LETTER

GENOMICS REVEALS ABUNDANT SPECIATION IN THE CORAL REEF BUILDING ALGA *POROLITHON ONKODES* (CORALLINALES, RHODOPHYTA)[1]

Paul W. Gabrielson iD *, Jeffery R. Hughey and Guillermo Diaz-Pulido*

An essential suite of coral reef ecosystem engineers is coralline red algae. Among these, the smooth, encrusting *Porolithon onkodes* has historically been considered the most important and common reef building species worldwide. We assess *P. onkodes* biodiversity by performing a genomic analysis of the lectotype specimen collected in 1892 from the Tami Islands, Gulf of Huon, east of New Guinea. Comparisons of DNA sequences from the lectotype specimen to those deposited in GenBank and to newly generated sequences from both field-collected and historical specimens demonstrate that at least 20 distinct species are passing under *P. onkodes*. We hypothesize that there were multiple evolutionary drivers including ecophysiology, hydrodynamic regimes, and biotic interactions as well as historical biogeography, which resulted in this high diversity of smooth, encrusting *Porolithon* species throughout the tropics. Our results emphasize the need to document the biodiversity, ecophysiology, and habitats of these tropical, reef-building algae in light of climate change and ocean acidification.

Porolithon onkodes is currently recognized worldwide as the major coral reef cementing and armoring coralline red alga, providing structural integrity to fore reef habitats (Maneveldt and Keats 2014). The importance of nongeniculate coralline red algae to coral reef formation was first recognized by Charles Darwin (1890), "Nothing can be more singular than the appearance at low tide of this 'flat' of naked stone, especially where it is externally bounded the smooth convex mound of Nulliporae appearing like a breakwater built to resist the waves." Ever since, throughout the tropical Pacific and Indian Oceans, *P. onkodes* has been cited as the most important species providing major cover on windward fore reefs from the intertidal reef ridge and continuing seaward through the reef crest and rim (Howe 1912, Setchell 1926, Taylor 1950, Doty and Morrison 1954, Marsh 1970, Littler and Doty 1975, Dean et al. 2015). Its ecological counterpart in the Caribbean Sea was known as *Porolithon pachydermum* (Adey 1967), until placed into synonymy under *P. onkodes* (Maneveldt and Keats

2014). Both of these *Porolithon* species are distinguished by their smooth, encrusting habitat in contrast to other fore reef corallines that form densely branched more or less hemispherical mounds. Beginning in the late 1960s and continuing to the present, the ecological niches thought to be occupied by *P. onkodes* have expanded to include back reef rubble-filled moats as well as slope habitats to 5–10 m depth (Womersley and Bailey 1970, Lee 1978, Adey et al. 1982).

Seven coralline species were described based on specimens from the Tami Islands, Gulf of Huon, collected by native divers in 1–3 m of water in March 1892 and communicated by the Lutheran missionary Rev. Georg Bamler, including the coral tine encrusting *Lithothamnion onkodes* (Heydrich 1897), basionym of *P. onkodes* (Fig. S1 in the Supporting Information). Much of this collection was destroyed in the bombing of the Berlin Museum during World War II, but before 1909, Heydrich sent a specimen of *L. onkodes* to his coralline competitor, Mikhail Foslie, in Trondheim, Norway, who placed it in

his new genus, *Porolithon* (Foslie 1909). Following earlier successes characterizing organellar genomes of red algal type specimens (Hughey et al. 2014, 2017, Boo et al. 2016), we analyzed the lectotype of *L. onkodes* and compared its DNA to recent field-collected specimens. The lectotype DNA was extracted from a 2×2 mm^2 fragment following Hernández-Kantún et al. (2016) and implementing the guidelines proposed by Hughey and Gabrielson (2012). Illumina 76 bp paired-end library and sequencing was carried out by *myGenomics* LLC using an in-house low-input protocol (http://www.mygenomics.com).

For all other samples, the DNA was extracted, amplified, and Sanger sequenced following Gabrielson et al. (2011) and Adey et al. (2015). Genomic data were assembled with the default de novo settings in CLC Genomics Workbench 9.5.2 (®2016 CLC bio, a QIAGEN Company, Germantown, MD, USA), Velvet 1.2.08 with a kmer of 59 (Zerbino and Birney 2008), and default settings in IDBA-UD (Peng et al. 2012). The three assemblers independently yielded the same results.

Genome gaps were closed by PCR and sequencing and by iterative mapping with Geneious 8.1.5 (BioMatters, Auckland, New Zealand). Genomes were annotated using NCBI ORFfinder and alignments obtained via BLASTX against the reference sequences of *Calliarthron tuberculosum* (GenBank accessions KC153978 and KR005619). tRNAs were identified using tRNAscan-SE 1.21 web server (Schattner et al. 2005) and rRNAs using RNAmmer 1.2 (Lagesen et al. 2007). Locally Collinear Block (LCB) alignments were generated using ProgressiveMauve with a seed of 21 with the "Use seed families" option selected (Darling et al. 2010). Sequences of the plastid genome (GenBank KY212106), mitogenome (GenBank KY212107), and nuclear ribosomal cistron (GenBank KY212108) were deposited in GenBank. DNA sequences of the *rbc*L gene and multiple genes (SSU, LSU, *psb*A, COX1, 23S, *rbc*L) representing published and unpublished *Porolithon*, and unidentified coralline algae were downloaded from GenBank (Table S1 in the Supporting Information) and aligned with MAFFT (Katoh and Standley 2013). Maximum likelihood analyses were executed with T-REX (Boc et al. 2012) and the GTR + gamma model with 1,000 fast bootstraps. The Bayesian analysis (Ronquist and Huelsenbeck 2003) was performed using the same model with default settings in Geneious. The *rbc*L tree was rooted with *Harveylithon* sp. (GenBank MF979962) and the multigene tree with *Harveylithon rupestre* (SSU, GenBank KM073303; *psb*A, KM407535). Phylogenetic trees were visualized with TreeDyn 198.3 at Phylogeny.fr (Dereeper et al. 2008).

Analysis of the lectotype of *Lithothamnion onkodes* resulted in the complete plastid (Table S2 in the Supporting Information) and mitochondrial (Table S3 in the Supporting Information) genomes as well as the ribosomal cistron. The complete plastid genome of *P. onkodes* is 170,468 bp in length and contains 228 genes (Table S2). It has a high level of gene synteny with the two previously published genomes of *Calliarthron tuberculosum* and *Sporolithon durum* (Janouškovec et al. 2013, Lee et al. 2016). The mitogenome of *Porolithon onkodes* is 27,882 bp and contains 46 genes (Table S3). The mitogenome is similar in gene content to other corallines (*C. tuberculosum*, *Corallina officinalis*, and *S. durum*); however, it differs in organization (Fig. S2 in the Supporting Information). Most noteworthy, ribosomal proteins *rps*12 and *rpl*20, the small subunit rRNA, and nad4L of the NR segment (Yang et al. 2015) are situated with the CY segment, and the tRNAs (R, Y, V, N) are flanking *orf*172 following the large subunit rRNA.

Maximum Likelihood (ML) phylogenetic analyses of the plastid-encoded *rbc*L gene (Fig. 1) and multigene trees (Fig. S3 in the Supporting Information), based on newly generated sequences or sequences from GenBank (Table S3), showed that over 20 species are passing under the name *Porolithon onkodes*. *rbc*L sequences from the 3′ end of the gene (*rbc*L-3P) were also obtained from the type specimens of *P. sandvicense* (basionym: *Lithophyllum dentatum* f. *sandvicensis*, type locality Sandwich Islands, now Hawaiian Islands), *P. oligocarpum* (basionym: *Lithophyllum oligocarpum*, type locality: Puerto Orotava [Puerto de la Cruz], Tenerife, Canary Islands), *P. pachydermum* (basionym: *Lithophyllum onkodes* f. *pachydermum*, type locality: Vestindien = Danish West Indies, now U.S. Virgin Islands), and *P. antillarum* (basionym: *Lithophyllum antillarum* type locality: Culebra Island, Puerto Rico). All of these species were recently proposed as synonyms of *P. onkodes* based on morpho-anatomical characters (Maneveldt and Keats 2014, Gallardo et al. 2016). Not one of them is *P. onkodes* (Fig. 1). *Porolithon sandvicense* and *P. oligocarpum* are each distinct species, whereas *Porolithon antillarum* and *P. pachy-dermum* are the same species, with *P. antillarum* having nomenclatural priority. Just as there are multiple species passing under *P. onkodes* in the Indo-Pacific, there are at least two other species passing under *P. antillarum* in the tropical western Atlantic (Fig. 1, represented by MF979946 and MF979960). There are four unnamed specimens in GenBank (LCB0701, LCB9658, LCB0712, LCB0678), all from Fiji and all with vague habitat data ("isolated by scuba diving or on the shore at low tide"), whose *psb*A (GQ917931, GQ917955, GQ917 964, GQ917969) and mitochondrial-encoded COI (GQ917588, GQ917571, GQ917592, GQ917 276) gene sequences indicate that they are *P. onkodes* (for *psb*A <0.4% diverged, Hind et al. 2016, for COI <4.5% diverged, Saunders 2005). None of the other *P. onkodes* GenBank sequences of any marker, including those listed as *Hydrolithon onkodes*, are *P. onkodes*, but all belong in *Porolithon* except KM369154 from New Zealand (Fig. 1). The distributions of the *Porolithon* species for which we have *rbc*L sequences are shown in Figure 2. Species level status is based on *rbc*L pair-wise sequence divergences >1% (Table S4 in the Supporting Information), a conservative value given that 0.5%–0.8% has been used to distinguish coralline species in other genera of Corallinales (Gabrielson et al. 2011, Hind et al. 2016).

It is clear from the DNA sequence data based on multiple genetic markers that the evolution of tropical and subtropical smooth, encrusting *Porolithon* species is not marked by morpho-anatomical discontinuities and that none of the morpho-anatomical studies of the past 100+ years has enabled us to recognize the numerous species passing under *P. onkodes*. Sequenced specimens, for which we also have good habitat data, indicate that species evolution has been complex with multiple, independent, evolutionary drivers yielding the current geographic and ecological
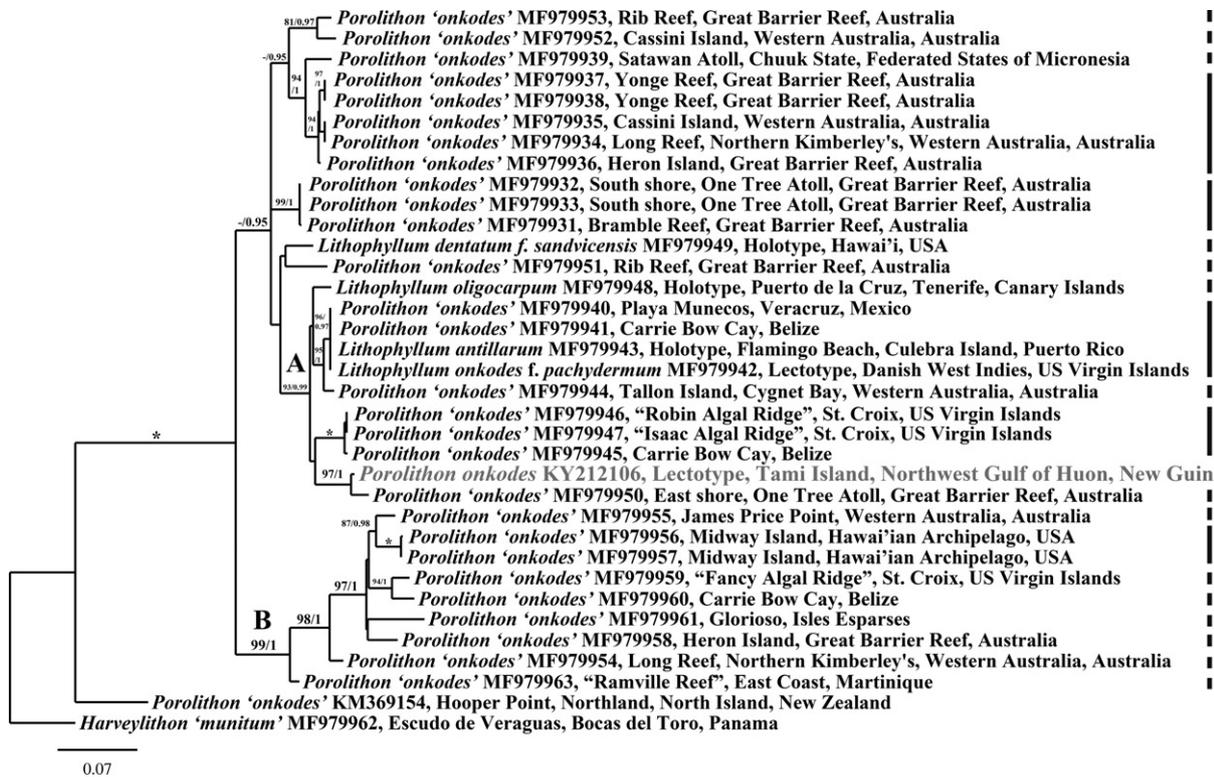
FIG. 1. Maximum likelihood phylogram of *Porolithon* "*onkodes*." Phylogenetic analysis of *rbc*L sequence data showing evolutionary relationship of *P. onkodes* to other sequences attributed to *P.* "*onkodes*." Numbers above nodes are bootstrap supports based on 1,000 replicates (<75% not shown) followed by Bayesian posterior probabilities (<0.90 not shown). Asterisks indicate full support in both analyses and dashes no statistical support; A and B label clades discussed in paper; vertical bars and numbers indicate species; corresponding numbers on Figure 2 show distributions.
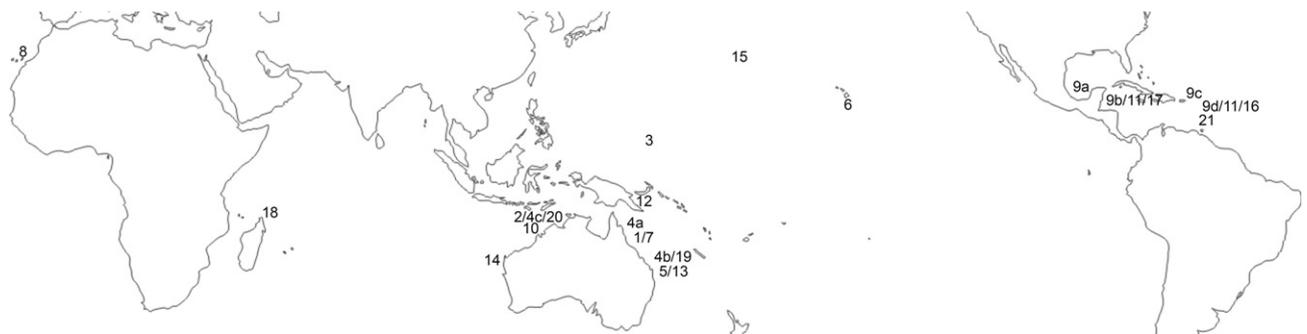

FIG. 2. Distribution of 21 species called *Porolithon onkodes* based on *rbc*L sequences. Numbers correspond to specimens in Figure 2 called *P. onkodes*; 12 is type specimen of *P. onkodes*. KM369154 from New Zealand was omitted as that specimen called *P. onkodes* belongs in another genus. Numbers with letters indicate species present at different localities. 4a = Yonge Reef; 4b = Heron Island; 4c = Cassini Island and the nearby Long Reef; 9a = Playa Munecos, Vera Cruz, Mexio; 9b = Carrie Bow Cay, Belize; 9c = Culebra Island, Puerto Rico; 9d = U.S. Virgin Islands.

distributions. The evidence for this complexity is revealed in the phylogenetic tree, from observations of field-collected specimens and from the literature.

Two well-supported clades (Fig. 1, A and B) contain smooth, encrusting *Porolithon* species from both the western Atlantic and

Pacific Oceans, rather than all western Atlantic Ocean species forming a clade and all Pacific Ocean species forming a separate clade. We hypothesize that the ancestors of these clades already were widely distributed in tropical seas and that their descendants reflect that historical distribution

pattern. This hypothesis can be tested by sequencing many more specimens with faster mutating markers, such as COI (Saunders 2005), to more precisely track the geographic patterns of these species. Rösler et al. (2017) proposed the Indo-Australian archipelago as the center of origin of coral reef

corallines. Our data support that it also was the center of diversification of these smooth, encrusting *Porolithon* species. Thus far, there is no evidence for a single, pantropical, smooth, encrusting *P.* "*onkodes*."

With respect to ecophysiology being a significant evolutionary driver, the results are mixed. Some species appear to have narrow habitat requirements, whereas others are broadly adapted to a range of PAR, hydrodynamic regimes, and desiccation. *rbc*L sequences MF979945, MF979946, and MF979947, which are identical, are all from intertidal specimens on the reef crest at different Caribbean Sea localities (St. Croix Island and Belize). Likewise, sequences MF979931, MF979932, and MF979933 of specimens from the Great Barrier Reef (GBR) occur intertidally on either the fore or back reefs, and these are the same species. These two Caribbean and GBR intertidal species occur in habitats with high levels of PAR and are subjected to desiccation at low tide. In contrast, sequences MF979937, MF979938, MF979934, MF979935, and MF979936 represent what may be a single, although genetically variable species (0.8–0.9 *rbc*L sequence divergence) that ranges from the intertidal reef crest to 10 m depth at exposed sites and from different localities either on the GBR or in Northwest Australia. This species appears broadly adapted to different PAR, wave exposure, and desiccation regimes. However, the two sample sites on the GBR are 1,300 km apart and even further from sample sites on the northwest coast of Australia. Additional collections are needed to determine if this is indeed one species.

In contrast, Payri et al. (2001) examined photoacclimation in "*Porolithon onkodes*" (as *Hydrolithon onkodes*) based on specimens collected from both the reef crest and from the lagoon, the latter from three different PAR exposures. The reef crest specimens, despite experiencing PAR levels comparable to the most exposed lagoon specimens, were distinct in both the total amount of pigment, more than twice any of the lagoon specimens, and had the highest concentrations of carotenoids. Likely the reef crest and lagoon specimens represented two or more distinct species of *Porolithon*, although alternative hypotheses, such as high physiological plasticity or local adaptation, cannot be ruled out. Variable hydrodynamic regimes rather than differences in PAR may influence species physiology and be important evolutionary drivers for some *Porolithon*. "*onkodes*" taxa.

Adding to this complex evolutionary picture is the presence of morphologically indistinguishable, but distinct species (MF979953, MF979938, MF979951, MF979958) that all occur in the same habitat on exposed fore reefs at 5 m depth, but occupy different clades throughout the tree (Fig. 1). The strength of biotic interactions, including herbivory and interspecific competition, has been hypothesized to limit macroalgal species diversity in tropical regions (Keith et al. 2014). Indeed, herbivory has been tightly linked to coralline abundance and radiation in tropical reef habitats where the thick, high-density calcium carbonate construction, as found in *Porolithon* species, was hypothesized to allow them to thrive in environments of intensive herbivory (Steneck 1985). Intensive herbivory may also have contributed to the diversification of these smooth, encrusting *Porolithon* species through variable allelopathic interactions, as suggested for the tropical macroalgal genus *Lobophora* (Vieira et al. 2017). Furthermore, coral reef environments have been suggested as centers of species diversification as they create complex mosaics of habitats (both in size and variety) and act as a refuge from high species extinction for a variety of reef organisms (Cowman and Bellwood 2011). It is likely that similar processes may explain the co-occurrence and diversification of smooth, encrusting *Porolithon* species in reef environments.

In light of global climate change and the sensitivity of *Porolithon* "*onkodes*" to ocean acidification and warming (Diaz-Pulido et al. 2012, 2014, Johnson and Carpenter 2012) and based on the evidence presented herein, it is essential that future studies examining the physiology, minerology, and response of *P.* "*onkodes*" to ocean acidification be conducted on specimens from the same habitat with respect to PAR, hydrodynamic regime and desiccation, and that these specimens be sequenced to establish their identity. Only then can we begin to understand evolution, biogeography, ecology, and physiology of this complex of species functioning as critical ecosystem engineers on tropical coral reefs.

PAUL W. GABRIELSON*, JEFFERY R. HUGHEY† and GUILLERMO DIAZ-PULIDO‡,§

*Herbarium and Biology Department, University of North Carolina - Chapel Hill, Coker Hall, CB 3280, Chapel Hill, North Carolina 27599-3280, USA
†Division of Mathematics, Science,

and Engineering, Hartnell College, 411 Central Ave., Salinas California, 93901, USA
‡School of Environment and Science, Australian Rivers Institute —Coast and Estuaries, Griffith University, Nathan Campus, 170 Kessels Road, Brisbane, Queensland, 4111, Australia
§Australian Research Council Centre of Excellence for Coral Reef Studies, Townsville, Queensland 4811, Australia

Adey, W. H. 1967. The algal ridges and coral reefs of St. Croix their structure and Holocene development. *Atoll Res. Bull.* 187:1–67.

Adey, W. H., Hernandez-Kantun, J. J., Johnson, G. & Gabrielson, P. W. 2015. DNA sequencing, anatomy and calcification patterns support a monophyletic, subarctic, carbonate reef-forming *Clathromorphum* (Hapalidiaceae, Corallinales. *Rhodophyta. J. Phycol.* 51:189–203.

Adey, W. H., Townsend, R. A. & Boykins, W. T. 1982. The crustose coralline algae (Rhodophyta: Corallinaceae) of the Hawaiian islands. *Smithson. Contribs. Mar. Sci.* 15:1–74.

Boc, A., Diallo, A. B. & Makarenkov, V. 2012. T-REX: a web server for inferring, validating and visualizing phylogenetic trees and networks. *Nucleic Acids Res.* 40:W573–9.

Boo, G. H., Hughey, J. R., Miller, K. A. & Boo, S. M. 2016. Mitogenomes from type specimens, a genotyping tool for morphologically simple species: ten genomes of agar-producing red algae. *Sci. Rep.* 6:35337.

Cowman, P. F. & Bellwood, D. R. 2011. Coral reefs as drivers of cladogenesis: expanding coral reefs, cryptic extinction events, and the development of biodiversity hotspots. *J. Evol. Biol.* 24:2543–62.

Darling, A. E., Mau, B. & Perna, N. T. 2010. ProgressiveMauve: multiple genomic alignment with gene gain, loss, and rearrangement. *PLoS ONE* 5:e11147.

Darwin, C. 1890. *On the Structure and Distribution of Coral Reefs. In* Bettany, G. T. [Ed.]. Ward, Lock and Co., London, 208 pp.

Dean, A. J., Steneck, R. S., Tager, D. & Pandolfi, J. M. 2015. Distribution, abundance and diversity of crustose coralline algae on the Great Barrier Reef. *Coral Reefs* 34:581–94.

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevent, F., Dufayard, J. F. et al. 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res.* 36: W465–9.

Diaz-Pulido, G., Anthony, K. R. N., Kline, D. I., Dove, S. & Hoegh-Guldberg, O. 2012. Interactions between ocean acidification and warming on the mortality and dissolution of coralline algae. *J. Phycol.* 48:32–9.

Diaz-Pulido, G., Nash, M. C., Anthony, K. R. N., Bender, D., Opdyke, B. N., Reyes-Vivia, M. & Trotzsch, U. 2014. Greenhouse conditions induce mineralogical changes and dolomite accumulation in coralline algae on tropical reefs. *Nat. Commun.* 5:3310.

Doty, M. S. & Morrison, J. P. E. 1954. Interrelationships of the organisms on Raroria aside from man. *Atoll Res. Bull.* 35:1–61.

Foslie, M. 1909. Algologiske notiser VI. *Kong. Norske Vidensk. Selsk. Skr.* 1909:1–63.

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

Gallardo, T., Bárbara, I., Afonso-Carrillo, J., Bermejo, R., Altamirano, M., Gómez Garreta, A., Barceló Martí, M. C., Rull LLuch, J., Ballesteros, E. & De la Rosa, J. 2016. Nueva lista crítica de las algas bentónicas marinas de España. A new checklist of benthic marine algae of Spain. *Algas, Boletín Informativo de la Sociedad Española de Ficología* 51:7–52.

Hernández-Kantún, J. J., Gabrielson, P. W., Hughey, J. R., Pezzolesi, L., Rindi, F., Robinson, N. M., Peña, V., Riosmena-Rodriguez, R. Le., Gall, L. & Adey, W. H. 2016. Reassessment of branched *Lithophyllum* spp. (Corallinales, Rhodophyta) in the Caribbean Sea with global implications. *Phycologia* 55:609–35.

Heydrich, F. 1897. Neue Kalkalgen von Deutsch-Neu-Guinea (Kaiser Wilhelms-Land). *Bibliotheca Bot.* 7:1–11.

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

Howe, M. A. 1912. The building of "coral" reefs. *Science* 35:837–42.

Hughey, J. R. & Gabrielson, P. W. 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* 90:1191–4.

Hughey, J. R., Gabrielson, P. W., Rohmer, L., Tortolani, J., Silva, M., Miller, K. A., Young, J. D., Martell, C. & Ruediger, E. 2014. Minimally destructive sampling of type specimens of *Pyropia* (Bangiales, Rhodophyta) recovers complete plastid and mitochondrial genomes. *Sci. Rep.* 4:5113.

Hughey, J. R., Hommersand, M. H., Gabrielson, P. W., Miller, K. A. & Fuller, T. 2017. Analysis of the complete plastomes of three species of *Membranoptera* (Ceramiales, Rhodophyta) from Pacific North America. *J. Phycol.* 53:32–43.

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

Johnson, M. D. & Carpenter, R. C. 2012. Ocean acidification and warming decrease calcification in the crustose coralline alga *Hydrolithon onkodes* and increase susceptibility to grazing. *J. Exp. Mar. Bio. Ecol.* 434–435:94–101.

Katoh, K. & Standley, D. M. 2013. MAFFT Multiple Sequence Alignment Software Version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30:772–80.

Keith, S. A., Kerswell, A. P. & Connolly, S. R. 2014. Global diversity of marine macroalgae: environmental conditions explain less variation in the tropics. *Global Ecol. Biogeogr.* 23:517–29.

Lagesen, K., Hallin, P., Rødland, E. A., Staerfeldt, H. H., Rognes, T. & Ussery, D. W. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* 35:3100–8.

Lee, R. K. S. 1978. Taxonomy and distribution of the melobesioid algae on Rongelap Atoll, Marshall Islands. *Can. J. Bot.* 45:985–1001.

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

Littler, M. M. & Doty, M. S. 1975. Ecological components of structuring the seaward edges of tropical Pacific reefs. *J. Ecol.* 63:117–29.

Maneveldt, G. W. & Keats, D. W. 2014. Taxonomic review based on new data of the reef-building alga *Porolithon onkodes* (Corallinaceae, Corallinales, Rhodophyta) along with other taxa found to be conspecific. *Phytotaxa* 190:216–49.

Marsh, J. A. 1970. Primary productivity of the reef-building calcareous red algae. *Ecology* 51:255–63.

Payri, C. E., Maritorena, S., Bizeau, C. & Rodierè, M. 2001. Photoacclimation in the tropical coralline alga *Hydrolithon onkodes* (Rhodophyta, Corallinaceae from a French Polynesian reef. *J. Phycol.* 37:223–34.

Peng, Y., Leung, H. C., Yiu, S. M. & Chin, F. Y. 2012. IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics* 28:1420–8.

Ronquist, F. & Huelsenbeck, J. P. 2003. MRBAYES 3: Bayesian phylogenetic

inference under mixed models. *Bioinformatics* 19:1572–4.

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

Saunders, G. W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Phil. Trans. R. Soc. B* 360:1879–88.

Schattner, P., Brooks, A. N. & Lowe, T. M. 2005. The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. *Nucleic Acids Res.* 33:686–9.

Setchell, W. A. 1926. Nullipore versus coral in reef formation. *Proc. Am. Phil. Soc.* 65:136–40.

Steneck, R. S. 1985. Adaptations of crustose coralline algae to herbivory: patterns in space and time. *In* Toomey, D. F. & Nitecki, M. H. [Eds.] *Paleoalgology.* Springer-Verlag, Berlin, pp. 352–66.

Taylor, W. R. 1950. *Plants of Bikini and Other Northern Marshall Islands.* University of Michigan Press, Ann Arbor, Michigan, 860 pp.

Vieira, C., Camacho, O., Sun, Z., Fredericq, S., Leliaert, F., Payri, C. & De Clerck, O. 2017. Historical biogeography of the highly diverse brown seaweed *Lobophora* (Dictyotales, Phaeophyceae). *Mol. Phylogenet. Evol.* 110:81–92.

Womersley, H. M. S. & Bailey, A. 1970. Marine algae of the Solomon Islands. *Phil. Trans. R. Soc. B* 259:257–352.

Yang, E. C., Kim, K. M., Kim, S. Y., Lee, J., Boo, G. H., Lee, J. H., Nelson, W. A. et al. 2015. Highly conserved mitochondrial genomes among multicellular red algae of the Florideophyceae. *Genome Biol. Evol.* 7:2394–406.

Zerbino, D. R. & Birney, E. 2008. Velvet: algorithms for de novo short read assembly using de brujin graphs. *Genome Res.* 18:821–9.

Supporting Information

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

Figure S1. Lectotype specimen of *Porolithon onkodes* (TRH A26-1494). Encrusting habit on coral tine and box lid with collection data.

Figure S2. Locally collinear blocks (LCBs) analysis of red algal mitogenomes. The figure depicts linearized alignments identifying conserved gene regions for Florideophyceae. Each mitogenome is oriented horizontally and homologous blocks are shown as identically colored regions linked across genomes. Regions inverted relative to *Porolithon onkodes* are shifted below the genome's center axis. Sequence similarities within an LCB are proportional to the heights of interior-colored bars. Large sections of white within blocks and gaps between blocks indicate lineage-specific sequences. The figure was drawn using Mauve 2.3.1.

Figure S3. Maximum likelihood phylogram of *Porolithon onkodes* based on multigene sequence data, showing evolutionary relationship of authentic *P. onkodes* to other sequences attributed to *P. onkodes*. Numbers along branches are bootstrap supports based on 1,000 replications (<75% support not shown).

Table S1. List of taxa with collection data and GenBank numbers for sequences used in this paper arranged from east to west under each name. Sequences included in concatenated analyses shown in italics.

Table S2. Plastid genome gene content of the lectotype of *Porolithon onkodes.*

Table S3. Mitogenome gene content of the lectotype of *Porolithon onkodes.*

Table S4. Pair-wise sequence divergences (%) for rbcL sequences reported in this study.