


MINIREVIEW

INTRASPECIFIC DIVERSITY AND GENETIC STRUCTURE IN THE WIDESPREAD
 MACROALGA *AGAROPHYTON VERMICULOPHYLLUM*¹

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Single-gene markers, such as the mitochondrial *cox1*, microsatellites, and single-nucleotide polymorphisms are powerful methods to describe diversity within and among taxonomic groups and characterize phylogeographic patterns. Large repositories of publicly-available, molecular data can be combined to generate and evaluate evolutionary hypotheses for many species, including algae. In the case of biological invasions, the combination of different molecular markers has enabled the description of the geographic distribution of invasive lineages. Here, we review the phylogeography of the widespread invasive red macroalga *Agarophyton vermiculophyllum* (synonym *Gracilaria vermiculophylla*). The *cox1* barcoding provided the first description of the invasion history and hinted at a strong genetic bottleneck during the invasion. Yet, more recent microsatellite and SNP genotyping has not found evidence for bottlenecks and instead suggested that genetically diverse inocula arose from a highly diverse source population, multiple invasions, or some mix of these processes. The bottleneck evident from *cox1* barcoding likely reflects the dominance of

one mitochondrial lineage, and one haplotype in particular, in the northern source populations in Japan. Recent *cox1* sequencing of *A. vermiculophyllum* has illuminated the complexity of phylogeographic structure in its native range of the northwest Pacific Ocean. For example, the western coast of Honshu in the Sea of Japan displays spatial patterns of haplotypic diversity with multiple lineages found together at the same geographic site. By consolidating the genetic data of this species, we clarify the phylogenetic relationships of a well-studied macroalga introduced to virtually every temperate estuary of the Northern Hemisphere.

Key index words: algae; COI; *cox1*; mitochondrial DNA; PCR; seaweed

Abbreviations: BI, Bayesian inference; COI or *cox1*, cytochrome oxidase subunit I; ML, maximum likelihood; mtDNA, mitochondrial DNA; RFLP, restriction fragment length polymorphism

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While ecological consequences of algal invasions have long been noted, we have less resolution on evolutionary and genetic patterns (e.g., Inderjit et al. 2006, Williams and Smith 2007, Andreakis and Schaffelke 2012). Where were/are the sources of a

particular algal invader? What are the reproductive system shifts that accompany invasions? Does rapid evolutionary adaptation occur during algal invasions? The answers to these fundamental questions are critical to understand the mechanisms that facilitate macroalgal invasions but will also shed light on how macroalgae may respond to other consequences of climate change (e.g., range shifts; Moran and Alexander 2014).

In the case of the widespread red macroalga *Agarophyton vermiculophyllum*, answers to these questions have emerged in the last decade. *Agarophyton vermiculophyllum* is native to the northwest Pacific and is found from southern China north along the coasts of the Korean Peninsula, Russia, and Japan (Xia 1999, Kim et al. 2010). In <100 y (Krueger-Hadfield et al. 2017a), this macroalga was introduced throughout the estuarine habitats along the coasts of Western North America (Goff et al. 1994, Bellorin et al. 2002, 2004, Saunders 2009, Krueger-Hadfield et al. 2017a, 2018), the eastern United States (Freshwater et al. 2006a,b, Thomsen et al. 2006, Kim et al. 2010, Gulbransen et al. 2012, Nettleton et al. 2013, Krueger-Hadfield et al. 2017a), northwestern Africa (Guillemin et al. 2008, Krueger-Hadfield et al. 2017a), and Europe (Mollet et al. 1998, Rueness 2005, Schaal et al. 2008, Weinberger et al. 2008, Kim et al. 2010, Sfriso et al. 2010, Krueger-Hadfield et al. 2017a,b). This species caused negative economic impacts (Freshwater et al. 2006a) and generated multiple ecological consequences in local estuaries (e.g., Nyberg et al. 2009, Thomsen et al. 2009, 2013, Byers et al. 2012, Hammann et al. 2013, Guidone et al. 2014, Gerstenmaier et al. 2016, Kollars et al. 2016, Bippus et al. 2018, Haram et al. 2018, Besterman et al. 2020). The success of this species likely depended on adaptive shifts in tolerance to temperature and salinity (Hammann et al. 2016, Sotka et al. 2018) and biofoulers (e.g., Wang et al. 2017, Bonthond et al. 2021).

Here, we provide a brief review of the literature on *Agarophyton vermiculophyllum*'s invasion history and patterns of phylogeographic structure in the native range that may shed light on the invasion process itself (see Geller et al. 2010 for a review on invasion histories and sources more broadly). We pay specific attention to the particular molecular tools that have been used and the challenge of integrating data from different studies to generate comprehensive insight. We hope that, by summarizing mitochondrial (*cox1*) data in one place, future research may further elucidate the mechanisms that facilitate invasions that will be highly relevant to the evolutionary ecology of haplodiplontic taxa.

THE INVASION HISTORY OF *AGAROPHYTON*
VERMICULOPHYLLUM

Although Saunders (2009) described the occurrence of *Agarophyton vermiculophyllum* in British

Columbia and Skriptsova and Choi (2009) described this species in the native range in Russia (see Table S1 in the Supporting Information), Kim et al. (2010) were the first to directly address the invasion history using ~1,200 bp of the *cox1* gene sequenced from thalli collected in the native range (China, South Korea, Russia, Japan) and the non-native range (the United States [California, North Carolina, Virginia], Morocco, France, Denmark, and Sweden). The non-native range was overwhelmingly dominated by a single haplotype that the authors referred to as Haplotype 6, suggesting that a strong genetic bottleneck occurred during the invasion. Because Haplotype 6 was found in the Sea of Japan, the authors concluded this region was the likely source of the invasion (Kim et al. 2010). They uncovered two haplotypes, 18 and 19, that were found in France and California, respectively, but were not detected in the native range populations they had sampled.

The limited sampling of Japan (Fig. S1a in the Supporting Information) by Kim et al. (2010) left the actual source(s) and/or region(s) of the invasion unresolved. The Pacific oyster *Magallana gigas* (synonym *Crassostrea gigas*) has been proposed as one of the main vectors transporting estuarine invaders throughout the world (Bonnot 1935, Ruesink et al. 2005). It is likely that sites in Japan served as important donor regions for the invasion of the Northern Hemisphere by *Agarophyton vermiculophyllum* (see also Miura et al. 2006), but, to determine source populations at a finer spatial scale, more high-resolution sampling throughout Japan was required. Krueger-Hadfield et al. (2016, 2017a) genotyped more than 2,900 thalli from 90 sites across the Northern Hemisphere, including 30 sites in Japan (Fig. S1, b and c). Using the available Kim et al. (2010) haplotypes, Krueger-Hadfield et al. (2017a) found two nucleotide positions (945th and 1,040th bp) that distinguished two major lineages, one with a 'C' and one with a 'T' at each of these two positions using a restriction fragment length polymorphism (RFLP) assay. There was clear phylogeographic structure with a break at ~35° N in the native range where the 'T' lineages were found at higher latitudes in Japan and they also dominated the non-native range (Figs. 1, S1). Haplotype 6, the most common haplotype among the non-native populations as well as populations north of ~35° N, belongs to the 'T' lineage. While this assay was based solely on two SNPs, it allowed Krueger-Hadfield et al. (2017a) to genotype all their thalli, thereby generating coarse patterns of genetic diversity throughout the Northern Hemisphere.

The authors bolstered this RFLP assay with sequences along the ~1,200 bp region of *cox1* from 201 thalli (Table S1). Krueger-Hadfield et al. (2017a) found Haplotype 18, previously only found in France (Kim et al. 2010), at the Mangoku-ura site

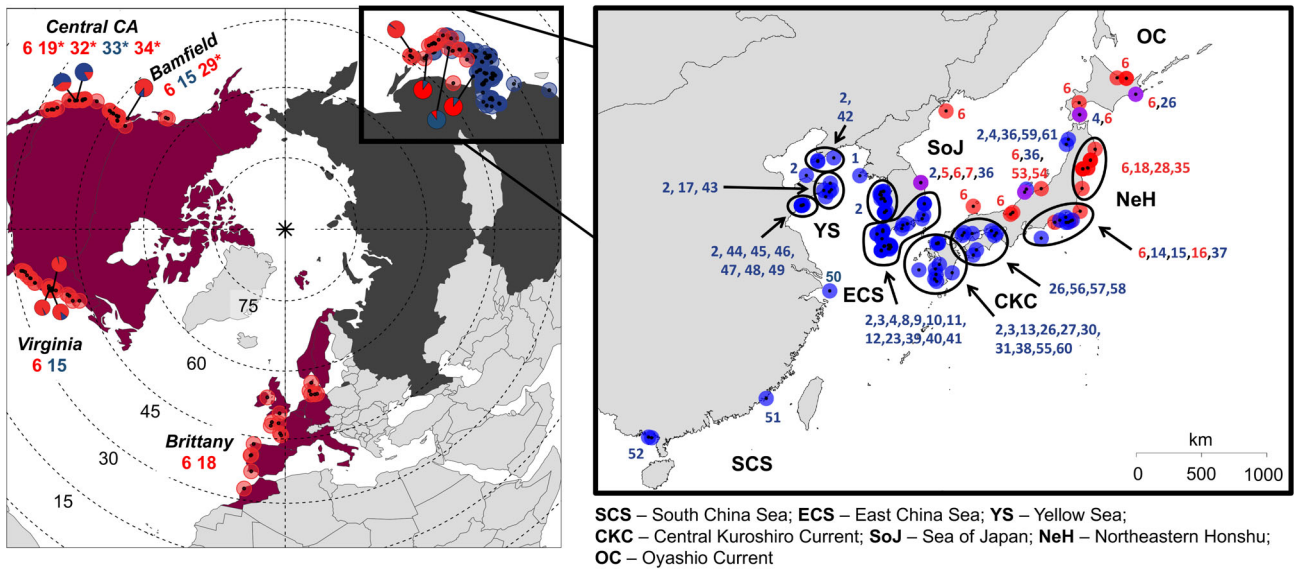


FIG. 1. Map of the Northern Hemisphere distribution of *Agarophyton vermiculophyllum*. Sampling sites are shown with a small black circle and the color denoting whether the site is composed of ‘C’ lineages (blue) or ‘T’ lineages (red) based on a combination of *cox1* sequencing ($n = 201$ thalli) and the RFLP assay ($n = 2,500$ thalli) from Krueger-Hadfield et al. (2017a). For sites that had both ‘C’ and ‘T’ lineages, pie charts depict the frequency of the two lineages based on Sanger sequencing data. In the non-native range (countries shown in maroon, we note that the Caspian Sea and Baltic coasts of Russia would be considered part of the non-native range), all sites were composed of thalli belonging to Haplotype 6 (T-2) unless otherwise noted for Bamfield, three sites in central California (Elkhorn Slough, two sites in Morro Bay), three sites in Virginia (Gargatha, Magotha, and Machipongo), and St. Pol de Léon in Brittany, France. Haplotypes with an * (19 [T-2], 29 [T-2], 32 [T-2], 33 [C-1], 34 [T-2]) have not yet been sampled in the native range (see discussion in Krueger-Hadfield et al. (2018) about sampling intensity). Inset: map of sites sampled in the native range following the same red and blue color scheme. Purple site circles denote sites where ‘C’ and ‘T’ haplotypes were. Haplotype numbers are shown next to the sites in which they were found, including collections of sites that are grouped by larger black circles. The biogeographic provinces are shown in the native range inset map according to Spalding et al. (2007).

in the Miyagi Prefecture, suggesting more sequencing would uncover the other ‘missing’ haplotypes currently found exclusively in the non-native range (Fig. 1). Nevertheless, *cox1* sequencing supported the strong genetic structure detected by Kim et al. (2010) in the native range (Fig. 1) in which the non-native range is overwhelmingly dominated by Haplotype 6 and the ‘T’ lineage. The one exception to this non-native pattern was along the coast of Virginia, USA, an area in which Gulbransen et al. (2012) found high haplotypic diversity. However, Gulbransen et al. (2012) amplified ~500 bp of *cox1*, only partially aligning with the full ~1,200 bp sequence of Kim et al. (2010; Table S1). Although Gulbransen et al. (2012) built on the haplotypic nomenclature started by Kim et al. (2010), singletons that differentiate other haplotypes were not sequenced, making it more challenging to resolve haplotypes and ambiguities (e.g., the haplotype called ‘13-14-16’ by Gulbransen et al. 2012). More thalli from Virginia need to be sequenced to verify the occurrence of Haplotypes 20–24 from Gulbransen et al. (2012) because Kim et al. (2010) and Krueger-Hadfield et al. (2017a) did not find the same levels of *cox1* diversity in this region.

Because several hundred kilometers of shoreline of northeastern Japan and nearly all of the non-native range are dominated by a few *cox1*

haplotypes, more polymorphic markers were necessary to more precisely resolve the source of the invasion as well as the genetic structure along North American and European coastlines. The 10 microsatellites used by Krueger-Hadfield et al. (2016, 2017a) and approximately 62,000 SNPs in Flanagan et al. (2021) found comparable levels of nuclear genetic diversity between native and non-native regions, suggesting highly genetically diverse inocula, a highly diverse source population, multiple invasions, or some mix of the three processes. Multiple analyses definitively identified the Miyagi Prefecture (e.g., sites Soukanzan and Mangoku-ura) as the main source of most of the Northern Hemisphere invasion (Figs. 1; S1; Krueger-Hadfield et al. 2017a, Flanagan et al. 2021). One exception occurs in the invaded region of the Salish Sea in Washington state and British Columbia which was sourced by a mixture of northern and southern Japanese populations.

Haplotype 6 dominates the higher latitude Japanese populations, including the main source site at Mangoku-ura. Yet, the lack of a strong bottleneck in microsatellite loci (Krueger-Hadfield et al. 2016, 2017a) and SNPs (Flanagan et al. 2021) suggests the mitochondrial bottleneck may largely be due to the spread of Haplotype 6 in the recolonization of higher latitudes following the last glacial maximum.

PHYLOGEOGRAPHIC STUDIES OF *AGAROPHYTON*
VERMICULOPHYLLUM IN THE NATIVE RANGE

In addition to the main goal in their paper of defining the invasion history, Krueger-Hadfield et al. (2017a) also used their molecular data to provide some preliminary assessments of phylogeographic structure in the native range. They combined the haplotypes from sequencing native thalli with the available haplotype data from Kim et al. (2010). Krueger-Hadfield et al. (2017a) found three shallow mtDNA clades, suggesting a correlation between demographic histories and the geography of the marginal seas: the South China Sea, the East China Sea (including the Yellow-Bohai Sea), and the Sea of Japan/East Sea (Figs. 1, S1). Based on the sites sampled by Kim et al. (2010) and Krueger-Hadfield et al. (2017a), there was a much stronger break at ~35°N (Fig. 1), as compared to other studies (e.g., Ho et al. 2014, Ni et al. 2014, He et al. 2015), where *Agarophyton vermiculophyllum* 'C' lineages were found at lower latitudes and 'T' lineages were found at higher latitudes (Figs. 1, S1). The RFLP *cox1* assay, *cox1* sequencing, microsatellite genotyping, and SNP genotyping support these historical and contemporary demographic hypotheses (see details in Krueger-Hadfield et al. 2017a, Flanagan et al. 2021).

Recently, Zhong et al. (2020) published ~1,200 bp sequences of the *cox1* gene, using the same primers and sequence length as Kim et al. (2010) and Krueger-Hadfield et al. (2017a). Although these data provide a clearer picture of phylogeographic structure in the native range, specifically along the coastlines of China and the Sea of Japan, the authors used a subset of the published *Agarophyton vermiculophyllum* haplotypes and generated a new haplotypic nomenclature. For example, the Zhong et al. (2020) Haplotype 1 corresponds to the invasive Haplotype 6 in previous studies (see Table S2 in the Supporting Information; Kim et al. 2010, Gulbransen et al. 2012, Krueger-Hadfield et al. 2017a). Here, we resolve these inconsistencies with a definitive set of known haplotypes and names and a translation key to connect the separate studies (Table S2). In addition, we provide new sequence data for 101 thalli sampled throughout the main Japanese islands from Kyushu to Hokkaido (Figs. 1, S1, Table S3, Appendix S1, Appendix S2 in the Supporting Information).

To assess the phylogeny of all known unique *cox1* haplotypes, we generated a Bayesian inference and maximum likelihood phylogeny and median joining networks using all available haplotypes (see Appendix S1 for more detailed information). Overall, the phylogenies had limited statistical support, with only two lineages receiving strong support (Fig. S2a in the Supporting Information). The phylogenies (Fig. S2) and haplotype network we generated (Fig. S3 in the Supporting Information) reflect

that the lineages defined in Zhong et al. (2020) are genetically rather shallow and, based on *cox1*, not confidently grouped into separate lineages. Nonetheless, four clusters can be observed corresponding to 'C' lineages (C-1, C-3, C-4), and one to the original 'T' lineage (T-2) still found exclusively north of ~35° N in the native range and throughout the non-native range (Figs. 1, S3). We have combined the Zhong et al. (2020) lineages 1-5 with our previous 'C' and 'T' designations to aid in translating information across studies (see also Table S2). These shallow genetic lineages roughly match to the marginal seas in the northwest Pacific that likely were refugia during the last glacial maximum (Fig. 1, Table S2; Wang 1999).

Zhong et al. (2020) also uncovered several native sites with mixed 'C' and 'T' lineages. Previous sequencing efforts (Krueger-Hadfield et al. 2017a) revealed a single site (Akkeshi) that had mixed 'C' and 'T' haplotypes (Haplotype 6 ['T-2'] or Haplotype 26 ['C-3']; Fig. S1, b and c). Haplotype 26 has now been detected in the Seto Sea (Figs. 1, S1), both from sequencing more thalli from more sites in this study and in Zhong et al. (2020). It will be intriguing to determine how Haplotype 26 came to be found in Akkeshi – perhaps contemporary movement of thalli within the native range as suggested by Krueger-Hadfield et al. (2017a). Further, Kim et al. (2010) found thalli from the site Donghae in South Korea to be composed of three 'T-2' haplotypes and further sequencing of thalli by Zhong et al. (2020) has revealed two 'C-5' haplotypes (Haplotypes 2 and 36; note these are the corrected haplotype names; Table S2). Sequencing along the Japanese west coast, largely unexplored by Kim et al. (2010) and Krueger-Hadfield et al. (2017a), has revealed sites with a mix of 'C' and 'T' lineages as well as sites with only 'C' lineages (Figs. 1, S1).

The strong genetic structure in Japan described in Krueger-Hadfield et al. (2017a) and Flanagan et al. (2021) are supported with all available *cox1* haplotypes along the eastern coast of Honshu. However, sampling along the western coast of Japan presents a more complex demographic history. Because the Sea of Japan was one of the marginal seas during the last glacial maximum (Wang 1999), it is not surprising that greater sampling effort has uncovered greater haplotypic diversity. The complex distributions of the 'C' and 'T' lineages warrant further sampling in this region (Figs. 1, S1), particularly with more polymorphic markers. Interestingly, Zhong et al. (2020) found the 'C' Haplotype 4 at Shinori near Hakodate in southwestern Hokkaido. Previous work detected only 'T' haplotypes by both sequencing and the RFLP assay (Krueger-Hadfield et al. 2017a). Although we note the location of Shinori is incorrect in one of Zhong et al. (2020) maps (site 3 in their fig. 1), it is possible that the widespread Haplotype 4 could be found in Shinori near the Tsugaru Strait, a phylogeographic break

between biogeographic provinces (Figs. 1, S1; Briggs and Bowen 2012). Each of the marginal seas in the northwestern Pacific likely served as a refugium resulting in intraspecific patterns that are due to different historical events that in turn lead to chaotic genetic patterns (Ni et al. 2014). More studies that incorporate all available genetic data are necessary for *Agarophyton vermiculophyllum*, encompassing the entire extant range along the coastlines of southern China into Russia in order to tease apart historical versus contemporary processes generating the ‘C/T’ break around 35° N as well as the complex ‘C/T’ lineage patterns in the Sea of Japan.

CONCLUSIONS

Our review of the current knowledge of the *Agarophyton vermiculophyllum* invasion highlights two salient points. First, the mitochondrial bottleneck was not detected with microsatellite or SNP genotyping (Krueger-Hadfield et al. 2016, 2017a, Flanagan et al. 2021). Flanagan et al. (2021) suggested that the main source site of Mangoku-ura was particularly diverse. Because this is a main region of oyster aquaculture, it is possible that the heterozygosity levels detected at Mangoku-ura could be due to more complex movement of *A. vermiculophyllum* in Japan prior to the invasion, either during recolonization following the last glacial maximum or more recent anthropogenic movement as a result of aquaculture. Second, more detailed genotyping of Japanese populations is warranted to understand the patterns of extant diversity. Such genotyping efforts will enable us to unravel the complex origins of phylogeographic structure in the native range. For example, Haplotype 6 is very common at higher latitudes in the native range. However, this mitochondrial bottleneck is also not reflected with nuclear markers in the native range (Krueger-Hadfield et al. 2017a, Flanagan et al. 2021). What are the mechanisms that drive such complex structure in the Sea of Japan, and do they occur in other taxa with low dispersal potential? Understanding the processes that generate the patterns of genetic structure in the native range of ubiquitous invaders is often limited due to poor sampling across the range. However, as we have highlighted here, there is now ample data to resolve patterns at multiple spatial and temporal scales in *A. vermiculophyllum* that will aid in our understanding of the forces that govern patterns of genetic diversity in invasive species.

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AUTHOR CONTRIBUTIONS

S.A. Krueger-Hadfield: Conceptualization (lead); data curation (lead); formal analysis (equal); funding acquisition (equal); writing-original draft (lead). **J.E. Byers:** Conceptualization (equal); writing-review & editing (equal). **G. Bonthond:** Formal analysis (equal); methodology (equal); writing-review & editing (equal). **R. Terada:** Methodology (equal); writing-review & editing (equal). **F. Weinberger:** Funding acquisition (equal); methodology (equal); writing-review & editing (equal). **E.E. Sotka:** Conceptualization (equal); writing-review & editing (equal).

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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:

Figure S1. (a) Site and haplotypes from Kim et al. (2010). Map of the native range showing sampling sites with a small black circle and the color denoting whether the site is composed of 'C' lineages (blue) or 'T' lineages (red) based on Krueger-Hadfield et al. (2017a). The marginal seas from Spalding et al. (2007) are shown with the following abbreviations: SCS – South China Sea; ECS – East China Sea; YS – Yellow Sea; CKC – Central Kuroshiro Current; SoJ – Sea of Japan; NeH – Northeastern Honshu; OC – Oyashio Current. (b) Map of the native range showing the sites sampled by Krueger-Hadfield et al. (2017a) shown in black and the new sites sampled in 2018 shown in red. (c) The sites included in Krueger-Hadfield et al. (2017a). Map of the native range showing sampling sites with a small black circle and the color denoting whether the site is composed of 'C' lineages (blue), 'T' lineages (red), or both (purple) based on Krueger-Hadfield et al. (2017a). Note that haplotype numbers are only written next to sites where sequences were obtained. Krueger-Hadfield et al. (2017a) sequenced 201 thalli but focused the majority of their effort on the RFLP assay that enabled them to assay all 2,500 thalli. Sites without a number are denoted solely by the RFLP assay distinguishing 'C' from 'T' lineages. The marginal seas from Spalding et al. (2007) are shown with the following abbreviations: SCS – South China Sea; ECS – East China Sea; YS – Yellow Sea; CKC – Central Kuroshiro Current; SoJ – Sea of Japan; NeH – Northeastern Honshu; OC – Oyashio Current. (d) The sites and haplotypes found in Zhong et al. (2020; 6.2 corresponds to a haplotype called Haplotype 4 by Zhong et al. that was not included in analyses in the current study due to occurring at the 3' end of the sequence). The map of the native range showing sampling sites with a small black circle and the color denoting whether the site is composed of 'C' lineages (blue), 'T' lineages (red), or both (purple) based on Krueger-Hadfield et al. (2017a). Collections of sites may be grouped by a larger black circle due to space limitations. The marginal seas from Spalding et al. (2007) are shown with the following abbreviations: SCS – South China Sea; ECS – East China Sea; YS – Yellow Sea; CKC – Central Kuroshiro

Current; SoJ – Sea of Japan; NeH – Northeastern Honshu; OC – Oyashio Current.

Figure S2. The Bayesian inference (BI) phylogeny based on all previously published haplotypes (Kim et al. 2010, Krueger-Hadfield et al. 2017, 2018, Zhong et al. 2020) plus new haplotypes from the present study. Haplotype numbers are in accordance with earlier work (Kim et al. 2010, Krueger-Hadfield et al. 2017, 2018) and when included in Zhong et al. (2020), under different numbers, these are displayed in parentheses. 'C' and 'T' lineages are denoted by blue and red, respectively, following Krueger-Hadfield et al. (2017a), and the *Agarophyton* outgroup is shown in gray. The 'C' and 'T' clades are labeled from 1–5 following Zhong et al. (2020). Posterior probability values for nodes are displayed on top of the corresponding branch. Bootstrap values from the maximum likelihood (ML) phylogeny have been integrated in the tree beneath the branches corresponding to nodes that were also resolved in the maximum likelihood analyses. Only BI probabilities > 0.5 and ML bootstrap values > 50 are displayed.

Figure S3. Median joining network based on mitochondrial *cox1* haplotypes from (a) Kim et al. 2010, 2018, and Zhong et al. (2020). Red and blue haplotypes have either a 'T' or a 'C', respectively, at the 945th and 1,040th bp and follow the designation of the two main lineages from Krueger-Hadfield et al. (2017a). Haplotypes shown in bold and underlined represent novel haplotypes from (a) Zhong et al. (2020) or (b) shown with an * are novel haplotypes from this study. The median joining network based on mitochondrial *cox1* haplotypes from (b) all haplotypes from Kim et al. (2010), Krueger-Hadfield et al. (2017a, 2018), Zhong et al. (2020), and this study. Red and blue haplotypes have either a 'T' or a 'C', respectively, at the 945th and 1,040th bp and follow the designation of the two main lineages from Krueger-Hadfield et al. (2017a). Haplotypes shown in bold and underlined represent novel haplotypes from (a) Zhong et al. (2020) or (b) shown with an * are novel haplotypes from this study.

Table S1. Studies using *cox1* to identify species, characterize phylogeographic structure, or both in *Agarophyton vermiculophyllum*. Other studies that have used different barcode markers for species identification or phylogeography are not included in this table (e.g., Rueness (2005) used *cox1-3* spacer, the RuBisCO spacer, and *rbcL*).

Table S2. *cox1* haplotypic key for *Agarophyton vermiculophyllum*. We converted the haplotypic names from Zhong et al. (2020) to the

established names used by Kim et al. (2010) and Krueger-Hadfield et al. (2017a, 2018). *Haplotype*, proposed name for *A. vermiculophyllum* *cox1* haplotypes; *Study*, the study from which the haplotype name originated; *GenBank Acc.*, the GenBank accession number associated with that haplotype; *C/T lineage*, the lineage to which the haplotype belongs based on the two RFLPs in the *cox1* sequence (see Krueger-Hadfield et al. 2017a); *Network group*, the group to which a given haplotype belongs based on Zhong et al. (2020) and Figure S3; *Region*, the biogeographical region based on Spalding et al. (2007) in which the haplotype has been found based on existing data including this study (WNA, western coast of North America;

EUSA, eastern coast of the United States; EU, all coastlines of Europe, including northwestern Africa); *Zhong et al. name*, the nomenclature used by Zhong et al. (2020), including the relevant GenBank accession number for mis-named haplotypes.

Table S3. List of sites, study, collection data, collectors, latitude, longitude, and the haplotypes generated in this study.

Appendix S1. Methods for *cox1* amplification.

Appendix S2. FASTA file of all described haplotypes for *Agarophyton vermiculophyllum*.