

CHIHARAEA AND YAMADAIA (CORALLINALES, RHODOPHYTA) REPRESENT REDUCED AND RECENTLY DERIVED ARTICULATED CORALLINE MORPHOLOGIES¹

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Phycologists have hypothesized that the diminutive fronds produced by species in the genera *Chiharaea* and *Yamadaia*, which are composed of comparatively few genicula and intergenicula, represent morphological intermediates in the evolution of articulated corallines from crustose ancestors. We test this “intermediate frond hypothesis” by comparing *rbcL* sequences from the generitype species *Chiharaea bodegensis* and *Yamadaia melobesioides* to sequences from other coralline genera. We demonstrate that *Chiharaea* includes two other NE Pacific species, *Arthrocardia silvae* and *Yamadaia americana*. *Chiharaea* species are characterized morphologically by inflated intergenicula and axial conceptacles with apical or acentric pores. Although relationships among the three species are unresolved, *Chiharaea bodegensis*, *C. americana* comb. nov., and *C. silvae* comb. nov. are distinguished from one another by DNA sequences, morphology, habitat, and biogeography. *Chiharaea* occurs together with *Alatocladia*, *Bossiella*, *Calliarthron*, and *Serraticardia macmillanii* in a strongly supported clade of nearly endemic north Pacific articulated coralline genera and species that have evolved relatively recently compared to other articulated corallines. In contrast, NW Pacific *Yamadaia melobesioides* belongs in a clade with *Corallina officinalis*, the generitype species of *Corallina*, and therefore we reduce *Yamadaia* to a synonym of *Corallina* and propose *Corallina melobesioides* comb. nov. We reject the ‘intermediate frond hypothesis’ and conclude that *Chiharaea* and *Yamadaia* are recently derived taxa that evolved from articulated coralline ancestors and represent a reduction in the number of genicula and intergenicula.

Key index words: *Arthrocardia*; *Chiharaea*; *Chiharaea americana* comb. nov.; *Chiharaea bodegensis*; *Chiharaea silvae* comb. nov.; *Corallina*

melobesioides comb. nov.; Corallinales; intermediate frond hypothesis; NE Pacific; *rbcL*; *Yamadaia*

Abbreviations: AK, Alaska; BC, British Columbia; CA, California; WA, Washington; NE, northeast; NW, northwest

The evolution of calcifying coralline algae (Corallinales and Sporolithales, Rhodophyta) has long been the subject of research because of the geological significance of biogenic limestone deposits (Johnson 1961, Wray 1977, Bosence 1991), the richness and depth of the coralline fossil record (e.g., Aguirre et al. 2000, 2010), the interplay between animal and coralline evolution (Steneck 1983), and the ecological importance of corallines in modern marine communities (Nelson 2009). According to the fossil record, calcifying macroalgae may have existed as early as the Cambrian (Wray 1977, Brooke and Riding 1998, Riding et al. 1998), and according to molecular clock and fossil data, ancestors to modern-day corallines (Corallinales and Sporolithales) likely emerged in the early Cretaceous, ~120–140 million years ago (mya; Aguirre et al. 2000, 2010). The fossil record further suggests that crustose coralline algae preceded articulated coralline algae, an evolutionary transition marked by the sudden appearance of calcified “intergenicular” segments less than 100 mya (Johnson 1961, Wray 1977, Mude and Kundal 2010). The hypothesis that articulated corallines evolved from crustose corallines has been consistently supported by DNA sequence analyses (Bailey and Chapman 1998, Broom et al. 2008, Aguirre et al. 2010, Gabrielson et al. 2011). These molecular results bolster early conclusions by Adey and Johansen (1972) and Cabioch (1972) based on “genicular” structure and development that articulated fronds arose from crustose ancestors at least three times, giving rise to the subfamilies Corallinoideae and Metagoniolithoideae and the tribe Amphiroideae (subfamily Lithophylloideae).

¹Received 26 June 2011. Accepted 16 December 2011.

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Given this evolutionary trajectory, previous researchers hypothesized that *Chiharaea* H. W. Johansen and *Yamadaia* Segawa, which produce extensive crusts with few diminutive upright fronds (1–6 tiers of intergenicula), may represent intermediate morphologies along the crust-to-upright continuum. For example, Johansen (1969) thought that *Yamadaia* (as *Yamadaea*) could have evolved from a bumpy crust through the development of a single geniculum subtending each excrescence; he positioned *Yamadaia* as phylogenetically ancestral to all other articulated Corallinoideae (see fig. 32 in Johansen 1969). Likewise, he thought that *Chiharaea* represented an ancestral genus due to its short fronds (fewer than seven tiers of intergenicula), but noted that the eccentric conceptacles in *Chiharaea* appeared to be a derived feature, obscuring phylogenetic placement of the genus (Johansen 1969). Cabioch (1971) echoed Johansen's hypothesis noting that *Chiharaea* and *Yamadaia* (as *Yamadaea*) were "morphologies of intermediate complexity," possibly representing the crust-to-upright transition within Corallinoideae. This 'intermediate frond hypothesis' is illustrated (Fig. 1) by adding *Chiharaea* and *Yamadaia* to a recently published coralline phylogeny (Gabrielson et al. 2011), according to the logic of Johansen (1969) and Cabioch (1971): crustose corallines (Step 1) gave rise to geniculate axes (Step 2) that ultimately led to erect, geniculate fronds composed of numerous intergenicula (Step 3).

Our approach to studying corallines is to unequivocally link name-bringing type specimens to field-collected material using a combination of DNA sequences, morphology, and biogeography

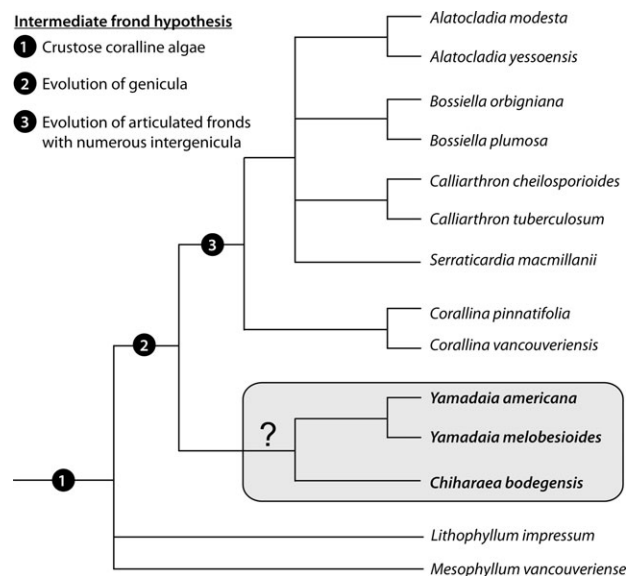


FIG. 1. Depiction of the "intermediate frond hypothesis" posed by Johansen (1969) and Cabioch (1971): (i) Crustose coralline algae gave rise to (2) geniculate axes and ultimately (3) erect, articulated fronds with numerous intergenicula. Diminutive axes produced by *Chiharaea* and *Yamadaia* species (shaded box) were thought to represent intermediates in the evolution of upright fronds from crustose corallinoid ancestors.

(Gabrielson et al. 2011). In this study, we: (i) determine the phylogenetic disposition of *Chiharaea* within the Corallinoideae, (ii) show that two other NE Pacific endemic species, *Yamadaia americana* E. Y. Dawson & R. L. Steele and *Arthrocardia silvae* H. W. Johansen also belong in *Chiharaea*, (iii) demonstrate that the generic type of *Yamadaia*, *Y. melobesioides* Segawa, belongs in *Corallina* and (iv) present evidence that *Chiharaea bodegensis*, *Yamadaia americana* and *Yamadaia melobesioides* are not intermediates between crustose and articulated coralline morphologies, but rather are derived taxa with diminutive upright fronds, representing a reduction in the number genicula and intergenicula and a concomitant reduction in erect stature.

MATERIALS AND METHODS

Specimens. Specimens for sequencing and morphological examination were field-collected, and most were desiccated in silica gel (Table S1 in the supplementary material). Voucher specimens were deposited in University of British Columbia (UBC), University of California at Berkeley (UC), and University of North Carolina at Chapel Hill (NCU – herbarium abbreviations follow Thiers 2011). Additional specimens from these herbaria were examined, but not sequenced; these are listed in Table S2 in the supplementary material. Habit photographs of specimens were taken with a light stand using a Canon Powershot A95 digital camera, a Prog.Res.3 digital camera, or an Olympus SZ61 dissecting microscope with a DP20 camera.

Molecular analysis. Specimen preparation, extraction, amplification, and sequencing followed Gabrielson et al. (2011). Nineteen rbcL sequences from the family Corallinoideae were analyzed with *Mesophyllum vancouveriense* (Foslie) Steneck et R. T. Paine from the sister family Hapalidaceae (Broom et al. 2008) serving as the outgroup (Table S1, Fig. 2). Models of sequence evolution and characteristics of the data set were obtained using jModelTest v. 0.1.1 (Posada 2008) and PAUP* v. 4 (Swofford 2003), and the phylogenetic analysis was performed using PAUP*.

A maximum likelihood (ML) analysis was performed using a GTR + I + G model of evolution and the following parameters were obtained from the Modeltest program: base frequencies (A = 0.3315, C = 0.1393, G = 0.1977, T = 0.3315), Nst = 6, rate matrix = (2.3591, 7.3347, 2.9966, 0.2681, 20.5319), gamma distribution shape (1.4411), and proportion of invariable sites (0.5798). Ten separate searches with random sequence additions using the tree bisection–reconnection (TBR) branch swapping algorithm were completed. The ML analysis had 250 replications of bootstrap resampling. Parsimony analysis (MP) was a branch and bound search of 1,000 random sequence additions with TBR and MULTREES branch swapping options used. Parsimony bootstrap values were determined based on analyses of 1,000 replications of simple sequence additions.

RESULTS

Molecular results. We analyzed an alignment of 1401 bp, of which 453 sites were variable (32%) and 344 sites were parsimony informative. Relationships among taxa were similar in both the ML and MP analyses; therefore, only the ML topology with bootstrap results from both methods is presented (Fig. 2). Three species occurred on the strongly supported branch that included the type species of

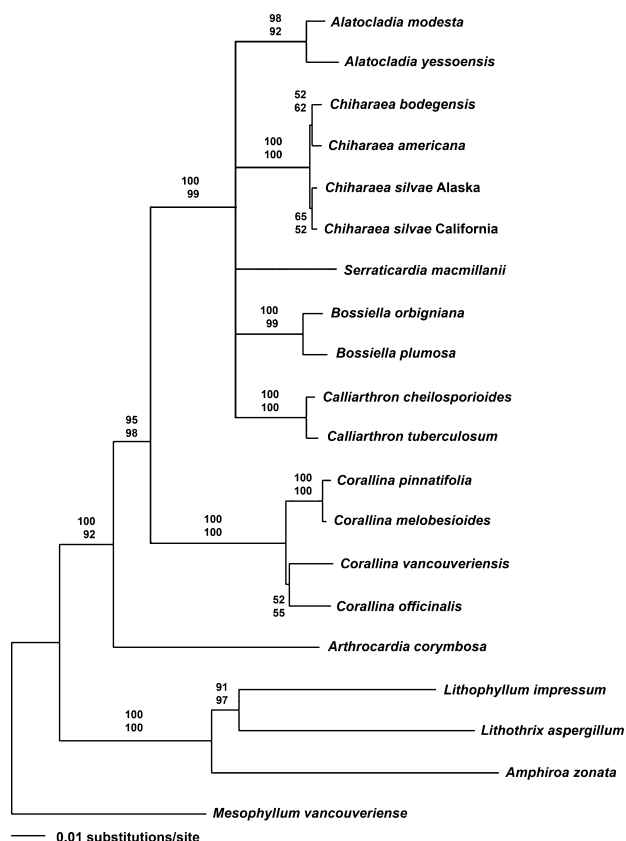


FIG. 2. Maximum likelihood tree inferred from *rbcL* sequences. At nodes, upper numbers are bootstrap values for maximum parsimony analysis; lower numbers are for maximum likelihood analysis.

Chiharaea, *C. bodegensis* (Fig. 2). Below we discuss each of these species, followed by notes on the genera in which two of these species were described.

Chiharaea bodegensis. We obtained three 1401 bp *rbcL* sequences from field-collected material identified as *C. bodegensis*: from the topotype locality of Bodega Head, Sonoma Co., CA (UC 1944755) from Tatoosh Island, WA (NCU 588182), and from Kyuquot, Vancouver Island, BC (UBC A88634) and two 668 bp sequences from Moss Beach, San Mateo Co., CA (UC 1966659) and Pacific Grove, Monterey Co., CA (NCU 593275, Table S1). All of these sequences were identical over aligned sites.

Yamadaia americana. We obtained a 1401 bp *rbcL* sequence from field-collected material identified as *Y. americana* from Point George, Shaw Island, WA (NCU 590280) and a 316 bp sequence from a herbarium sample identified as *Y. melobesioides* from Monterey Bay, CA (UC 1468482, Table S1). Both of these sequences were identical over aligned sites. The Point George specimen from near the holotype locality of *Y. americana* is in a clade with *C. bodegensis*, the generitype of *Chiharaea*.

Arthrocardia silvae. We obtained four 1401 bp *rbcL* sequences from field-collected material variously identified as *A. silvae* or *Corallina* spp. These came

from Attu Island, AK (NCU 586655), Cape Kaguyak, AK (NCU 586657), Port Renfrew (Botany Beach), Vancouver Island, BC (NCU 590266) and Bodega Head, Sonoma Co., CA (UC 1966669). Two 702 bp *rbcL* sequences were obtained from field-collected specimens at Sitka, AK (UBC A88800) and Malcolm Island, BC (UBC 88796, Table S1). All of the material north of CA, from southern BC to the westernmost Aleutian Island had identical sequences over aligned sites; the Bodega Head specimen differed by 4 bp (0.28%). All sequences fell into a clade with *C. bodegensis*, the generitype of *Chiharaea* (Fig. 2), and not with material identified as *Arthrocardia corymbosa* (Lamarck) Decaisne from South Africa, the generitype of *Arthrocardia* Decaisne (see below).

Yamadaia melobesioides. We obtained a 1401 bp *rbcL* sequence from a herbarium specimen identified as *Y. melobesioides* from Chiba Prefecture, Japan collected by T. Masaki (UBC A88595). This collection was made on the Boso Peninsula, ~100 km east of the type locality on the Izu Peninsula; the species has been recorded only from these two peninsulas, to the east and west of Tokyo Bay, respectively (Baba 2000). This specimen is in a clade with *Corallina* spp. and is not related to *Y. americana* or to *Chiharaea* (Fig. 2).

Arthrocardia corymbosa. We obtained a 1401 bp *rbcL* sequence from a specimen we identified as *A. corymbosa* from South Africa (NCU 593142). This specimen was collected ~800 km east of the presumed type locality of the Cape of Good Hope, South Africa. It is in a clade sister to *Corallina* and is not related to *Chiharaea* (Fig. 2).

Morphological Results and Taxonomy.

Chiharaea bodegensis H. W. Johansen (1966: 51–61, Figs. 1–11).

Holotypus: UC 1318216, California, Sonoma Co., Bodega Head, 80 m south of Horseshoe Cove, 29.xi.1963, *leg.* H. W. Johansen.

Morphology. Plants are epilithic with an extensive basal crust from which arise scattered to densely packed recumbent uprights branched to two or three orders (Fig. 3a) and fewer than six intergenicula in length (Fig. 3d). Erect axes commonly appear as rosettes when young (Fig. 3, b and c), but usually only one of the first order of intergenicula forms branches. Vegetative intergenicula are compressed when young (Fig. 3b) and inflated when mature (Fig. 3, c and d). Conceptacles are immersed in terminal intergenicula, causing them to appear swollen; most have superficially displaced acentric pores (Fig. 3d).

Chiharaea americana (E. Y. Dawson et R. L. Steele) Martone, S. C. Lindstrom, K. A. Miller et P. W. Gabrielson **comb. nov.**

Basionym: *Yamadaia americana* E. Y. Dawson et R. L. Steele, *Nova Hedwigia*, 8: 1–3, pls. 1–2 (1964).

Holotypus: UC 1885061/AHFH 75382 (in UC), Washington, Strait of Juan de Fuca, Hein Bank, 13.vii.1963, 10–12 m depth on cobble, *leg.* R. L. Steele.

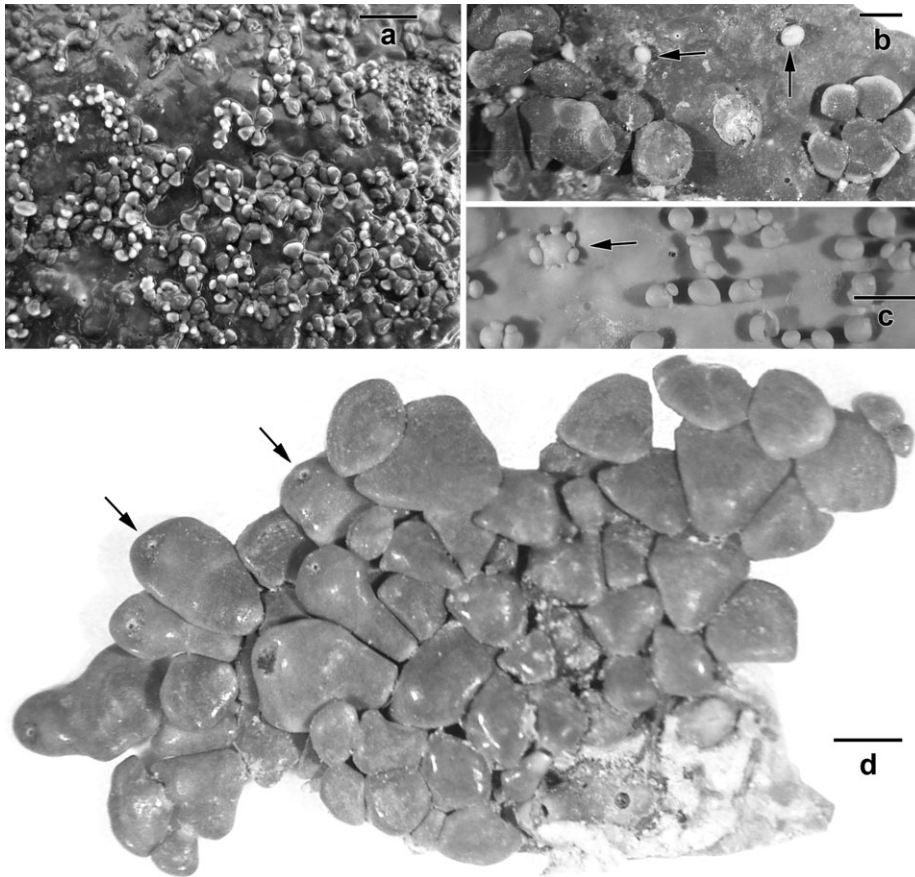


FIG. 3. *Chiharaea bodegensis*. (a) UC 1944755. Habitat of topotype material from Bodega Bay, Sonoma Co., CA showing extensive crustose base with numerous uprights. Scale bar = 1 cm (b) UBC A88634. Branched uprights on left and right with two white-tipped developing intergenicula (arrows). Right branched upright with conspicuous rosette pattern of intergenicula. Scale bar = 1 mm (c) UBC A66936. Inflated, upright intergeniculum characteristic of some specimens. Basal intergeniculum of uprights bearing 1–5 branches, one showing rosette pattern (arrow) seen in (b). Scale bar = 700 μ m (d) UBC A88648. Fertile, branched upright bearing on some branches terminal conceptacles, each with a conspicuous acentric pore (arrows). Scale bar = 1 mm.

Morphology. Plants are mostly epilithic and with an extensive crustose base commonly with a distinct white, scalloped margin (Fig. 4, a and c) and with or without sporadic scattered uprights (Fig. 4, a–c). Upright axes are composed of 1–3 unbranched (Fig. 4, b–d) to very rarely once branched intergenicula. Immature upright axes are terete to apically inflated with white, flat-topped rounded to truncate apices (Fig. 4, b and c), whereas mature upright axes have terminal, spindle-shaped and inflated intergenicula when reproductive (Fig. 4, c and d). Each immersed conceptacle has a single pore that is apical (Fig. 4c) or acentric (Fig. 4d).

Chiharaea silvae (H. W. Johansen) Martone, S. C. Lindstrom, K. A. Miller et P. W. Gabrielson **comb. nov.**

Basionym: *Arthrocardia silvae* H. W. Johansen, *Phycologia* 10: 241–243, figs. 1–3 (1971).

Holotypus: UC 1499979, California, Mendocino Co., mouth of Jackass Creek, 11.vi.1968, on tops of rocks mid- and low intertidal, *leg.* P. C. Silva, H. W. Johansen & A. Q. Howard.

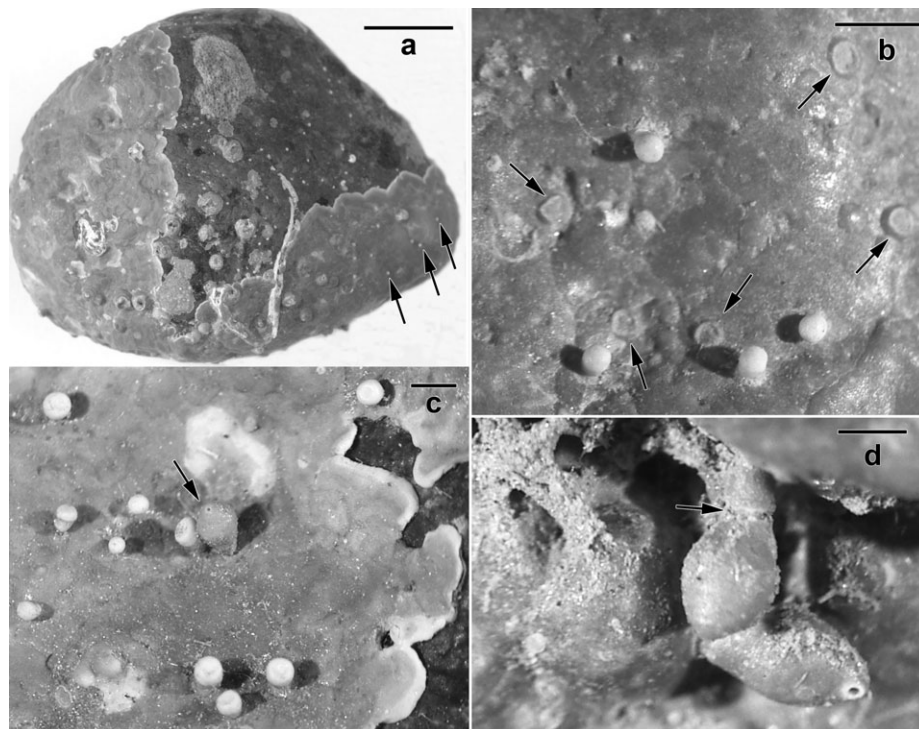
Morphology. Plants are epilithic and commonly found singly or in small clusters or occasionally form a dense sward (Fig. 5a). Upright axes are erect to recumbent, 10 or more intergenicula tall and branched to two orders, arising from an indistinct crustose base or a crustose base appears to be

entirely lacking (Fig. 5b). Branching is opposite or alternate, mostly distichous, and second order branches typically have only a single intergeniculum (Fig. 5b). Intergenicula are somewhat inflated throughout but especially at branch apices. Apical intergenicula are characteristically rounded to truncate (Fig. 5b). Fertile intergenicula are usually terminal, but occasionally bear short branches in the same positions as vegetative intergenicula (Fig. 5c). Conceptacles are immersed in the middle of these intergenicula, causing them to appear swollen (Fig. 5, c–e). Pores of conceptacles are apical or distinctly acentric, displaced superficially (Fig. 5, c–e).

***Chiharaea* revised generic diagnosis.** When Johansen (1966) described *Chiharaea* and *C. bodegensis*, he provided a combined generic and species diagnosis. With the addition of two more species to the genus, it is appropriate to provide a revised generic diagnosis.

Plants epilithic with a smooth (lacking excrescences), completely adherent, monomerous crustose base that may be expansive to 20 cm diameter and bearing numerous to only a few uprights or so reduced that the crust is difficult to discern and only upright axes are evident; erect to recumbent geniculate axes, when present, ranging from one to 20+ intergenicula tall, unbranched or branched to two or three orders; morphology of intergenicula typically inflated when mature, may appear terete or

FIG. 4. *Chiharaea americana* (*Yamadaia americana*). (a) NCU 590280. Cobble nearly covered by crustose base with white margin and very inconspicuous uprights (arrows). Scale bar = 1 cm (b, c and d) Scale bar = 1mm (b) UBC A81318. Four developing white-tipped uprights; scars (arrows) on crustose base where upright axes were present. (c) UBC A62465. Basal crust with white margin, developing uprights showing rounded, white-tipped intergenicula, and single fertile intergeniculum (arrow) with apical conceptacle pore. (d) UBC A81318. Two fertile uprights from basal crust, one with one intergeniculum with acentric conceptacle pore; other upright with two intergenicula (arrow indicates geniculum between two intergenicula of upright).



compressed when young; genicula unizonal (i.e., composed of a single tier of uncalcified cells); intergenicula multizonal; conceptacles all axial and immersed, present only on erect intergenicula; conceptacles axial with pores apical or acentric; carposporangial filaments arising from fusion cell anywhere on its upper surface.

Chiharaea species key.

1. Crustose base barely apparent to absent; upright axes with >8 tiers of branched intergenicula; mid-low intertidal ... *C. silvae*.
1. Crustose base expansive; uprights, if present, with 1–7 tiers of unbranched or branched intergenicula; low intertidal to subtidal ... 2.
2. Upright axes with 1–3 tiers of unbranched or very rarely branched intergenicula; subtidal (5–25 m) ... *C. americana*.
2. Upright axes with up to 7 tiers of branched intergenicula; low intertidal to shallow subtidal (4 m) ... *C. bodegensis*.

DISCUSSION

Generic relationships of Chiharaea, Arthrocardia and Yamadaia. For the first time, *Chiharaea*, *Yamadaia* and *Arthrocardia* are included in a single molecular analysis, and all are represented by *rbcL* sequences from generitype species. All are members of the subfamily Corallinoideae, as has previously been shown by an *rbcL* gene sequence of the generitype species of *Chiharaea* (Gabrielson et al. 2011) and an 18S rRNA gene sequence of a non-generitype

species of *Arthrocardia* (Bailey and Chapman 1996). No previously published DNA analysis included the genus *Yamadaia*.

DNA sequence data support recognition of *Chiharaea* as a distinct genus (Fig. 2). *Chiharaea* occurs in a clade with very strong bootstrap support (ML 99%; MP 100%) with other mainly NE and NW Pacific coralline endemics, including *Alatocladia* (Yendo) H. W. Johansen, *Calliarthron* Manza, *Serraticardia* (Yendo) P. C. Silva and *Bossiella* P. C. Silva. It should be noted that our molecular phylogenetic understanding of *Serraticardia* is based on the *rbcL* sequence of *S. macmillanii* (Yendo) P. C. Silva, not the generitype, *S. maxima* (Yendo) P. C. Silva.

Arthrocardia is supported as a distinct genus (Fig. 2), and this conclusion is consistent with previous studies using 18S rRNA (Bailey and Chapman 1996) and *psbA* gene sequences (Broom et al. 2008). *Arthrocardia* is sister to all other genera of tribe Corallineae, a conclusion that is strongly supported by both *rbcL* (Fig. 2, ML 92%, MP 100% bootstrap support) and 18S rRNA gene sequence analyses (Bailey and Chapman 1996, 1998, Bailey et al. 2004). In a Bayesian analysis of *psbA* gene sequences from New Zealand corallines, Broom et al. (2008, fig. 2) concluded that unnamed *Arthrocardia* species were sister to *Corallina*, but no broader conclusions were drawn because other genera of the tribe Corallineae were not analyzed.

In contrast, *Yamadaia* is not supported as a distinct genus, based on an *rbcL* gene sequence of the generitype species, *Y. melobesioides*. It is found in a clade with NW Atlantic *Corallina officinalis*

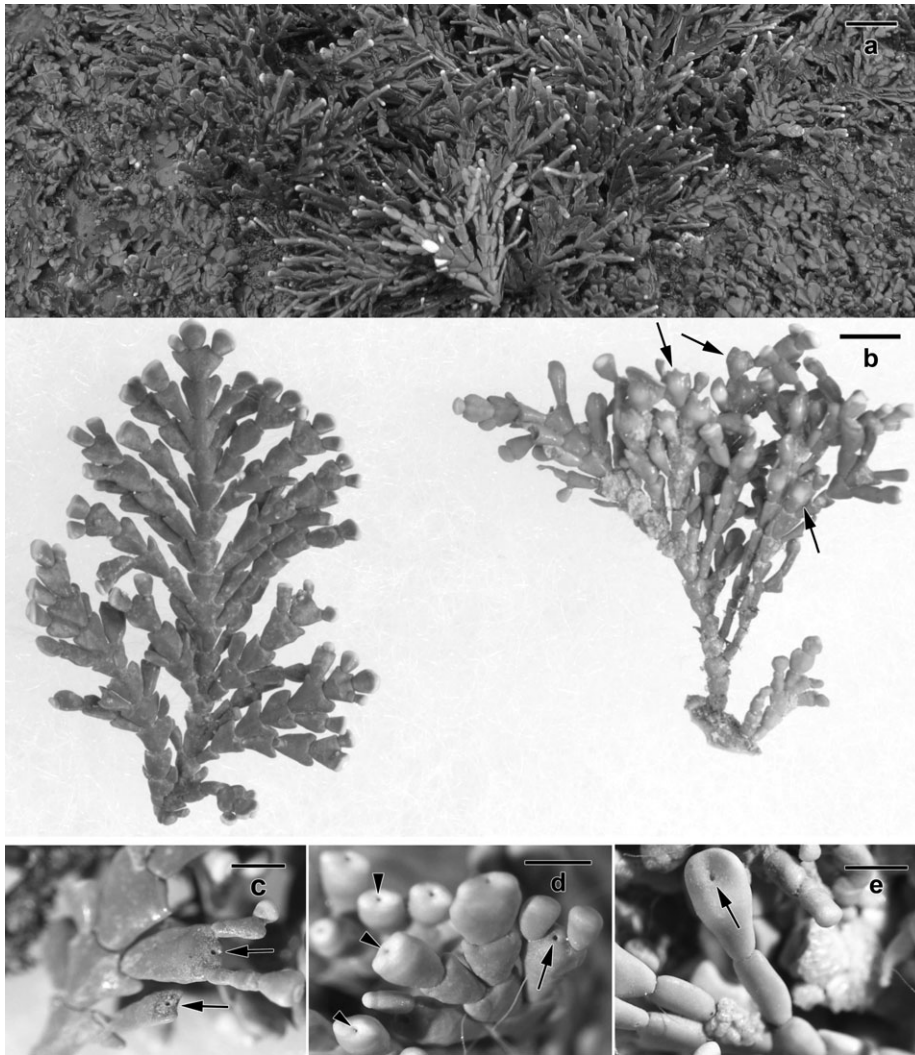


FIG. 5. *Chiharaea silvae* (*Arthrocardia silvae*). (a) UBC A88796. *In situ* habit showing dense sward of thalli. Thallus in bottom middle is rotated so lightly pigmented underside is shown. Scale bar = 1 cm (b) NCU 590266. Left upright with alternate or opposite distichous branches; right upright with small crustose basal portion and fertile, swollen intergenicula (arrows). Scale bar = 4 mm (c, d and e) Scale bar = 1 cm (c) Two fertile intergenicula with acentric conceptacle pores (arrows); upper fertile intergeniculum is intercalary and bears two branches. (d) Rightmost fertile intergeniculum intercalary and with acentric conceptacle pore (arrow); other fertile intergenicula terminal, each with an apical conceptacle pore (arrowheads). (e) Fertile intergeniculum with conspicuous acentric conceptacle pore (arrow).

L. sensu Walker et al. (2009) and NE Pacific *C. pinnatifolia* (Manza) E. Y. Dawson and *C. vancouveriensis* Yendo (Fig. 2; The names of the latter two have been verified by sequencing type material [P.W. Gabrielson, personal communication]). This clade has very strong bootstrap support (MP and ML 100%). Type material of *C. officinalis*, the generitype of *Corallina*, has not been sequenced and the application of this name to specimens sequenced from England is based solely on morphology (Walker et al. 2009). Nevertheless, based on our current understanding of *Corallina*, *Yamadaia* belongs in this clade and thus must be considered a synonym of *Corallina*. Herein, we transfer *Y. melobesioides* to *Corallina*, proposing the new combination ***Corallina melobesioides*** (Segawa) Martone, S. C. Lindstrom, K. A. Miller et P. W. Gabrielson **comb. nov.** (Basionym: *Yamadaia melobesioides* Segawa, *Botanical Magazine Tokyo* 68: 241–247, figs. 1–6 [1955]). We note that Segawa (1955), in his original description of *Yamadaia*, suggested that the genus was related most closely

to *Corallina* based on both reproductive and anatomical characters.

Rejecting the intermediate frond hypothesis. Contrary to the hypothesis posed by Johansen (1969) and Cabiocch (1971), our data show that *Chiharaea bodegensis*, *C. americana* and *Corallina* (*Yamadaia*) *melobesioides* are not intermediate forms in the evolution of the subfamily Corallinoideae. Rather, all of these diminutive coralline taxa likely evolved from upright articulated ancestors. This conclusion is bolstered by the apparent evolution of this lineage first from a common ancestor with the articulated genus *Arthrocardia* and then from a common ancestor with the articulated genus *Corallina* (Fig. 2). It is most parsimonious to assume that these common ancestors also were articulated corallines, otherwise *Arthrocardia*, *Corallina*, and other corallinoids would have evolved independently from crusts. Thus, we conclude that *Chiharaea bodegensis*, *C. americana* and *Corallina* (*Yamadaia*) *melobesioides* are derived taxa, whose morphological evolution is characterized by a partial loss of genicula and intergenicula and thus a reduction of upright stature.

The potential for loss of genicula over the course of coralline evolution was first proposed by Johansen (1969, fig. 33) in reference to conceptacle evolution. He suggested that lateral conceptacles (e.g., in *Bossiella* and *Calliarthron* species) could have evolved from axial conceptacles (e.g., in *Corallina* species) by a loss of genicula subtending reproductive apical intergenicula (see Johansen 1969, fig. 32). Here, we apply this concept more broadly, suggesting that genicular loss may also lead to a reduction of upright fronds. Moreover, data presented here show that a loss of genicula occurred independently in the genus *Chiharaea* and in *Corallina* (*Yamadaia*) *melobesioides* and that the morphological similarity of upright fronds produced by *Chiharaea americana* and *Corallina* (*Yamadaia*) *melobesioides* represents convergent evolution. This repeated loss of genicula and intergenicula is intriguing and suggests that a complete return to a crustose morphology – a complete evolutionary reversal – might be possible.

Chiharaea. With the transfer of *Arthrocardia silvae* and *Yamadaia americana* to *Chiharaea*, the formerly monotypic genus now contains three species, all with very to somewhat reduced numbers of intergenicula compared to most other species of articulated corallines. Like Johansen (1966), however, we have had difficulty identifying a suite of morphological and/or anatomical characters that uniquely characterize *Chiharaea*. Gabrielson et al. (2011) reviewed the history of using conceptacle position or conceptacle origin to distinguish genera of articulated corallines, particularly with respect to *Calliarthron* and *Alatocladia*, and they concluded that this character alone was insufficient to distinguish genera. The combination of a reproductive character, namely the presence (in *Alatocladia*) or absence (in *Calliarthron*) of sterile paraphyses in tetrasporangial conceptacles, and a shared vegetative character, intergenicula with intertwined medullary filaments, uniquely defined these genera and distinguished them from other Corallinoideae; these observations are supported by DNA-based phylogenies.

Herein, we characterize *Chiharaea* by a vegetative character (the morphology of intergenicula) and a reproductive character (location of the conceptacle pore). All three species have intergenicula that can best be characterized as inflated when mature and terete or compressed when immature. This is most evident in intergenicula of *C. americana* and in the distal intergenicula of *C. silvae*, but even the more laterally expanded intergenicula of *C. bodegensis* are inflated and not compressed or winged compared to intergenicula in *Alatocladia*, *Calliarthron*, *Serraticardia* and most species of *Bossiella*.

All three *Chiharaea* species are characterized by conceptacles with either apical or acentric pores. (Johansen [1966] used the term eccentric to describe conceptacle pores not aligned with the longitudinal axis of an intergeniculum, but we

prefer the term acentric, meaning off-center.) Indeed, Johansen (1966) used this character (as “eccentric”) to distinguish his new genus *Chiharaea* from *Yamadaia*, but later recognized the presence of both apical and acentric conceptacle pores in *C. americana* (as *Y. americana*) when compared to strictly apical pores in *Y. melobesioides*, the generitype of *Yamadaia* (Garbary et al. 1981). When he described *Arthrocardia silvae*, Johansen (1971) compared his species to *Arthrocardia duthieae* H. W. Johansen from South Africa. That species originally was described as a monotypic genus, *Duthiea setchellii* Manza (1937: 48), based solely on, in Manza’s words, “Conceptacular pores slightly lateral (slightly below the apices)”. (Tandy [1938] proposed *Duthiophycus* to replace *Duthiea* Manza, a name already used for a genus of a grass.) Johansen (1969), however, regarded this character by itself to be insufficient to elevate a species to generic rank, noting that *Alatocladia yessoensis* (Yendo) P. W. Gabrielson, K. A. Miller et Martone [as *Calliarthron yessoense* (Yendo) Manza] also had conceptacles with acentric pores. He reduced *Duthiophycus* to a synonym of *Arthrocardia*. Thus, the location of the conceptacle pore had been recognized by Manza for *Arthrocardia* (as *Duthiea*), but its application to *Chiharaea* has been made evident only by DNA sequence data.

Chiharaea species. Molecular, morphological, biogeographic, and habitat data support recognition of three *Chiharaea* species: *C. bodegensis*, *C. americana* and *C. silvae*. They differ from one another by seven or eight base pairs (of 1401 analyzed) in *rbcL* sequences (0.5%–0.57% divergence), similar to the sequence divergence value of 0.5% for the two species of *Calliarthron*, another NE Pacific genus of Corallinoideae (Gabrielson et al. 2011). Both MP and ML analyses gave only weak bootstrap support, 54 and 60%, respectively, for grouping *C. bodegensis* and *C. americana* as sister species separate from *C. silvae* (Fig. 2). These two species share the following characters: (i) an extensive crustose base, (ii) uprights with fewer than eight tiers of intergenicula and (iii) one to several conceptacles per intergeniculum. Likewise, bootstrap support for recognizing more than one species within *C. silvae* was weak in both MP and ML analyses, 65 and 52%, respectively (Fig. 2).

Each of these species is morphologically distinct – *C. bodegensis* recumbent and branched to two or three orders, but with fewer than eight tiers of intergenicula, *C. americana* erect and mostly unbranched with only one to three tiers of intergenicula, and *C. silvae* erect to recumbent, branched to two to three orders and with 10–25 tiers of intergenicula. *Chiharaea bodegensis* is found in the low intertidal where it is frequently covered by other foliose algae or surfgrass or rarely in the shallow subtidal; *C. americana* is subtidal occurring at depths of 5–25 m; *C. silvae* occurs in the mid- to low intertidal. *Chiharaea bodegensis* has the narrowest range from

Price Island, BC south to Monterey Co., CA (Fig. 6). *Chiharaea americana* occurs from the Queen Charlotte Islands, BC, south to Monterey Co., CA, with a recent report (Aguilar-Rosas and Pacheco-Ruiz 1995) from Baja California, Mexico (Fig. 6). *Chiharaea silvae* has the widest distribution from the westernmost of the Aleutian Islands, Attu Island, eastward to the Gulf of Alaska and south to Monterey Co., CA. (Fig. 6). See Tables S1 and S2 for documented specimens of *Chiharaea* species.

Chiharaea bodegensis. All *rbcL* sequences for *C. bodegensis* from throughout its range, including a sequence from topotype material, are identical. Johansen's (1966) original description clearly describes the morphology, anatomy, and habitat of this alga. Our observations agree with his and with those of Lebednik (1976), who noted that this species occurs on exposed coasts. All of our additional collections are from exposed, outer coast localities, with no collections from the extensive inland waterways east and south of Vancouver Island. We extend the distribution of *C. bodegensis* north to Price Island along the central coast of BC (Fig. 6). *Chiharaea bodegensis* likely is more common than records indicate, but is difficult to locate and collect due to its occurrence in the low intertidal along exposed coasts frequently beneath foliose algae and surfgrass.

Chiharaea americana. Dawson and Steele (1964) provided a detailed description of their new species from subtidal specimens from WA (Strait of Juan de Fuca and adjacent waterways). They placed it in *Yamadaia* due to morphological similarities with the genotype, *Y. melobesioides* from Japan, including an extensive crustose base and erect axes of only one (*Y. melobesioides*) or sometimes two intergenicula (*Y. americana*). They distinguished the two species by the larger size of the erect fertile axes in *Y. americana*.

Hollenberg and Abbott (1966: 63) reported *Y. melobesioides* based on specimens dredged from 15 to 17 m in Monterey Bay, CA. They identified their material as *Y. melobesioides* because erect axes had only one intergeniculum as opposed to one or two reported for *Y. americana*. Garbary et al. (1981) compared the two species of *Yamadaia* (as *Yamadaea*) using material from Japan and from the NE Pacific and concluded that there were numerous morphological differences between erect axes in the two species including their arrangement (abundant and densely aggregated in *Y. melobesioides*, sparse and scattered in *Y. americana*), height, diameter, and shape (shorter, narrower and obovate in *Y. melobesioides*, taller, wider and clavate in *Y. americana*), number of intergenicula (1 in *Y. melobesioides*, 1–2 in *Y. americana*), conceptacle pore position (apical in *Y. melobesioides*, apical and "eccentric" in *Y. americana*), and presence (in *Y. melobesioides*) or absence (in *Y. americana*) of trichocytes. On the basis of their morphological examination of material from Monterey Bay, they concluded that these specimens were *Y. americana* and not *Y. melobesioides*. Aguilar-Rosas

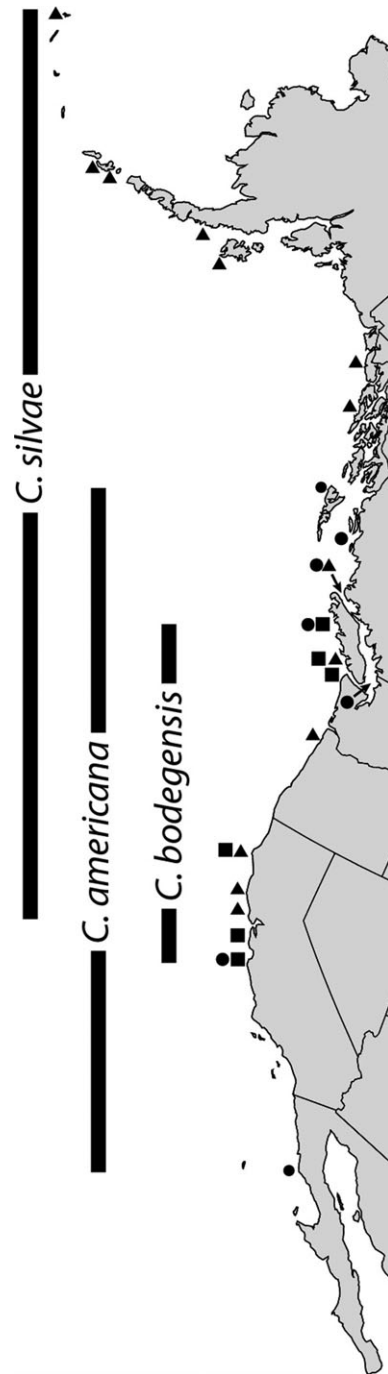


FIG. 6. Map showing distribution of *Chiharaea* species. Vertical lines at left show overall pattern of distribution of each species; symbols on map indicate individual records of each species. Note *C. americana* (*Yamadaia americana*) has very disjunct distribution in southern part of its range. Squares = *C. bodegensis*, circles = *C. americana*, triangles = *C. silvae* (*Arthrocardia silvae*).

and Pacheco-Ruiz (1995) reported *Yamadaia americana* subtidally (12–23 m) from two sites in northern Baja California, Mexico. They reported erect axes with up to three intergenicula and that the basal intergeniculum sometimes branched, bearing two terminal intergenicula.

The *rbcL* gene sequence of material from WA that we identified as *Y. americana* clearly places this species in *Chiharaea*. We did not attempt to sequence holotype material because it was preserved in formaldehyde, but this species is so distinct morphologically that its identity is clear. The partial *rbcL* gene sequence of a specimen dredged from Monterey Bay (Table S1, and cited by Hollenberg and Abbott 1966) is identical over aligned sites to the WA specimen and confirms the morphological observations of Garbary et al. (1981) that the CA material and the WA material are conspecific and are not *Corallina* (*Yamadaia*) *melobesioides*. We have not sequenced the Baja California, Mexico material, but the morphological characters indicate that these specimens belong in *C. americana*.

Chiharaea americana is strictly subtidal from 6 to 25 m depth and occurs primarily on bedrock and cobble, although it also can be epizooic on mussels and limpets. We have not found it on pebbles. Qualitative observations around San Juan Island, WA indicate that *C. americana* is the most common subtidal articulated coralline on cobble and bedrock in 6–25 m and perhaps deeper, and we expect that this is true at least throughout northern WA and BC. Collections in herbaria do not reflect its commonness because it appears to be a crustose coralline alga due to the paucity of uprights, and even when these are present, they are mostly inconspicuous (Fig. 4a) until the material is examined under a dissecting microscope, and it occurs only on larger cobble and bedrock making it difficult to collect. With the combination of habit and habitat characters provided herein, we expect that this species will be collected and identified more widely from NE Pacific subtidal habitats.

Chiharaea silvae. Johansen (1971) placed his new species in *Arthrocardia* on the basis of its pinnate branching in vegetative parts and dichotomous branching in its fertile parts, beautifully illustrated by Widdowson and Coon (1974, fig. 1) and because carposporangial filaments can arise anywhere on the fusion cell. Johansen (1971) noted, however, that it was difficult to understand the presence of an *Arthrocardia* species in northern CA, when all of the other reported species occur in the southern hemisphere. Our *rbcL* gene sequencing results address Johansen's concerns about *Arthrocardia* in CA by clearly demonstrating that *A. silvae* belongs in *Chiharaea* and not *Arthrocardia*. It is not surprising that Johansen did not associate his species with *Chiharaea* due to its nearly inconspicuous crustose base, in contrast to the expansive crust of *C. bodegensis*. However, once one knows the generic placement from DNA sequencing, then the morphological similarities are more readily apparent – particularly the inflated intergenicula and the presence of axial conceptacles with terminal or acentric pores.

That *Arthrocardia silvae* was not described until 1971 is not too surprising because the plant is more common and conspicuous in the Aleutian Islands

than further south in BC, WA and northern CA, where plants are smaller and more likely to occur as scattered individuals and to resemble depauperate specimens of *Corallina* species.

We obtained a 316 bp *rbcL* sequence from a fragment of the holotype of *Arthrocardia silvae*, but the sequencing reaction failed, indicating that the product was of poor quality, non-specific, or a mixture of several taxa. The type specimen probably was preserved in formaldehyde before being dried, and this may account for the difficulty in analyzing this sample. Nevertheless, the morphology of the type specimen, including inflated intergenicula and axial conceptacles with terminal or acentric pores, clearly matches other field-collected material that has been sequenced. Only one field-collected specimen from CA has been sequenced, and its sequence differs by four bp from all other northern specimens, ranging from southern BC north to Kodiak Island, AK and through the Aleutian chain to Attu Island, which have identical sequences. This 0.29% sequence divergence is not sufficient to recognize the northern specimens as a distinct species, especially as we do not know if, or how much, sequence variation is present in specimens from Oregon and CA. Additional specimens from these localities need to be sequenced to determine if additional taxonomic changes are needed.

New collections of *Chiharaea silvae* allow us to expand its range northward from BC (Scagel et al. 1989) to Kodiak Island, AK and westward to the last island of the Aleutian chain, Attu (Fig. 6). The presence of *C. silvae* on Attu Island raises the possibility that this species also is present in the Commander Islands and perhaps on the Kamchatka Peninsula; it should be sought in both localities.

CONCLUSIONS

This study demonstrates that the formerly monospecific genus *Chiharaea* includes three NE Pacific species, *C. bodegensis*, *C. americana* and *C. silvae*, and that the NW Pacific taxon *Yamadaia melobesioides* belongs in *Corallina*. Thus, the morphological similarity of *Corallina* (*Yamadaia*) *melobesioides* and *Chiharaea americana* reflects convergent evolution. Such morphological ambiguity underscores the importance of DNA comparisons in resolving phylogenetic relationships among genera and species of coralline algae and in determining vegetative and reproductive characters that are phylogenetically informative. We reject the 'intermediate frond hypothesis' posed by both Johansen (1969) and Cabioch (1971), and conclude that *Chiharaea bodegensis*, *C. americana* and *Corallina* (*Yamadaia*) *melobesioides* evolved from upright articulated coralline ancestors via a reduction in the number of genicula and intergenicula and a concomitant reduction in stature.

We thank Laura Anderson, Ted Klenk, Kevin Miklasz and Kevin Britton-Simmons for collections. Jackie Sones assisted

with our collections at the Bodega Marine Reserve. Collections made by SCL were supported by funding from Cook Inlet Regional Citizens Advisory Council, Hart Crowser, and NSERC and were facilitated by Amy Deveau, Jochen Halfar, Jonathan Houghton, Susan Saupe and Nancy Turner. A portion of this study was done while PWG was a visiting professor at the Friday Harbor Laboratories, University of Washington. The manuscript was improved by comments from Katy Hind and two anonymous reviewers. This research was funded in part by a Natural Sciences and Engineering Research Council (NSERC) grant to PTM and by a family trust to PWG.

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Supplementary Material

The following supplementary material is available for this article:

Table S1. List of specimens sequenced, including herbarium number, reason for their inclusion, collection data and GenBank accession number.

Table S2. List of specimens examined morphologically including, herbarium accession number and collection data.

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