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NOTE

MISLEADING MORPHOLOGIES AND THE IMPORTANCE OF SEQUENCING TYPE SPECIMENS FOR RESOLVING CORALLINE TAXONOMY (CORALLINALES, RHODOPHYTA): *PACHYARTHRON CRETACEUM* IS *CORALLINA OFFICINALIS*[1]

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Coralline red algae play a key role in the ecology of near shore marine ecosystems and are increasingly being used to study the effects of climate change in the marine environment. Corallines are very difficult to identify to species, and even to genus, using morpho-anatomy, likely complicating studies of their ecology, physiology, and biodiversity. We sequenced a 296 base pair fragment of chloroplast DNA from a 187-year-old isolectotype specimen of *Pachyarthron cretaceum*, a morphologically distinct geniculate species, to demonstrate that coralline morphology is often misleading and that species names can only be applied unequivocally by comparing DNA sequences from type material with sequences from field-collected specimens. Our results indicate that *Pachyarthron cretaceum* is synonymous with *Corallina officinalis*.

***Key index words:* geniculate coralline algae; historical DNA; systematics; taxonomy; type specimen**

Coralline algae are important components of marine ecosystems worldwide and are used increasingly by researchers to document the biological effects of climate change (e.g., Halfar et al. 2008, Diaz-Pulido et al. 2011, Ries 2011, Kroeker et al. 2013, Martin et al. 2013, McCoy 2013). Unfortunately, coralline algae are notoriously difficult to identify, leading to potentially confusing conclusions in ecological studies and an over- or underestimation of species diversity. Moreover, biodiversity conservation strategies inevitably depend upon correct species determinations (Rubinoff 2006, Krishnamurthy and Francis 2012).

Classical methods of anatomy and morphology have proven insufficient to distinguish some species, and even some genera, of coralline algae (Gabrielson et al. 2011, Martone et al. 2012, Hind and Saunders 2013a,b). Although DNA sequencing has the potential to revolutionize coralline systematics at all taxonomic ranks, efforts to date have not fully capitalized on this potential. Species names continue to be applied based on anatomy and morphology without DNA sequencing, an approach that is problematic in any lineage with known taxonomic uncertainty and high (or low) levels of morphological variability (e.g., Harvey et al. 2009, Da Nóbrega Farias et al. 2010, Selivanova and Zhigadlova 2010, Robinson et al. 2013). In other instances, species names have been applied to specimens without the incorporation of type specimen DNA sequences (Bailey and Chapman 1998, Broom et al. 2008, Le Gall et al. 2009, Kato et al. 2011, and Hind and Saunders 2013a). What is needed is a method that allows us to unequivocally assign species names to genetically delineated groups, to produce accurate identifications that can be used by the entire scientific community. We examined the currently recognized species *Pachyarthron cretaceum* (Postels & Ruprecht) Manza, the type and only species of this morphologically distinct genus, to determine whether it is indeed monotypic and how it is related to other coralline taxa.

We sequenced DNA from a 187-year-old isolectotype specimen of *Corallina cretacea* Postels and Ruprecht (1840: 20) (type locality Unalaska Island, Alaska [Fig. 1], collected on the von Lütke expedition [1826–1829] and housed in the Swedish Museum of

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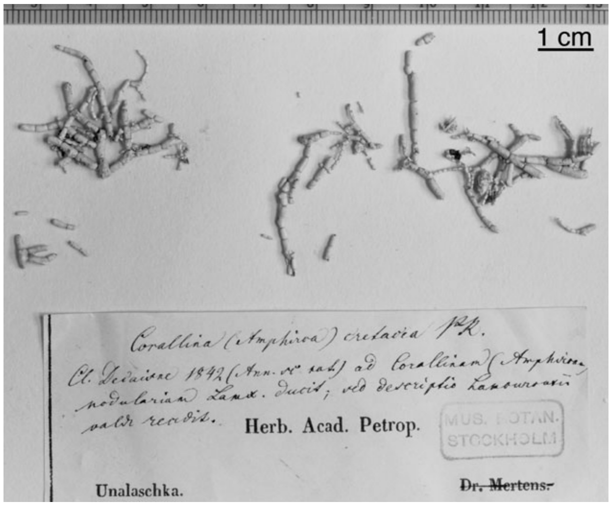

FIG. 1. Isolectotype specimen (A2609 in the Swedish Museum of Natural History (S)) of *Corallina cretacea*, with the original label from Herb. Acad. Petrop. (now the V. L. Komarov Botanical Institute) citing the type locality, Unalaska Island, and Dr. Mertens (crossed out), who was on the expedition. The collector was G. Kastalsky (Woelkerling et al. 2008). Fragments from this sheet were sequenced.

Natural History (S), Thiers (2014)), the basionym of *P. cretaceum*. We targeted a 296 base pair (bp) portion (c. 20%) from the 3′ end of the *rbc*L gene (*rbc*L-296) using the primer pair F1152Cor (Gabrielson et al. 2011) and RrbcS (Freshwater and Rueness 1994). Our extraction protocol followed Hughey et al. (2001) as modified by Gabrielson et al. (2011) and the recommendations of Saunders and McDevit (2012) and Hughey and Gabrielson (2012). The isolectotype sequence was compared to *rbc*L sequences (ranging from 296 to 1,353 bp) from field-collected material that morphologically resembled the isolectotype specimen and from other species that have been confused with it, viz. *Calliarthron tuberculosum* (Postels & Ruprecht) E.Y. Dawson and *Corallina officinalis* Linnaeus, including var. *chilensis* (Decaisne) Kützing (Hind and Saunders 2013a).

Both maximum likelihood and Bayesian analyses were run for a concatenated *rbc*L and *psb*A (2,204 bp) alignment that included coralline genera and species for which we have DNA sequence-based identifications based on type or topotype material (Fig. 2). Phylogenetic methods followed Hind and Saunders (2013a) for both the Bayesian and maximum likelihood analyses with the exception that maximum likelihood analyses were run using RAxML (Stamatakis 2006) and partitioned by both gene and codon.

The *rbc*L-296 sequence from the *P. cretaceum* isolectotype specimen was identical over its length to sequences of eight *P. cretaceum* specimens collected along the North Pacific rim from Bering Island, Russia, to Foster Island, British Columbia, Canada, including a topotype specimen from Unalaska Island, Alaska. These specimens had the morphology of *P. cretaceum* (sensu Manza (1937)) and had *rbc*L sequences that differed from North Atlantic *C. officinalis* Linnaeus (1758), the type of the genus *Corallina* (including the epitype specimen from Devon, England, Brodie et al. 2013), by only 1 or

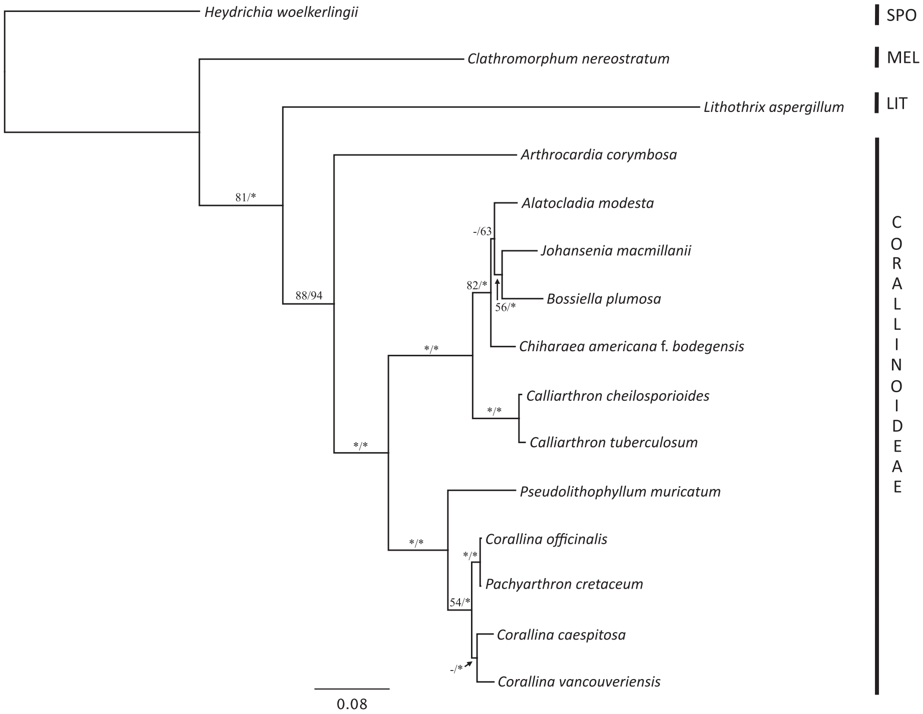

FIG. 2. Phylogram inferred by Bayesian analysis of concatenated *rbc*L and *psb*A sequence data demonstrating that specimens identified as *Pachyarthron cretaceum* resolve solidly with *Corallina officinalis*. Support values are listed as bootstrap values and posterior probabilities for maximum likelihood and Bayesian analyses respectively. Asterisks denote nodes that are strongly supported (posterior probabilities = 1.0, bootstrap values ≥95%) in all analyses. Scale bar refers to substitutions per site. Outgroup taxa were chosen from subfamilies within the Corallinales and Sporolithales. SPO, Sporolithales; MEL, Melobesiodeae; LIT, Lithophylliodeae.

2 bp over their sequence lengths (Fig. 2, Table S1 in the Supporting Information). *psb*A sequences from four of the contemporary *P. cretaceum* specimens (Table S1) were identical to *psb*A sequences from North Atlantic *C. officinalis*. Six other specimens, ranging from Iniskin Bay, Alaska to Nanaimo, British Columbia were field identified as *C. officinalis* var. *chilensis*; their *rbc*L and/or *psb*A sequences also were identical to, or differed by 1–2 bp from, Atlantic *C. officinalis* (Table S1). Sato et al. (2009) had earlier indicated in an abstract that *P. cretaceum* resolved in a clade with species in the genus *Corallina* using SSU, *rbc*L, and *psb*A sequences. In addition, five specimens that were sequenced for *psb*A also were sequenced for the mitochondrial cytochrome c oxidase subunit 1 (CO1-5P). Pacific (including Japan, see Table S1) and Atlantic *C. officinalis* specimens consistently were delimited using the CO1-5P, demonstrating that these populations can be differentiated using this marker (Hind and Saunders 2013a).

Manza (1937), in his revision of articulated coralline genera, proposed *Pachyarthron* to accommodate species with the following characters: (1) conceptacles all lateral and scattered over intergenicula, (2) genicula unizonal, (3) intergenicular medullary cells multizonal with cells in transverse zones of uniform length, and (4) intergenicula terete. Woelkerling et al. (2008) argued on the basis of three morpho-anatomical characters [branching pattern (irregular), intergenicular shape (terete), and conceptacle position (lateral for female gametophytes and tetrasporophytes, and axial and lateral for male gametophytes)] that *Pachyarthron* was a distinct genus widely distributed in the North Pacific as well as in Argentina, Chile, and Australia. More recently, Gabrielson et al. (2011) and Hind and Saunders (2013a) pointed out that conceptacle position is not a useful character to distinguish among genera of Corallinoideae. In addition, Hind and Saunders (2013a) noted that species belonging to different genera of Corallinoideae in the NE Pacific, namely *Calliarthron tuberculosum* and *C. officinalis* var. *chilensis*, could exhibit irregular branching patterns and/or terete intergenicula like *Pachyarthron*. The *rbc*L-296 was unequivocal in identifying the isolectotype material of *C. cretacea* as belonging to *C. officinalis* (Fig. 2).

Unfortunately, the lectotype material is currently inaccessible in the Komarov Botanical Institute herbarium in St. Petersburg (LE) and was not available for DNA analyses. However, a topotype specimen (NCU 593281) that was morphologically similar to both isolectotype and lectotype material also has the same *rbc*L-296 sequence, and the other taxa (noted above) with which *P. cretaceum* could be confused, do not occur in the region where the topotype collection was made. The identities of specimens cited in Woelkerling et al. (2008) from outside the North Pacific as belonging to *P. cretaceum* remain unconfirmed and require DNA sequence data to resolve their identities.

On the basis of our findings, we synonymize *Pachyarthron* Manza (1937) with *Corallina* L. (1758) and *C. cretacea* Postels and Ruprecht (1840) with *C. officinalis* L. (1758) following the rule of priority (McNeill et al. 2012, Article 11.4).

Our findings highlight the uncertainty of generic and specific concepts applied to both geniculate and nongeniculate coralline algae: we simply do not yet know what morpho-anatomical characters are informative and at what rank those characters apply without reference to an independent data set, such as DNA sequences. Although we have made good progress in resolving the evolutionary relationships of higher rank taxa of Corallinales, e.g., families and subfamilies, using DNA sequence data (Bailey and Chapman 1998, Le Gall et al. 2009, Kato et al. 2011), we are just beginning to understand how to define genera and species—mostly by setting aside morpho-anatomical characters that have been used historically and replacing them with sequence-delineated taxa. To date, DNA sequence data coupled with biogeography are better at delimiting geniculate coralline genera than anatomical/morphological characters, as, for example, *Alatocladia* (Yendo) H.W. Johansen, *Calliarthron* Manza, *Chiharaea* H.W. Johansen (Gabrielson et al. 2011, Martone et al. 2012, Hind and Saunders 2013b), *Johansenia* K.R. Hind & G.W. Saunders, and *Ellisolandia* K.R. Hind & G.W. Saunders (Hind and Saunders 2013a), and this appears to be true for nongeniculate corallines as well (Kato et al. 2011). Sequencing type material was essential to understanding the true identity of *P. cretaceum*, and this may prove to be true for other species. In particular, many of the purportedly widespread and disjunct nongeniculate coralline species, such as *Heydrichia woelkerlingii* R.A. Townsend, Y.M. Chamberlain & Keats (South Africa and New Zealand), *Lithothamnion muelleri* Lenormand ex Rosanoff (south Australia and tropical to boreal NE Pacific), and *Mesophyllum erubescens* (Foslie) Me. Lemoine (Brazil and tropical W Pacific) may represent collections of several distinct species that are morphologically similar. Widespread taxa like *C. officinalis*, which is herein confirmed to occur in both the North Pacific and the North Atlantic, tend to be the exception. However, this particular geographical pattern (N. Atlantic/N. Pacific) has been reported for a growing number of species (see Lindstrom 2001 for an early summary).

These results demonstrate that coralline anatomy and morphology are often misleading, and reference to DNA sequences from type material of many species likely will be needed to establish identities and to clarify relationships. We cannot overstate the importance of DNA sequencing of type material as well as diverse contemporary collections for resolving coralline taxonomy, and we hope that future studies will heed our advice. Correct taxonomic determinations will help us better characterize local and global patterns of biodiversity and predict impacts of climate change on coralline algae and the ecosystems that depend upon them. Furthermore, taxonomic revisions based on molecular data raise new and exciting questions about coralline ecology, biogeography, morphogenesis (e.g., branching patterns, intergenicular shape, conceptacle positions), and the phenotypically plastic response of corallines to environmental conditions, which underlies morphological variation and, ultimately, taxonomy. The morphotypes named *C. officinalis* and *P. cretaceum*, which represent a single species, would be excellent candidates to investigate such morphogenetic control mechanisms.

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Supporting Information

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

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