geq$ 0.98) in all analyses. Support values are not indicated for all nodes (i.e. bootstrap values $\leq$ 60, posterior probabilities $\leq$ 0.74). Scale bar refers to substitutions per site.

Haida Gwaii, British Columbia, Canada (GWS013058 and GWS012917, GenBank accession nos. JQ422194 and KJ637840, respectively), were 0.6% divergent from *B. californica* (a result consistent with the COI-5P and ITS results). These results suggested that the plastid of *B. californica* may have introgressed into populations of *Bossiella* clade 3 in southern waters and that this introgression did not extend to northern populations. A consequence of this interpretation was that plastids and mitochondria may have had different patterns of inheritance in *Bossiella*, a pattern also reported recently for the coralline genus *Chiharaea* Johansen (Hind & Saunders 2013a).

A phylogram was generated from a combined data set of the COI-5P and *psb*A gene regions using Bayesian and ML analyses (Fig. 2). Single gene analyses were run with the same resulting topology as the combined analysis (data not



Figs 3–5. Morphology of *Bossiella californica*.
Fig. 3. Specimen (GWS020111) showing dorsiventral habit and intergenicula lacking midribs. Scale bar = 1 cm.
Fig. 4. *Bossiella californica* (GWS019219) has fewer than 10 conceptacles on the ventral surface of the intergenicula. Scale bar = 1 mm.
Fig. 5. Image of holotype specimen of *Amphiroa californica* (PC0028660). Image provided courtesy of the Muséum National d'Histoire Naturelle (PC). Scale bar = 1 cm.

shown). Overall, the Bayesian analysis resolved the same topology as ML with equal or higher support for each node. One exception was that in the ML analysis, species in the genera *Johansenia* and *Alatocladia* resolved as sister to the lineage containing *Bossiella* with weak support. The genus *Bossiella* was monophyletic with all eight 'cryptic' species morphologically assignable to the generitype, *Bossiella plumosa* (Manza) P.C. Silva, resolving in the lineage with all other *Bossiella* species.

Taxonomic results

Bossiella was proposed by Silva (1957, p. 46) as a substitute name for *Bossea* (*Bo.*) Manza (Manza 1937a), which unfortunately was a later homonym of *Bossea* Reichenbach (1841), a genus of Geraniaceae. The type species is *Bossiella plumosa* (Manza) P.C. Silva (1957, p. 47). Wynne & Schneider (2007) treated *Bossiella* as a synonym of *Pachyarthron* Manza (1937a) and transferred many species to the latter genus, based on Johansen's (1969) assertion that 'no fundamental differences exist' to distinguish these genera. Woelkerling et al. (2008) argued that *Pachyarthron* was a distinct genus based on morphoanatomy. Neither interpretation was supported by DNA sequencing of an isotype of *Corallina cretacea* Postels & Ruprecht [basionym of *Pachyarthron cretaceum* (Postels & Ruprecht) Manza] as demonstrated by Hind *et al.* (2014). We therefore used the generic name *Bossiella*. Morphological attributes useful for distinguishing *Bossiella* from related genera are treated in detail elsewhere (Hind & Saunders 2013b).

Bossiella californica (Decaisne) P.C. Silva 1957, p. 46

HOMOTYPIC SYNONYMS: *Cheilosporum californicum* (Decaisne) Yendo 1902: 715; *Amphiroa tuberculosa* f. *californica* (Decaisne) Setchell & N.L. Gardner 1903, pp. 361—362; *Bossea californica* (Decaisne) Manza 1937b, p. 561; *Pachyarthron californicum* (Decaisne) Schneider & M.J. Wynne 2007, p. 321.

HETEROTYPIC SYNONYMS: *Bossea angustata* W.R. Taylor 1945, p. 193, pl. 59; *Bossea corymbifera* Manza 1937b, p. 562; *Bossiella corymbifera* (Manza) P.C. Silva 1957, p. 47; *Bossea pachyclada* W.R. Taylor 1945, pp. 194–195, pl. 58; *Bossiella pachyclada* (W.R. Taylor) P.C. Silva 1957, p. 47; *Pachyarthron pachycladum* (W.R. Taylor) Schneider & M.J. Wynne 2007, p. 231.

TYPE LOCALITY: Monterey, California, USA.

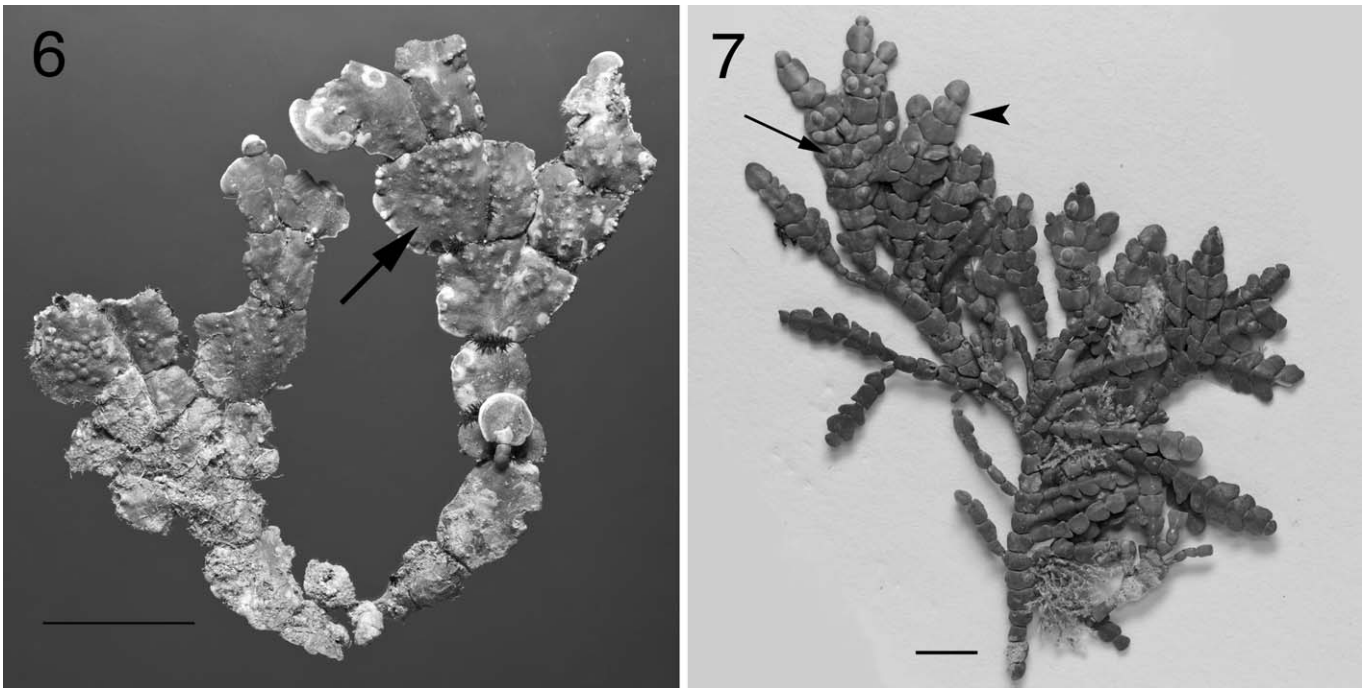
The thallus was up to 12 cm tall, dorsiventral and pyramidal to corymbose in shape, and it arose from a barely discernable crustose base. There were one to several branches arising from the crust. The basal axes were typically terete to compressed and unbranched, above compressed and dichotomously to somewhat irregularly branched (Fig. 3). The midrib was absent to inconspicuous. Conceptacles were 1 to 10 per intergeniculum (Fig. 4) and were often scattered on the ventral surface.

Bossiella californica was found predominantly in tidepools in the low, mid- and upper littoral zones; although, two subtidal specimens were collected, one each from California (GWS022085) and British Columbia (GWS019566). Based on sequenced specimens, *B. californica* occurred from Haida Gwaii, British Columbia, Canada, to Isla Cedros, Baja California Norte, Mexico.

Bossiella schmittii (Manza) H.W. Johansen

HOMOTYPIC SYNONYMS: *Bossiella californica* ssp. *schmittii* (Manza) H.W. Johansen 1971, p. 389; *Pachyarthron californicum* subsp. *schmittii* (Manza) Schneider & M.J. Wynne 2007, p. 231.

TYPE LOCALITY: Point Loma, San Diego County, California, USA, 38–44 m in depth.



Figs 6, 7. Morphology of *Bossiella schmittii* and *Bossiella dichotoma*.
Fig. 6. Dorsiventral habit of *Bossiella schmittii* (GWS004585). Arrow indicates intergeniculum with greater than 10 conceptacles. Scale bar = 1 cm.
Fig. 7. Specimen of *Bossiella dichotoma* (GWS003242) from this study displaying a mixture of dichotomous and pinnate branching. Arrowhead indicates distinctive rounded branch tips that are useful for identification. Arrow indicates paired, dome-shaped conceptacles. Scale bar = 5 mm.

The thallus was up to 12 cm long, typically recumbent and flattened dorsiventrally or occasionally erect. Branches arose from an inconspicuous basal crust. Branching was mixed pinnate and dichotomous. Basal intergenicula were subterete to flattened and rapidly became flattened and winged and were commonly somewhat convex. Axial intergenicula were 1.5–5.1 mm in length. The ventral surface was frequently lighter in colour than the dorsal surface. A midrib was typically present on the ventral surface. There were 10–50 conceptacles per intergeniculum (Fig. 6), primarily on the dorsal surface.

Bossiella schmittii was epilithic and subtidal. All of our sequenced collections were from 4–20 m of depth; however, this species was reported to 85 m of depth (Dawson 1949). The type locality, San Diego County, California, USA, is the southern limit of this species, and we expand its distribution northward to Haida Gwaii, British Columbia, Canada. It is frequently encountered subtidally in the protected and exposed waters of Barkley Sound, the Strait of Juan de Fuca and northern Puget Sound.

Bossiella dichotoma (Manza) P.C. Silva 1957, p. 47

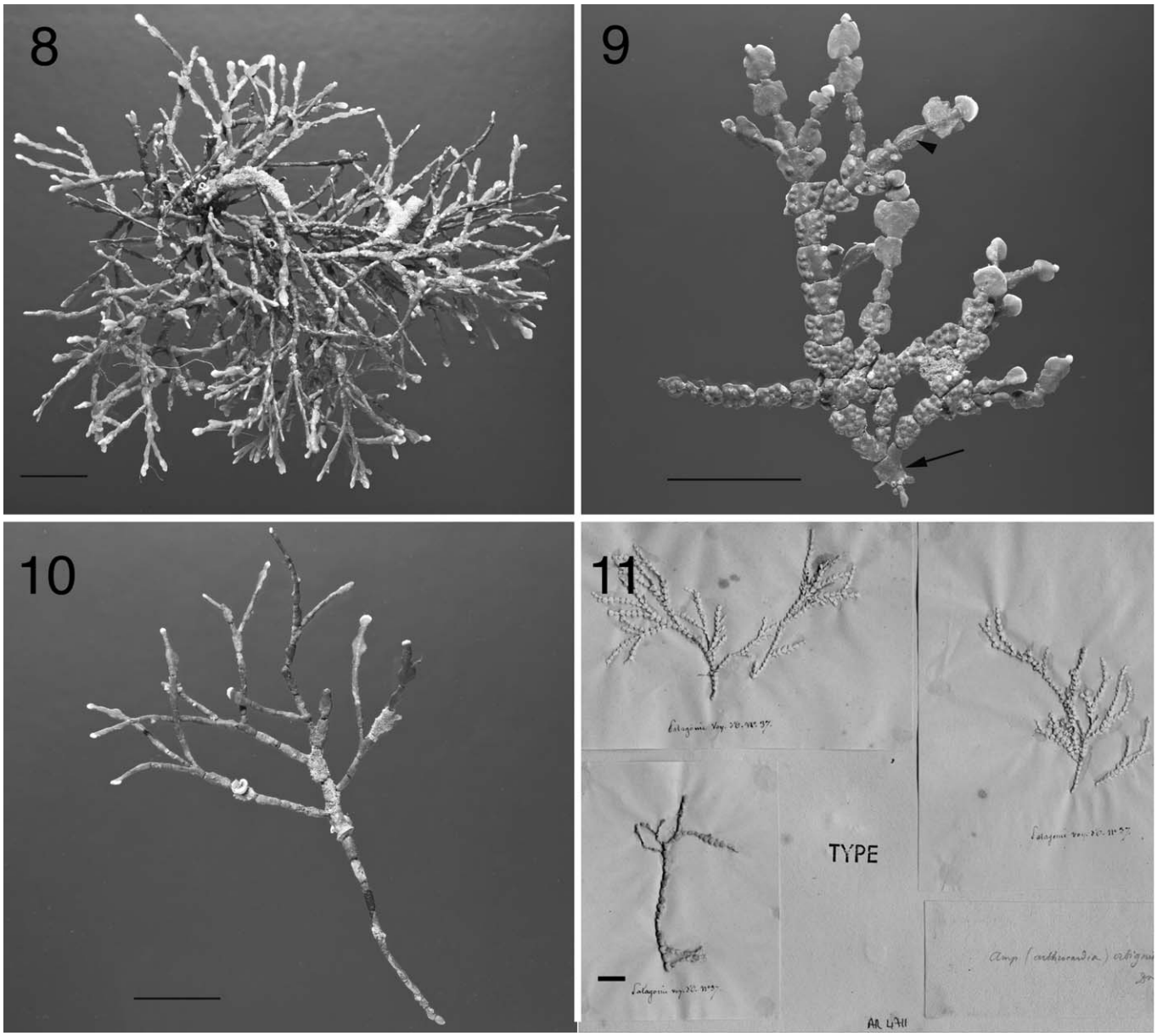
HOMOTYPIC SYNONYMS: *Bossiella orbigniana* subsp. *dichotoma* (Manza) H.W. Johansen 1971, p. 394; *Pachyarthron orbignianum* subsp. *dichotomum* (Manza) Schneider & M.J. Wynne 2007, p. 231.

HETEROTYPIC SYNONYMS: *Bossea cooperi* E.Y. Dawson & P.C. Silva in Dawson 1953, pp. 158–159, fig. 2; *Bossiella cooperi* (E.Y. Dawson & P.C. Silva) P.C. Silva 1957, p. 47; *Pachyarthron cooperi* (E.Y. Dawson & P.C. Silva) Schneider & M.J. Wynne 2007, p. 231; *Bossea gardneri* Manza 1937b, p. 563; *Bossiella gardneri* (Manza) P.C. Silva 1957, p. 47; *Bossiella dichotoma* var. *gardneri* (Manza) E.Y. Dawson 1960, p. 45; *Pachyarthron gardneri* (Manza) Schneider & M.J. Wynne 2007, p. 231; *Bossea insularis* E.Y. Dawson & P.C. Silva in Dawson 1953, pp. 159–160; pl. 8, figs 5–6; pl. 25, fig. 2; *Bossiella insularis* (E.Y. Dawson & P. C. Silva) P. C. Silva 1957, p. 47; *Bossea interrupta* Manza 1937b, p. 563; *Bossiella interrupta* (Manza) P.C. Silva 1957, p. 47; *Pachyarthron interruptum* (Manza) Schneider & M.J. Wynne 2007, p. 231; *Bossea ligulata* E.Y. Dawson 1953, pp. 156–157; pl. 8, fig. 8; pl. 26, fig. 2; *Bossiella ligulata* (E.Y. Dawson) P.C. Silva 1957, p. 47. *Bossea sagittata* E.Y. Dawson & P.C. Silva in Dawson 1953, pp. 157–158; pl. 8, fig. 1; pl. 32, figs 1–2; *Bossiella sagittata* (E.Y. Dawson & P.C. Silva) P.C. Silva 1957, p. 48.

TYPE LOCALITY: Moss Beach, San Mateo County, California, USA.

The thallus was erect, 15 (30) cm tall and arose from an inconspicuous crustose base. Branching overall was predominantly distichous, fastigiate to spreading and sometimes corymbose, commonly dichotomous, or sometimes pinnate or a mixture of both to two to four orders (Fig. 7). Basal intergenicula were terete to subterete and measured 0.7–2.0 mm long and 0.6–1.8 mm in diameter. Intergenicula gradually became flattened and winged, measuring 0.4–1 mm long, and varied from sagittate to wing-nut shaped. A midrib was typically present. There were generally one to four conceptacles per intergeniculum, and often two were aligned on either side of the midrib.

This species occurred from Haida Gwaii, British Columbia, Canada, to Baja California Norte, Mexico, and throughout its range at exposed to semiexposed sites. In



Figs 8–11. Morphology of *Bossiella orbigniana*.
Fig. 8. Specimen GWS010063, a tetrasporophytic plant. Scale bar = 1cm.
Fig. 9. Specimen GWS010226 displaying intergenicula with gaps between successive segments. Arrow indicates flattened basal intergeniculum. Arrowhead indicates midrib. Note clusters of secondarily derived conceptacles on lower intergenicula. Scale bar = 1 cm.
Fig. 10. Specimen GWS010258. Note all intergenicula are rectangular and maintain contact between successive intergenicular segments. Scale bar = 1 cm.
Fig. 11. Three fragments of collections of *Amphiroa orbigniana* (PC0028696) from Decaisne (1842). The bottom-left specimen was designated as the lectotype (AR4711). Image provided courtesy of the Muséum National d'Histoire Naturelle (PC). Scale bar = 1.5 cm.

the northern part of its range it was epilithic in high and mid-intertidal pools and in the low intertidal; whereas, from Monterey south it was more common subtidally, although at some sites in Baja California Norte, Mexico, it was also present in the mid- to low intertidal. By DNA sequence, we have confirmed this species as far south as Isla Guadaloupe, Baja California Norte, Mexico, but it was reported further south from Isla Cedros and from several localities on the mainland another 200 km to Bahia Ascuncion, Baja California Norte, Mexico, by Dawson and Silva (in Dawson 1953, p. 160, as *Bossea insularis*).

Bossiella orbigniana (Decaisne) P.C. Silva 1957, p. 47

HOMOTYPIC SYNONYMS: *Bossea orbigniana* (Decaisne) Manza 1937b, pp. 563–564; *Amphiroa tuberculosa* f. *orbigniana* (Decaisne) Setchell & N.L. Gardner 1903, p. 362; *Bossiella orbigniana* ssp. *orbigniana*: 392; *Pachyarthron orbignianum* (Decaisne) Schneider & M.J. Wynne 2007, p. 231.

TYPE LOCALITY: Patagonia.

The thallus was erect, 3–10 cm tall, arose from a reduced crustose base and was epilithic or epizoic. Branching was distichous (Figs 8–11), dichotomous especially at the apices (Figs 8, 10) or irregular pinnate (Figs 10, 11) to irregular (Fig. 9). The plant axis branched at regular (Fig. 8) to irregular (Fig. 9) intervals, and branches spread prominently (Figs 8–11). Basal intergenicula were generally subterete or flattened, 0.5–2.5 mm in diameter and 0.7–3.2 mm in length. Axial intergenicula were 0.4–4.0 mm broad and 1.8–4 mm long and varied from rectangular (Figs 8, 10) to sagittate (Figs 9, 11). A midrib was either inconspicuous or present. There were one to six conceptacles per intergeniculum that were generally scattered and not aligned on either side of midrib (if present). Secondarily derived conceptacles were clustered in lower branches (Fig. 9).

The only sequenced specimen of *B. orbigniana* from the southern hemisphere is from Quintay, Chile (District V, GenBank *rbc*L: HQ322279; Gabrielson *et al*. 2011); we assumed all other reports were based on either herbarium records or field-collected specimens that were identified using morphology alone. In Chile, *B. orbigniana* was reported from District V through Magallanes (District XII; Ramírez & Santelices 1991); in Argentina, it was reported from 38°20′ to 54°42′S (Boraso & Zaixso 2011); and in North America, it was confirmed by DNA sequencing from Los Angeles, California, to Sitka, Alaska. Boraso and Zaixso (2011) gave its habitat in Argentina as 0–10 m in depth, implying that it occurred in the intertidal, but it appeared to be subtidal in Chile. In the northern part of its northeast Pacific range (Haida Gwaii, British Columbia, Canada), it was epilithic or epizoic in the high, mid- and low intertidal in pools or on bedrock. It was also common subtidally at 4–12 m, but this may represent a sampling bias, as it was also found at 22 and 24 m by anchor dredge in Gwaii Haanas National Park Reserve, British Columbia, Canada, and offshore of Los Angeles Harbor, California, USA, respectively. In California, our collections were strictly subtidal, consistent with populations from Chile. Approximately 8% of our collections of *B. orbigniana* were found attached to live snails.

Bossiella heteroforma K.R. Hind, P.W. Gabrielson & G.W. Saunders *sp. nov.*

Figs 12, 13

DESCRIPTION: Thalli epilithic with crustose base bearing erect fronds, generally 3–10 cm high and with primarily dichotomous branching (Fig. 12). Intergenicula of fronds with or without gaps between successive segments, variable in shape, terete and longer than wide or compressed and wider than long and somewhat sagittate or rectangular. Midrib sometimes present on dorsal surface (Fig. 12). Basal intergenicula terete (Fig. 13) 1.1–2.3 mm long and 0.5–1.2 mm diameter. Upper axial intergenicula variable in shape, 1.9–3.1 mm long and 0.7–1.8 mm diameter.

HOLOTYPE: GWS010088 deposited in Connell Memorial Herbarium (UNB). Image of holotype shown in Fig. 12. Collected from Island south of Clotchman Island, Spanish Pilot Group, Tahsis, British Columbia, Canada (49.61454, –126.58255), 23 May 2008; subtidal (17 m) on rock; *leg*. K. R. Hind and D. McDevit. Holotype DNA barcode: GenBank KJ591789, COI-5P.

ETYMOLOGY: Many of our collections of *B. heteroforma* had a mixture of both sagittate and rectangular intergenicula, hence the name meaning 'different forms'.

DISTRIBUTION: From Haida Gwaii, British Columbia, Canada, to Monterey, California, USA (see Table S1).

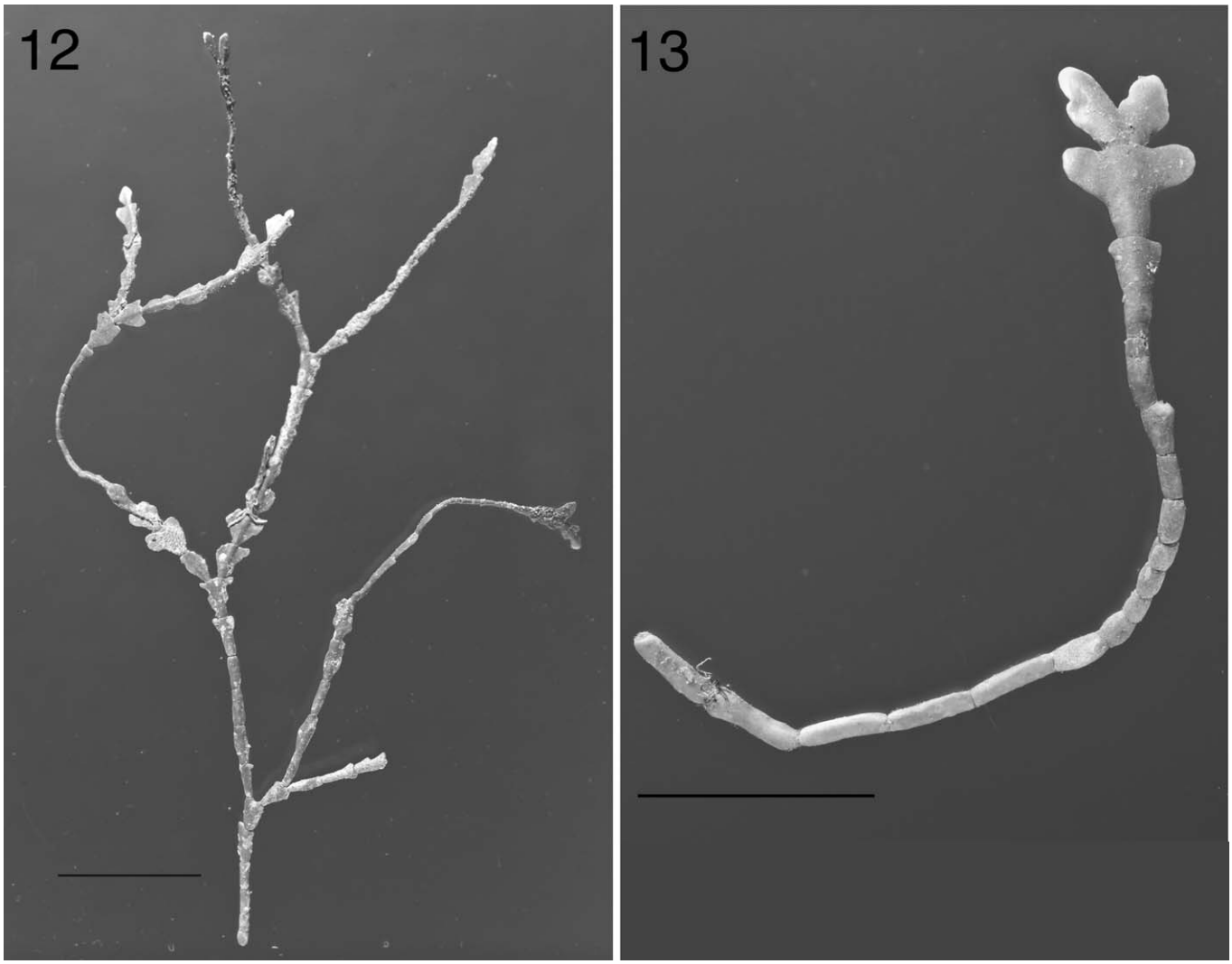
DISCUSSION

We used MAAT to assess the number of *Bossiella* species present in the northeast Pacific Ocean, coupled with sequencing type specimens to assign species names (Gabrielson *et al*. 2011). Previously, four species were recognised by the regional taxonomic keys for this area (Johansen 1971; Gabrielson *et al*. 2012); however, molecular sequencing resolved 17 distinct species groups. In this study, we focused our analyses on the two more or less dichotomously branched morphospecies, *B. californica* and *B. orbigniana* (Johansen 1971), which are typically easy to distinguish from those *Bossiella* species that are pinnately branched (*B. plumosa* and *B. chiloensis*). All loci that we sequenced (*psb*A, *rbc*L and COI-5P) segregated five species: *B. californica*, *B. dichotoma*, *B. orbigniana*, *B. heteroforma* and *B. schmittii*. There is no molecular support from any locus to recognise *B. schmittii* as a subspecies of *B. californica* or *B. dichotoma* as a subspecies of *B. orbigniana*.

Differentiating *Bossiella* species using morphological characters

Johansen (1971) reduced the number of species in *Bossiella* from 11 to four. He argued that many of the characters used in identification had overlapping values between two or more species and that these characters were readily modified by the environment. Forty years later, we can use MAAT to assess which morphological characters are useful to segregate species with the advantage of an *a priori* understanding of species diversity and genetic boundaries. In our analyses, measurements of some characters, such as intergenicular length and thickness, overlapped; whereas, other characters, such as habitat, were unique for particular species (Table 1). For example, the main difference between *B. californica* and *B. schmittii* is their habitat (intertidal vs subtidal, respectively). This provides a simple way to assign specimens to these species that otherwise may appear similar. At the same time, MAAT also provided insight into the phenotypic plasticity observed within a species. For example, *B. orbigniana* has a range of morphological forms that do not appear to be dictated by depth, exposure or substrate preference (Figs 8–11).

Johansen (1971) used three key features to distinguish species of *Bossiella*. These were branching pattern and intergenicular size and shape. We found that each of these characters can be used to differentiate between broad morphological types but that they are not useful in segregating species. For example, branching pattern (pinnate or dichotomous) typically will segregate major morphological groups (11 species had mostly or exclusively pinnate branching, three had more or less dichotomous branching and three had a mixture of dichotomous and pinnate branching within an



Figs 12, 13. Morphology of *Bossiella heteroforma sp. nov.*
Fig. 12. Image of the holotype specimen (GWS010088) displaying a combination of rectangular and sagittate intergenicula and sparse dichotomous branching. Scale bar = 5 mm.
Fig. 13. Specimen GWS008716 displaying a mixture of terete and serrate intergenicula as well as barrel-shaped basal segments. Scale bar = 5mm.

individual specimen) but is not a useful character for species identification.

Johansen (1971) used intergenicular size to differentiate between dichotomously branched species, but we found significant overlap in intergenicular length among *B. californica*, *B. dichotoma*, *B. orbigniana* and *B. heteroforma* (Table 1). *Bossiella schmittii* is the only species that can be identified using intergenicular length, but only for specimens with intergenicula greater than 3 mm (Table 1). Johansen (1971) also used intergenicular shape to differentiate between *B. orbigniana* subsp. *orbigniana* and *B. orbigniana* subsp. *dichotoma*, but we found this character to be variable within species and individuals (e.g. *B. orbigniana*) and thus not useful for species identification.

Placement and number of conceptacles were useful characters for identifying specimens of *B. californica* and *B. schmittii*, but this character was not informative for other *Bossiella* species. Few collections of male and female gametophytes have been made for *Bossiella* species, most likely because they are ephemeral, and therefore little taxonomic importance has been placed on the internal anatomy of conceptacles for these taxa.

At present, morphological characters are of very limited value in distinguishing among these *Bossiella* species, but, when combined with habitat and distribution data, frequently the choice can be narrowed to two species. To be certain of identifications, however, molecular sequencing of one of the loci used herein is needed.

Distributions of *Bossiella* species

Our molecular sequence data allow us to clearly distinguish species of *Bossiella* and provide the first accurate assessment of their distributions. We show that *Bossiella* species have extensive ranges in the eastern Pacific with all dichotomously branched species present from northern British Columbia

Table 1. Morphology and habitat assessment of dichotomously branched *Bossiella* species examined in this study. n/a = not available.

Character	*B. californica*	*B. dichotoma*	*B. orbigniana*	*B. heteroforma*	*B. schmittii*
Habitat	upper, mid-, or low intertidal (in pools)	upper, mid-, or low intertidal (in pools), subtidal (California only)	low intertidal, typically shallow subtidal	subtidal (12–17 m)	subtidal (3–17 m)
Shape of branch tips	sometimes rounded	typically rounded	variable, rectangular	typically narrow, slender	typically rectangular, sometimes rounded
Midrib presence	typically absent	typically present	sometimes present	sometimes present	typically present
Number of conceptacles per intergeniculum	typically <10	1–4, typically 2 arranged on either side of midrib	1–6, not regularly arranged on either side of midrib	n/a	typically >10
Position of conceptacles	typically ventral	typically dorsal	typically dorsal	n/a	typically dorsal
Intergenicular length (mm)	0.9–2.8	0.4–1.0	1.8–4.0	1.9–3.1	1.5–5.1
Intergenicular thickness (mm)	0.1–0.5	not measured	not measured	not measured	0.2–0.5
Intergenicular gaps extending to midrib	absent	absent	sometimes present	present	absent
Basal intergenicula	typically flattened	typically terete	flattened or terete	typically terete	typically flattened

south to at least Monterey Bay, California, and two species, *B. californica* and *B. dichotoma*, confirmed as far south as Baja California Norte, Mexico. Most surprising to us was the confirmed presence of *B. orbigniana* on the northeast Pacific coast (from Los Angeles County, California, USA, to Sitka, Alaska, USA); this antiboreal species was said to be widely distributed in southern Argentina (Boraso & Zaixso 2011) and from central to southernmost Chile (Ramírez & Santelices 1991; Boraso & Zaixso 2011). To our knowledge, along with *B. orbigniana*, only two other red algal species have been confirmed by DNA sequence data to occur in boreal and antiboreal waters of the eastern Pacific Ocean: *Mastocarpus latissimus* (Harvey) S.C. Lindstrom, Hughey & Martone (Lindstrom *et al.* 2011) and *Callophyllis variegata* (Bory de Saint-Vincent) Kützing (Clarkston & Saunders 2013).

Additionally, this is the only red algal species, along with *Mastocarpus latissimus* (Lindstrom *et al.* 2011), where a name, confirmed by sequencing type material, is correctly applied in boreal and antiboreal waters of the eastern Pacific region. More collections need to be assessed using population-level markers to gain insight into these confirmed disjunct distributions. This study, along with Gabrielson *et al.* (2011), Hind and Saunders (2013a, b), and Martone *et al.* (2012), provides much-needed baseline data for ecological and physiological studies of geniculate corallines in the northeast Pacific Ocean related to climate change and ocean acidification.

Taxonomic discussion

Subsamples of the holotype specimen (PC0028660) for *Bossiella californica* were not available for DNA analysis. We applied the name *B. californica* because its morphological characters match the holotype (PC0028660; see Fig. 5) as well as topotype material described and illustrated as *B. californica* ssp. *californica* by Johansen (1971, fig. 1). Specifically, our specimens were found primarily in the intertidal zone, had robust intergenicula that generally lacked a central midrib and displayed a reduced number of conceptacles ($\leq$ 10) per intergeniculum compared with a similar species [i.e. *B. schmittii* (Manza) H.W. Johansen].

We successfully amplified *rbc*L 135 from holotype material of *Bossea corymbifera* (Manza 1940, pl. 13; sequence available in BOLD) and from *Bo. pachyclada* (Taylor 1945, pl. 58; sequence available in BOLD) but were unsuccessful with *Bo. angustata* (Taylor 1945, pl. 59). The *Bo. corymbifera* and *Bo. pachyclada* sequences were identical to each other and differed by 1 bp from field-collected material identified as *B. californica*. When Manza (1937b) first described *Bo. corymbifera*, he compared his new species to *Bo. californica* and noted that the former was shorter in stature (3–5 cm vs 5–12 cm), the intergenicula were smaller in length and width and the conceptacles were fewer in number per intergeniculum (two to four, mostly two vs two to eight, mostly six). Likewise, Taylor (1945, p. 195) compared his new species, *Bo. pachyclada*, with *Bo. californica*, noting that the former had more terete to subterete intergenicula basally, distally the intergenicula were more flattened and the conceptacles were larger and fewer per intergeniculum (two to four). Additionally, Taylor (1945, p. 193) compared *Bo. angustata* to *Bo. orbigniana* and *Bo. gardneri*; whereas, Dawson and Silva (in

Dawson 1953) placed *Bo. angustata* into synonymy under *Bo. pachyclada*, arguing that the primary stipe was coarse and terete like *Bo. pachyclada* and that the secondary proliferous branches of *Bo. angustata* resembled other specimens from the same locality that they considered to belong to *Bo. pachyclada*. Later, Johansen (1971) placed *B. pachyclada* and *Bo. angustata* in synonymy with *B. californica* and suggested that *B. pachyclada* represented a morphological form of *B. californica* that occurs south of Point Conception, California. None of the evidence (molecular or morphological) is conclusive that *Bo. angustata*, *B. corymbifera* and *B. pachyclada* represent the same species as *B. californica*, particularly as we have not sequenced collections south of Monterey County, California, aside from the short and, in this case, inconclusive *rbc*L 135 sequences from the holotypes of *Bo. corymbifera* and *Bo. pachyclada*. However, none of our sequences from contemporary specimens that we field identified as *B. californica* or *B. corymbifera* from Monterey County showed enough variation to warrant recognition of more than one species.

Regarding *Bossiella schmittii*, Manza (1937b) described *Calliarthron schmittii* from fragmentary specimens (Manza 1940, pl. 5) dredged from 38.4–44 m of depth. Manza further explained that this species differed from all others in the genus by having fronds that appeared to be creeping and intergenicula that were convex and dorsiventral with conceptacles restricted to the dorsal surface. Johansen (1969) transferred *C. schmittii* to *Bossiella* as *B. schmittii* and subsequently reduced it to a subspecies of *B. californica* (Johansen 1971, as *B. californica* ssp. *schmittii*).

We confirmed the application of the name *B. schmittii* based on *rbc*L 135 of the holotype (sequence available in BOLD), which was an exact match to field-collected material (although only one sequence direction of the holotype was successful). DNA sequencing of field-collected material confirmed Johansen's placement of this species in *Bossiella*, but the substantial genetic divergence, for all of the gene regions examined and relative to all other *Bossiella* species (Fig. 1), caused us to re-elevate this subspecies to specific rank. Johansen (1971) emphasised that intergenicular thickness was important in differentiating between *B. californica* and *B. californica* subsp. *schmittii*, but we did not find this to be a taxonomically informative character (Table 1). We observed that lengths of intergenicula for *B. californica* were on average shorter (mean = 1.6 mm) than for *B. schmittii* (mean = 2.9 mm), but there was overlap in the ranges (Table 1). Intergenicular length was able to differentiate these species only when values for a specimen were greater than 3 mm, as observed for the holotype of *B. schmittii*. See Table 1 for a summary of the differences between *B. californica* and *B. schmittii*.

Regarding *Bossiella dichotoma*, Manza (1937b) described three new species, *Bossea dichotoma*, *Bo. gardneri* and *Bo. interrupta*, all with central California type localities. Dawson & Silva (in Dawson 1953) suggested that *Bo. gardneri* and *Bo. dichotoma* were conspecific, and subsequently Dawson (1960) reduced *B. gardneri* to varietal status as *B. dichotoma* var. *gardneri*. Johansen (1971) placed all dichotomously branched *Bossiella* specimens into one species, *B. orbigniana*, and recognised two subspecies, '*orbigniana*' and '*dichotoma*', the latter accommodating *B. dichotoma* var. *gardneri*. The two subspecies were distinguished by intergenicular shape – in *B. orbigniana* subsp. *orbigniana*, most intergenicula narrow proximally near to the midrib so that a gap appeared to be present between successive intergenicula; whereas, in *B. orbigniana* subsp. *dichotoma*, intergenicula did not narrow proximally, and no gap appeared to be present between successive intergenicula. For all markers tested in this study, *B. dichotoma* was no more closely related to *B. orbigniana* than it was to any other species of *Bossiella*, with a minimum sequence divergence value of 0.9% in *rbc*L, 0.9% in psbA and 7.0% in COI-5P, comparable to sequence divergence values between other *Bossiella* species.

The *rbc*L 135 was obtained for type specimens of all predominantly or even somewhat dichotomously branched *Bossea* species with type localities in the northeast Pacific area that might have affinities with *B. dichotoma*, all of which had been placed in synonymy with *B. chiloensis* (Decaisne) H.W. Johansen (type locality: Isla Chiloe, Chile) or *B. orbigniana* subsp. *orbigniana* (type locality: Patagonia) by Johansen (1971). These were *Bo. cooperi*, *Bo. gardneri*, *Bo. insularis*, *Bo. interrupta*, *Bo. ligulata* and *Bo. sagittata* as well as *Bo. dichotoma*. The *rbc*L 135 sequences for *Bo. cooperi* (sequence available in BOLD), *Bo. dichotoma* (sequence available in BOLD), and *Bo. gardneri* (sequence available in BOLD) were exact matches to each other and *Bo. insularis* (sequence available in BOLD), and *Bo. ligulata* (sequence available in BOLD), differed by 1 bp, and all of these sequences matched contemporary *rbc*L haplotypes of *B. dichotoma*. *Bossea interrupta* (sequence available in BOLD) and *Bo. sagittata* (sequence available in BOLD) differed by 1–2 bp from the others and did not match haplotypes of contemporary *Bossiella* collections or other type specimen *rbc*L sequences, including *Amphiroa orbigniana* Decaisne (basionym of *B. orbigniana*; see below). A holotype fragment for DNA analysis was not available from *Amphiroa chiloensis* Decaisne [basionym of *Bossiella chiloensis* (Decaisne) H.W. Johansen]. However, specimens in the northeast Pacific that we considered most similar morphologically to the holotype of *B. chiloensis* differed substantially in DNA sequence and morphology from *B. dichotoma*. Although *B. dichotoma* had the highest frequency of single nucleotide polymorphisms (SNPs) in *rbc*L among *Bossiella* species, these SNPs did not rise to the level of recognising distinct species, nor did they correlate with the differences in intergenicular shape recognised by Johansen (1971). Therefore, we included *Bo. interrupta* and *Bo. sagittata* among the synonyms of *B. dichotoma*, but topotype field collections are needed for both of these species to confirm their synonymy.

Regarding *Bossiella orbigniana*, Decaisne (1842), as part of his original description of *Amphiroa* (*Arthrocardia*) *orbigniana*, wrote 'Hab. Patagoniae S. Carlos Chiloensisque littora', and this was repeated by Johansen (1971). In PC there are two sheets, AR 4711 and AR 4712, representing two different collections. AR 4711 has three smaller sheets pinned to it, each with the handwritten label 'Patagonie d'O. n°. 37′ and each bearing at least one individual (Fig. 11). These specimens apparently were collected in Patagonia by Alcide d'Orbigny, where he traveled for the Paris Museum between 1826 and 1833. AR 4712, a single sheet, bore five individuals; a handwritten label that read, 'Coraline. Sur le rivage attacheé a une pierre jetté par la mer. San Carlos de Chiloe, Janvier'; and below that a printed label, 'Herb. Mus. Paris'., on which

was written 'Amphiroa Arthrocardia Orbigniana Dne' and below that 'Chile' and 'M. Gay'. These specimens were apparently collected by a French entomologist living in Chile, Claudius Gay, at San Carlos de Chiloe, the name for the city of Ancud on Chiloe Island, prior to 1834. AR 4711 was labeled 'type' in 1967 by Johansen (unpublished) and 'lectotype' by de Reviers and Woelkerling in 2000 (also unpublished). Although Decaisne's (1842) protologue included 'littora', there is no indication of habitat on AR 4711, and the handwritten label on sheet AR 4712 indicated that this collection was not found attached, an observation that agreed with all reports as well as our own collecting experience in Chile that this species grew subtidally. It is unfortunate that the AR 4711 specimen, with a vague locality (Patagonia) and no habitat data, was designated as the lectotype rather than the AR 4712 specimen with a specific locality, San Carlos de Chiloe, and habitat data.

From several intergenicula of one of the lectotype individuals of *A. orbigniana* (Fig. 11; bottom left individual AR 4711), we sequenced *rbc*L 135 (sequence available in BOLD), which was diagnostic for *B. orbigniana* compared to all other eastern Pacific *Bossiella* species. Over that sequence length, *rbc*L 135 was an exact match to a contemporary specimen from Quintay, Chile (Gabrielson *et al.* 2011), and to sequences generated from North American collections (Table S1). Using Article 9.17 (McNeill *et al.* 2012), we narrow the lectotype to the bottom-left individual on sheet AR 4711 that was sequenced. Individuals on sheet PC AR 4712 are paratypes.

Collins *et al.* (1898; *Phycotheca Boreali-Americana*) were the first to use the name *B. orbigniana* (as *A. orbigniana*) for a northern hemisphere specimen (Collins *et al.* 1898; #398) from La Jolla, California, USA, and this was followed by Manza (1940), Smith (1944, p. 236), Doty (1947) and Dawson & Silva (in Dawson 1960) for material ranging from Oregon, USA, to Baja California Norte, Mexico. We attempted to sequence *rbc*L 296 from 11 of these historical specimens but were successful with only five: two G. M. Smith specimens dredged from Monterey Bay (sequence available in BOLD), *P B-A* (Collins *et al.* 1898; #398), the holotype of *B. cooperi* (sequence available in BOLD) and a Dawson specimen from Cabo Colnett, Baja California Norte, Mexico (UC 940202; sequence available in BOLD). The G. M. Smith specimens were *B. orbigniana*; the other three were *B. dichotoma*.

Despite its historical usage, this is the first unequivocal evidence that *B. orbigniana* is present in the northeast Pacific Ocean and, moreover, disjunctly distributed over 2800 km of coastline. Genetic studies of both northern and southern hemisphere populations are needed to understand this distribution.

Because *Bossiella heteroforma* and *B. orbigniana* were morphologically similar and because *B. orbigniana* and *B. dichotoma* had been synonymised (Johansen 1971), we discuss these three species here. In southern northeast Pacific waters (i.e. California), all three species occurred subtidally, and only *B. dichotoma* occurred intertidally. In northern waters (i.e. Canada), *B. heteroforma* was found subtidally from 12 to 17 m of depth and often in areas with high currents; whereas, *B. orbigniana* typically was found intertidally, where it was common, and in the shallow subtidal [rarely below 10 m except for three out of 39 subtidal specimens collected at 17 m (GWS022331, GWS022333) and 22 m (GWS028200), respectively] (Table 1). Only one subtidal specimen (GWS10528) of *B. dichotoma* was found (6 m) in Canada. *Bossiella heteroforma* appeared to be the least abundant (only nine specimens collected; Table S1).

One ecological observation worth noting was the presence of epiphytes on our collections of *B. heteroforma*. Six of our collections that were genetically identified as *B. heteroforma* had animal epiphytes (bryozoans and tunicates) growing externally on their thalli. At Monterey Shale Beds, California (Table S1), at 17 m of depth, one specimen each of *B. heteroforma* and *B. orbigniana* were collected. The specimen of *B. heteroforma* (GWS022338) was covered in tunicates; whereas, the morphologically similar *B. orbigniana* specimen (GWS022331) did not have animal epiphytes. This suggested that although there are few morphological differences between these two species, there may be biochemical differences that can be detected by marine invertebrates.

The most noticeable morphological feature useful to identify *B. heteroforma* was the mixture of both sagittate intergenicula, where gaps extended to the midrib, and rectangular intergenicula, without gaps (Fig. 12), in one individual. *Bossiella orbigniana*, the species most closely resembling *B. heteroforma*, often had either rectangular or sagittate intergenicula within an individual but not both. When sagittate intergenicula were present in *B. orbigniana*, they usually predominated throughout the entire plant, from the basal intergenicula to the plant apex (Fig. 11, top left specimen). In California it appeared that the sagittate form (with gaps extending to the midrib) was most common; whereas, the rectangular form (without gaps) was more common in the north. The lectotype specimen of *B. orbigniana* had more or less rectangular-shaped intergenicula (Fig. 11, bottom left individual), but our collections of this species displayed a wide range of intergenicular shapes and sizes (Figs 8–10). There appeared to be no correlation with these intergenicular shapes to habitat, depth, substrate preference, or exposure. For *B. dichotoma*, we found that gaps between successive intergenicula rarely occurred; if gaps were present, they were usually near the base of the main axis below the first level of branching.

Basal intergenicula of *B. dichotoma* and *B. heteroforma* were always terete (Figs 7 and 12, respectively); whereas, basal intergenicula of *B. orbigniana* sometimes were flattened (Fig. 9, Table 1). *Bossiella dichotoma* main axial intergenicula were 1 mm or less; whereas, *B. orbigniana* and *B. heteroforma* intergenicula were greater than 1.8 mm (Table 1). Additionally, the shapes of branch tips in *B. dichotoma* were more or less attenuated (Fig. 7); whereas, in *B. orbigniana* branch tips varied in shape (can be rectangular, attenuated, or triangular), and in *B. heteroforma* they were typically narrow and slender (Fig. 12). A midrib was typically present in *B. dichotoma*; however, this trait was variable in both *B. heteroforma* and *B. orbigniana* (Table 1). Conceptacles (one to four) in *B. dichotoma* were typically arranged on either side of the midrib (when present); however, in *B. orbigniana*, conceptacles (one to six) were not regularly arranged (Fig. 9, Table 1). Reproductive specimens of *B. heteroforma* were not observed during this study, and further anatomical observations are warranted. Morphological identifications of these species were difficult, as their overall branching patterns are similar and

variable; however, as detailed above, slight morphological and habitat variations did occur.

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SUPPLEMENTAL DATA

Supplementary data associated with this article can be found online at http://dx.doi.org/10.2216/13–239.1.s1.

REFERENCES

Benson D.A., Karsch-Mizrachi I., Lipman D.J., Ostell J. & Wheeler D.L. 2005. GenBank. *Nucleic Acids Research* 33: D34–D38.

Boraso A. & Zaixso J.M. 2011. Atlas de sensibilidad ambiental de la costa y el Mar Argentino. *Algas marinas bentónicas*: 1–28.

Broom J., Hart D., Farr T., Nelson W., Neill K., Harvey A. & Woelkerling W. 2008. Utility of *psb*A and nSSU for phylogenetic reconstruction in the Corallinales based on New Zealand taxa. *Molecular Phylogenetics and Evolution* 46: 958–973.

Cianciola E.N., Popolizio T.R., Schneider C.W. & Lane C.E. 2010. Using molecular-assisted alpha taxonomy to better understand red algal biodiversity in Bermuda. *Diversity* 2: 946–958.

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: 27–46.

Collins F.S., Holden I. & Setchell W.A. 1898. *Phycotheca Boreali-Americana: A Collection of Dried Specimens of the Algae of North America*, vol. Fasc. 6. Malden, Massachusetts.

Dawson E.Y. 1949. Contributions toward a marine flora of the southern California Channel Islands. I–III. *Occasional Papers of the Allan Hancock Foundation* 8: 1–57.

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

Dawson E.Y. 1960. New records of marine algae from Pacific Mexico and Central America. *Pacific Naturalist* 1: 31–52.

Decaisne J. 1842. Memoire sur les Corallines ou polypiers calciferes. *Annales des Sciences Naturelles; Botanique 2* 18: 96–128.

Doty M. 1947. The marine algae of Oregon. Part II. Rhodophyta. *Farlowia* 3: 159–215.

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

Gabrielson P.W., Miller K. & Martone P. 2011. Morphometric and molecular analyses confirm two distinct species of *Calliarthron* (Corallinales, Rhodophyta) a genus endemic to the northeast Pacific. *Phycologia* 50: 298–316.

Gabrielson P.W., Lindstrom S.C. & O'Kelly C.J. 2012. *Keys to the seaweeds and seagrasses of southeast Alaska, British Columbia, Washington, and Oregon*, vol. 8. PhycoID, Hillsborough, North Carolina. 192 pp.

Guindon S. & Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704.

Hamsher S.E., Evans K.M., Mann D.G., Poulickova A. & Saunders G.W. 2011. Barcoding diatoms: Exploring alternatives to COI-5P. *Protist* 162: 405–422.

Hind K.R. & Saunders G.W. 2013a. 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.

Hind K.R. & Saunders G.W. 2013b. 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: 103–114.

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

Huelsenbeck J.P. & Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

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

Johansen H.W. 1969. *Morphology and systematics of coralline algae with special reference to* Calliarthron. University of California Press, Berkeley. v96 pp.

Johansen H.W. 1971. *Bossiella*, a genus of articulated corallines (Rhodophyceae, Cryptonemiales) in the eastern Pacific. *Phycologia* 10: 381–396.

Johansen W.H. & Colthart B.J. 1975. Variability in articulated coralline algae (Rhodophyta). *Nova Hedwigia* 26: 135–149.

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: 661–683.

Maddison W. & Maddison D. 2003. MacClade 4.06. Sinauer Associates, Sunderland, Massachusetts.

Manza A.V. 1937a. The genera of the articulated corallines. *Proceedings of the National Academy of Sciences of the United States of America* 23: 44–48.

Manza A.V. 1937b. Some north Pacific species of articulated corallines. *Proceedings of the National Academy of Sciences of the United States of America* 23: 561–567.

Manza A.V. 1940. A revision of the genera of articulated corallines. *Philippine Journal of Science* 71: 239–316.

Martone P.T., Lindstrom S.C., Miller K.A. & Gabrielson P.W. 2012. *Chiharaea* and *Yamadaia* (Corallinales, Rhodophyta) represent reduced and recently derived articulated corallline morphologies. *Journal of Phycology* 48: 859–868.

McNeill J.C., Barrie F.R., Buck W.R., Demoulin V., Greuter W., Hawksworth D.L., Herendeen P.S., Knapp S., Marhold K., Prado J., Prud'homme Van Reine W.F., Smith G.F., Wiersema J.H. & Turland N.J.S. 2012. *International code of nomenclature for algae, fungi, and plants (Melbourne code) adopted by the eighteenth International Botanical Congress Melbourne, Australia, July 2011*. Koeltz Scientific Books, Koenigstein, Germany. 208 pp.

Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256.

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.

Ratnasingham S. & Hebert P. 2007. BOLD: the Barcode of Life Data System. *Molecular Ecology Notes* 7: 355–364.

Reichenbach H.G.L. 1841. *Der deutsche Botaniker, Erster Band. Das herbarienbuch*. Dresden, Germany. 240 pp.

Saunders G.W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* 360: 1879–1888.

Saunders G.W. 2008. A DNA barcode examination of the red algal family Dumontiaceae in Canadian waters reveals substantial cryptic species diversity. 1. The foliose *Dilsea-Neodilsea* complex and *Weeksia*. *Botany-Botanique* 86: 773–789.

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.

Schneider C.W. & Wynne M.J. 2007. A synoptic review of the classification of red algal genera a half century after Kylin's 'Die Gattungen der Rhodophyceen'. *Botanica Marina* 50: 197–249.

Setchell W.A. & Gardner N.L. 1903. *Algae of northwestern America*, vol. 1. 500 pp.

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

Smith G.M. 1944. *Marine algae of the Monterey Pennisula*. Stanford University Press, Stanford, California. 622 pp.

Taylor W.R. 1945. *Pacific marine algae of the Allan Hancock Expeditions to the Galapagos Islands*, vol. 12. University of Southern California Press, Los Angeles. 528 pp.

Wheeler Q.D. 2004. Taxonomic triage and the poverty of phylogeny. *Philosophical Transactions of the Royal Society, Series B: Biological Sciences* 359: 571–583.

Woelkerling W.J., Millar A.J.K., Harvey, A. & Baba M. 2008. Recognition of *Pachyarthron* and *Bossiella* as distinct genera in the Corallinaceae, subfamily Corallinoideae (Corallinales, Rhodophyta). *Phycologia* 47: 265–293.

Wynne M.J. & Schneider C.R. 2007. A synoptic review of the classification of red algal genera a half century after Kylin's 'Die Gattungen der Rhodophyceen'. *Botanica Marina* 50: 197–249.

Yendo K. 1902. Coralline verae of Port Renfrew. *Minnesota Botanical Studies* 2: 711–722.

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