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The Introduction of the Asian Red Algae *Melanothamnus japonicus* (Harvey) Díaz-Tapia & Maggs in Peru as a Means to Adopt Management Strategies to Reduce Invasive Non-Indigenous Species

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Abstract: Early detection of non-indigenous species is crucial to reduce, mitigate, and manage their impacts on the ecosystems into which they were introduced. However, assessment frameworks for identifying introduced species on the Pacific Coast of South America are scarce and even non-existent for certain countries. In order to identify species' boundaries and to determine the presence of non-native species, through morphological examinations and the analysis of the plastid ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*-5P) gene, we investigated the phylogenetic relationships among species of the class Florideophyceae from the coast of Ancash, Peru. The *rbcL*-5P dataset revealed 10 Florideophyceae species distributed in the following four orders: Gigartinales, Ceramiales, Halymeniales, and Corallinales, among which the Asian species, *Melanothamnus japonicus* (Harvey) Díaz-Tapia & Maggs was identified. *M. japonicus* showed a pairwise divergence of 0% with sequences of *M. japonicus* from South Korea, the USA, and Italy, the latter two being countries where *M. japonicus* has been reported as introduced species. Our data indicate a recent introduction event of *M. japonicus* in Peru, and consequently, the extension of its distribution into South America. These findings could help to adopt management strategies for reducing the spread and impact of *M. japonicus* on the Pacific Coast of South America.

Keywords: DNA barcode; Florideophyceae; non-indigenous species; *Melanothamnus japonicus*; *rbcL*

1. Introduction

The deliberate or accidental introduction of non-indigenous species has been described as one of the four greatest threats to marine ecosystems [1]. Even though diverse international guidelines have been established to reduce the human-mediated exchange of species [2–4], on a global scale, the rate of first records is increasing, with the highest rates being observed in recent years [5]. For marine algae, the annual rate of introduced species has rapidly increased as they are more difficult to regulate and are associated with increasing trade [5–8]. Currently, macroalgae represent approximately 12.5% of the world's introduced species [9], a value that could be underestimated since different studies have shown that many macroalgal introductions go unnoticed due to cryptic introductions, i.e., introduced species that are morphologically indistinguishable but genetically different from native species [10–13]. Common cryptic introductions include those from the phylum Rhodophyta, one of the phyla containing most introduced algae species reported worldwide [11,12,14–19]. Within this phylum, the species *Melanothamnus japonicus* (Harvey) Díaz-Tapia & Maggs causes great concern since it has been successfully established in many non-native areas without being noticed due to its morphological similarity with

native *Melanothamnus* species [20–22]. Molecular evidence for the introduction of *M. japonicus* has been shown in Italy [18], the USA, Spain, Australia, and New Zealand [11,22], without reports for South American countries. Even though *M. japonicus* has not been reported on the Pacific and Atlantic coasts of South America, ten *Melanothamnus* species have been identified in these regions, with Peru and Chile being the countries with the highest number of native species. The species reported in Peru and Chile are *M. peruviansis* (D.E.Bustamante, B.Y.Won, M.E.Ramirez & T.O.Cho) Díaz-Tapia & Maggs, *M. sphaerocarpus* (Børgesen) Díaz-Tapia & Maggs, *M. savatieri* (Harriot) Díaz-Tapia & Maggs, *M. ramireziae* (D.E.Bustamante, B.Y.Won & T.O.Cho) Díaz-Tapia & Maggs (only reported for Peru), and *M. unilateralis* (Levring) Díaz-Tapia & Maggs (only reported for Chile) [23–26]. It is noteworthy to mention that the identification of many of these species, along with the newly identified Rhodophyta species for South America [27–31], was performed thanks to the use of molecular tools, highlighting the importance of molecular analysis for revealing the hidden biodiversity in those regions where the phycological studies have recently begun to increase [25].

Even though the introduction of non-indigenous *Melanothamnus* species such as *M. japonicus* can directly affect native species [22], their presence has not been evaluated in South America, a region where the determination of non-indigenous seaweed introductions has been hindered by insufficient knowledge of its endemic diversity [32]. The lack of studies focused on monitoring the presence of non-indigenous species can strongly affect this region since, except for Chile and Guyana, all South American countries show a low proactive capacity to manage alien species. A proactive capacity demonstrates that a country monitors the presence of non-indigenous species and establishes measures to tackle them [33]. Therefore, if a South American country has an undetected non-indigenous species, it is unlikely that it will be able to contain the emerging invasion. Indeed, Barrios et al. [34] showed that the introduction of *Kappaphycus alvarezii* (Doty) L.M.Liao, a native species from the Philippines, to Cubagua Island, Venezuela, was one of the main causes of coral bleaching, an effect that could not be controlled using the current mitigating approaches, such as the manual removal of the invasive algae. Although studies that have evaluated the negative impacts caused by non-indigenous algae species are scarce for South America, globally, different authors have shown that the introduction of non-native seaweed species negatively impacts local communities. For instance, Cebrian et al. [35] showed that *Womersleyella setacea* (Hollenberg) R.E.Norris, an exotic species in the Mediterranean, decreases the survival of coralligenous assemblages significantly. Smith et al. [36] also showed that *Acanthophora spicifera* (M.Vahl) Børgesen, an alga introduced in Hawaii, displaces most native species where it is abundant. Therefore, early detection of non-indigenous species is crucial to control the potential negative impacts on native communities.

In South America, besides a low proactive capacity, Peru is the only country that also shows a low reactive capacity regarding the degree to which a national action plan exists to reduce the impacts of non-indigenous species invasions [33]. Indeed, there is no current Peruvian law regarding management actions to control marine species introductions or prioritizes the studies focused on monitoring possible introduction events. For example, even though the Peruvian port of Chimbote in Ancash is a potential hotspot for introduced species: it receives many international ships [37] and has been the focus of illegal fishing from Asian ships [38], studies to determine the presence of exotic species are non-existent. The presence of Asian ships in Chimbote's Port is especially worrisome since they have been identified as the vectors driving the high degree of species exchange between Asian and South American ports [39], increasing the likelihood of introducing unnoticed species in this region.

To evaluate the presence of non-indigenous species along the coast of Ancash, Peru, we examined species diversity by performing morphological examinations and analyzing the phylogenetic relationships among the species of the class Florideophyceae using the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL-5P*) genetic marker.

We report the introduction of the Asian species *M. japonicus* in Peru for the first time, and consequently, the extension of its distribution into South America. Moreover, we provide further evidence regarding the phylogenetic relationships among native Florideophyceae species, enriching our knowledge of the biodiversity of native aquatic species from this country.

2. Materials and Methods

Forty-seven specimens of the class Florideophyceae collected along the coast of Ancash, Peru (from 8°59'23.64" S, 78°39'12.60" W to 10°1'42.96" S, 78°11'8.88" W, Table S1), were examined in the present study. The collected organisms were identified within the currently recognized species using identification keys, original description, and species' redescrptions [28,29,40–48]. Organisms that could not be identified at the species level were reported as "Genus sp." The microscopic observations were made in manual sections stained with Orange G (Azer Scientific, Morgantown, PA, USA) as follows: The samples were placed in the dye for 3 min, transferred to acid alcohol for 5 s (3% concentrated HCl), and mounted in 100% glycerin. Photomicrographs were taken with a Canon PowerShot G1X camera (Canon USA, Huntington, NY, USA). Voucher specimens were deposited in the Herbarium Truxillense of the Universidad Nacional de Trujillo, Trujillo, Peru. The data of the samples, vouchers, and GenBank accession numbers for the *rbcL*-5P gene sequences are listed in Table S1.

2.1. Molecular Analysis

DNA extractions were performed on silica gel dried tissue. A small amount of tissue (approximately 5 mg) was placed in a microcentrifuge tube containing 800 µL of cetyltrimethylammonium bromide (CTAB) buffer (2% CTAB, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl, and 20 mM EDTA) and 10 µL of proteinase K (Promega, Madison, WI, USA). The procedure was carried out following the protocol of Zuccarello and Lokhorst [49]. The primers for the amplification and sequencing of the *rbcL*-5P gene were F7-R753 [50]. The PCR master mix reaction consisted of 7.99 µL water PCR grade, 1.88 µL KAPA *Taq* Buffer B (10X), 3.6 µL MgCl₂ (25 mM), 0.9 µL dNTPs (2.5 mM), 0.18 µL each primer (50 µM each), 3.6 µL TBT-PAR 5X (1 mg mL⁻¹ BSA, 1% Tween 20, 8.5 mM Tris HCl pH 8 [51]), 0.18 µL KAPA *Taq* DNA polymerase (5 U µL⁻¹), and 1.8 µL genomic DNA. The PCR conditions consisted of 4 min of initial denaturation at 95 °C and 28 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 45 s. The PCR reaction was finalized with a 5 min final extension at 72 °C. The success in the amplification was determined by electrophoresis in 1% agarose gels. Successfully amplified samples were cleaned using exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific, Waltham, MA, USA). The PCR products were sent to Macrogen, USA for bidirectional sequencing reactions via capillary electrophoresis using the ABI3730XL automated DNA sequencer (Applied Biosystems®, Foster, CA, USA). All forward and reverse nucleotide sequences were edited and aligned using Sequencher 4.1.4 (Gene Codes Corporation, Ann Arbor, MI, USA). The sequences were aligned using the default alignment strategy (L-INS-i) of the multiple alignments program for nucleotide sequences (MAFFT 7.402) [52] and edited manually where required. The alignment was trimmed to 622 bp to reduce missing data and erroneous base calls at the ends of the sequences [53].

To determine the identity of each species, we searched a representative sequence of the *rbcL*-5P gene for each clade in the Barcode of Life Database (BOLD) Systems (<https://www.boldsystems.org/>, (accessed on 10 February 2021)) and the database of the National Center for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov/>, (accessed on 10 February 2021)), using the BOLD identification system (IDS) and the basic local alignment search tool (BLAST), respectively. The final identification of species was only accepted if they demonstrated more than a 99% similarity regarding the sequences available in both databases. The data presented in this study are openly available in GenBank and

the dataset with code “DS-LGFYR” on BOLD systems (dx.doi.org/10.5883/DS-LGFYR, (accessed on 10 February 2021)).

2.2. Phylogenetic Analysis

To determine phylogenetic relationships, a dataset of 78 *rbcL*-5P sequences was constructed, including 47 own sequences and 23 sequences with the highest identity obtained through the standard nucleotide BLAST search. For comparative purposes, six specimens representing five species native to the North Pacific were also included. The final alignment included 78 specimens with 622 nucleotide positions. The selection of the best model of nucleotide substitution was based on the Bayesian information criterion (BIC) through jModelTest 2 [54]. The chosen model was the general time-reversible model with a proportion of invariable sites and a gamma distribution (GTR + Γ + G). The statistical support, bootstraps, and subsequent Bayesian probabilities were calculated using maximum likelihood (ML) and Bayesian inference (BI) methods. Phylogenetic inference via ML was performed with RAxML-HPC2 on XSEDE 8.2.12 [55] through the CIPRES Science Gateway (<http://www.phylo.org/>, (accessed on 10 February 2021)) [56] using the GTRGAMMA model. Branch support was evaluated using bootstrapping with 1000 replicates. BI was performed using MrBayes 3.2.6 [57]. The BI analyses were run using the Metropolis coupled Markov chain Monte Carlo (MCMC) algorithm. Two independent runs were performed with four MCMC chains (three hot and one cold) for 50,000,000 generations. The trees were sampled every 50,000 generations. The convergence of both runs was tested using Tracer 1.6 [58] to see if the executions reached an effective sample size greater than 200. To calculate the potential scale reduction factor and posterior probabilities, we established a burn-in value to discard the first 25% of trees. The ML and BI trees were compared to evaluate the consistency of the results.

3. Results

For accurate species identification, both a detailed morphological examination and the use of molecular tools are necessary. Thus, we performed a preliminary survey to examine species diversity of the class Florideophyceae from the coast of Ancash, Peru, by performing microscopic observations and inferring their phylogenetic relationships through ML and BI. Our morphological identification and *rbcL*-5P phylogenetic analysis showed the presence of the Asian species *M. japonicus* in the port of Chimbote, Peru, for the first time. The morphological observations and phylogenetic analysis are described below.

3.1. Morphological Observations

Epiphytic. Solitary or in aggregates. Thallus 2–6 cm height, reddish-brown to dark brown. Tangled prostrated axes with a delicate texture, fixed to the substrate by rhizoids from which erect axes arise with few side branchlets (Figure 1a). The main erect axes are prominent and composed of four pericentral cells. Branching points occurred at intervals of 5–12 axial cells in the main axes. Scar cells developed between distal terminations of the pericentral cells (Figure 1b). Apices with a prominent apical cell of $8 \times 5 \mu\text{m}$ average size (Figure 1c). Apical cells were divided transversally (Figure 1c). Branches emerged in connection with trichoblasts (Figure 1c,d). Trichoblasts were delicate, few, 20–30 μm long, and emerged from adventitious branches (Figure 1c,d). Tetrasporangia spirally arranged (Figure 1e), slightly bulky, 40–60 μm in diameter, and tripartitely divided (Figure 1f). Female and carpogonial branches were not observed.

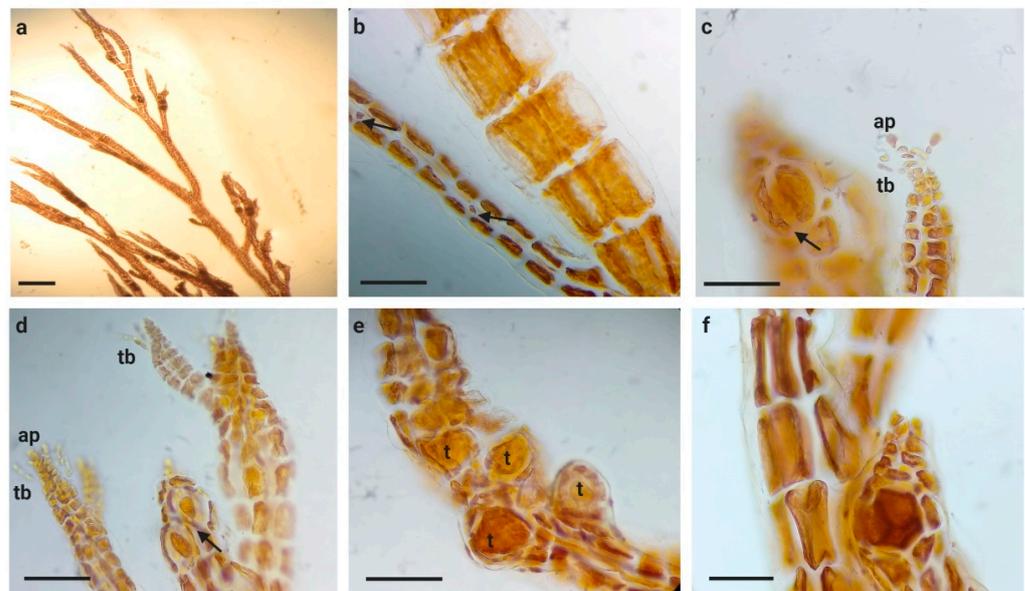


Figure 1. Habit, vegetative, and male structures of *Melanothamnus japonicus*. Microscopic observations were made in manual sections stained with Orange G, *Azer Scientific*. (a) Male thallus, scale bar = 1.5 mm; (b) Scar cells (arrows) on an erect axis, scale bar = 70 μm ; (c) Adventitious lateral arising from main axes with short trichoblasts (tb) and apical cell (ap); an apex with young tetrasporangia surrounded by two presporangial cover cells, scale bar = 70 μm ; (d) Apex of thallus with short trichoblasts and apical cell, an apex with young tetrasporangia (arrow), scale bar = 70 μm ; (e) Lateral branch with young tetrasporangia (t) in irregular series, scale bar = 70 μm ; (f) Surface view of a segment with tetraspores tripartitely divided and surrounded by cover cells, scale bar = 70 μm .

3.2. Molecular Phylogeny

To analyze species diversity in the class Florideophyceae from Ancash, Peru, and subsequently, to identify non-indigenous species from this region, we performed phylogenetic analyses using *rbcL*-5P sequences from 78 Florideophyceae specimens. The phylogeny resolved a monophyletic lineage corresponding to the class Florideophyceae with ML bootstraps (MLB) of 100% and a Bayesian posterior probability (BPP) of 1.00 (Figure 2). Within this class, we found the non-indigenous species *M. japonicus*, which showed a pairwise divergence of 0% with sequences from South Korea (the native range of this species), the USA, and Italy. The *rbcL*-5P phylogeny also showed that within the *Melanothamnus* group, the clade containing *M. japonicus* was a sister taxon (100% MLB and 1.00 BPP) to the clade containing native *Melanothamnus* species from Japan and South Korea, such as *M. harlandii* (Harvey) Díaz-Tapia & Maggs, *M. decumbens* (T.Segi) Díaz-Tapia & Maggs, *M. flavimarinus* (M.S.Kim & I.K.Lee) Díaz-Tapia & Maggs, and *M. yendoii* (T.Segi) Díaz-Tapia & Maggs. The sequences of native Peruvian species, such as *M. peruviansis* and *M. ramireziae*, instead, formed a distinct clade (87% MLB and 1.00 BPP), basal to the one containing *M. japonicus*. Therefore, it is highly likely that *M. japonicus* has been introduced to Peru by Northeast Pacific populations.

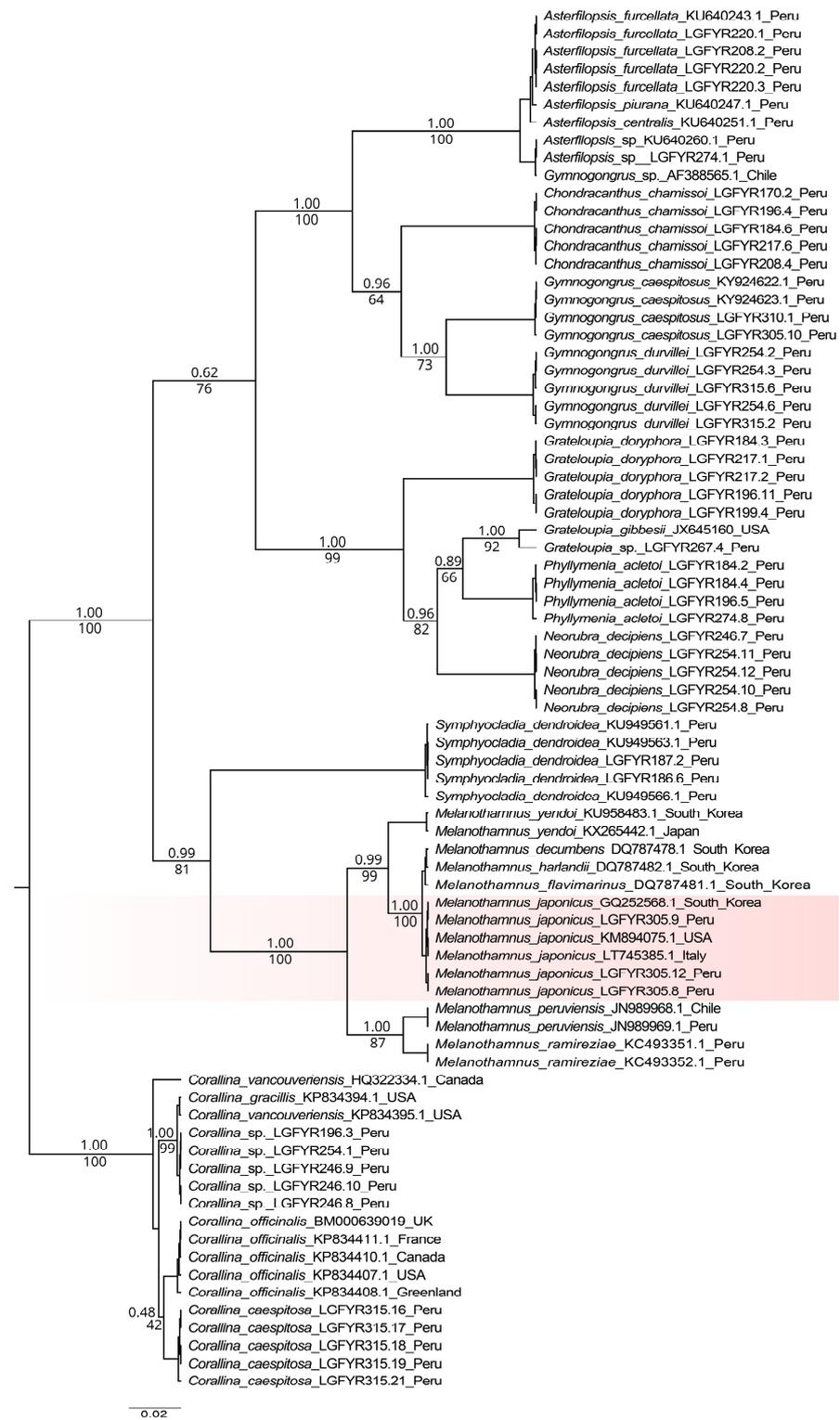


Figure 2. Bayesian inference tree of Florideophyceae species from Peru. The Bayesian inference analysis was performed for 50,000,000 generations using ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*-5P) sequences of Florideophyceae species collected along the coast of Ancash, Peru, and phylogenetically close species with available *rbcL*-5P sequences. The phylogenetic tree shows the sequences of *Melanothamnus japonicus* from Peru, the USA, and Italy in a strongly supported clade with sequences of *Melanothamnus* species native to Japan and South Korea, the native range of this species. The clade containing *M. japonicus* is shown in red. Bayesian posterior probability (BPP) and maximum likelihood bootstrap (MLB) values are indicated above and below the branches, respectively.

The *rbcL*-5P phylogeny also revealed a non-identified species from the genus *Corallina*, *Corallina* sp. This species showed high sequence similarity with sequences of *C. vancouveriensis* Yendo from the USA (0.3% pairwise divergence). Despite the low sequence divergence between *Corallina* sp. and *C. vancouveriensis*, an assignation to the species level could not be made since *Corallina* sp. also showed 2.5% pairwise divergence with *C. vancouveriensis* from Canada. Even though the sequence from Canada has the same species name as the sequence from the USA, it formed a different clade that was phylogenetically closer to *C. caespitosa* R.H.Walker, J.Brodie & L.M.Irvine from Peru. *Corallina* sp. also showed 1.8% and 2.1% pairwise divergence with sequences of *C. caespitosa* and *C. officinalis* Linnaeus, respectively. It is also important to note that even though *C. officinalis* has been reported for Peru, we could not find DNA sequences of this species from Peru in either BOLD Systems or GenBank databases, highlighting the absence of genetic studies for the identification of *Corallina* species from Peru.

Overall, among the 47 Florideophyceae specimens examined, the morphological and genetic information revealed the first report of a non-indigenous macroalgal introduction in Peru, i.e., *M. japonicus*, which extends the current distribution of *M. japonicus* into the Pacific coasts of South America.

4. Discussion

The morphological and phylogenetic analysis of this study showed the presence of the Asian species *M. japonicus* on the Pacific coast of South America for the first time. The morphological features of *M. japonicus* were in agreement with those described for *P. japonica*, the lectotype of *M. japonicus* characterized by Masuda et al. [59] and Kudo and Masuda [60]. *M. japonicus* was characterized by having four pericentral cells, few trichoblasts being composed of uninucleate cells, with branching points occurring at intervals of 5 to 12 axial cells in the main axes, and tripartitely divided tetrasporangia. The morphological identification was further confirmed by the molecular analysis, which showed a 100% similarity between the sequences of *M. japonicus* from Peru, South Korea, the USA, and Italy; the latter two being regions where *M. japonicus* has been reported as introduced [18,21,22]. It is important to note that to report a species as introduced, it is necessary to verify its genetic variation, which in most cases represents a subset of the genetic variation found in the native range of the species [61]. Even though the *rbcL*-5P sequences of *M. japonicus* collected in Chimbote's Port were identical, stating the presence of this species corresponds to an introduction event, based solely on the absence of intraspecific variation could not be performed, since this study has only three collections for comparison and utilized a molecular marker that, despite its ability to reconstruct phylogenetic relationships, is generally incapable of detecting intraspecific genetic variation [62,63]. A sampling design including other Peruvian coastal regions and the generation of supplementary molecular data using more variable markers, such as the mitochondrial cytochrome oxidase subunit 1 (*COI*-5P) gene [50,64], are still needed in order to determine whether there is intraspecific variation within the *M. japonicus* population present in Peru and whether it represents a subset of the genetic variation found within *M. japonicus* in its native range. Nevertheless, our phylogenetic analysis did show the sequences of *M. japonicus* and species such as *M. harlandii*, *M. decumbens*, *M. flavimarinus*, and *M. yendoi*, native to the Northeast Pacific, in a fully supported clade that diverged from the clade containing *Melanothamnus* species native to Peru. Hence, this study shows the introduction of *M. japonicus* into the Pacific coasts of South America and confirms the rapid settlement of this species outside its native range.

Although additional studies are required to determine the introduction vector for *M. japonicus*, this species was found in an important international port in Ancash, Peru. Several studies have shown that global shipping is the primary source of non-indigenous species [20,65]. For instance, using DNA metabarcoding on samples of ballast water taken from ships arriving at the Bay of Biscay, Ardura et al. [66] showed that about 22% of the total algae found in ballast waters were non-indigenous to that location. Importantly,

it has also been shown that among the algal species found in ballast water, the species from the genus *Polysiphonia*, a phylogenetically close genus to *Melanothamnus*, are able to survive prolonged periods in the ships' tanks and even increase in density [67]. Moreover, most algal spores have the ability to germinate when they are exposed to long periods of darkness (e.g., the conditions in the ships' tanks), an ability that appears to be unrelated to a particular taxonomic group or life-history style [68]. Like ballast water, ships' hulls also transport many sessile algal species between ports [69,70]. In fact, compared to ballast water and ship sediments, most non-indigenous species tend to be found on ships' hulls [71]. Because the oceanographic conditions of Chimbote are favorable for industrial vessels to enter, both ballast water and hull fouling are the most likely vectors for the introduction of *M. japonicus*. It is also important to note that using a species flow network based on ballast water exchange, Xu et al. [39] showed that the ships coming from the Pacific cluster (from Asian and Oceanian regions) are the most common vectors for the non-indigenous species invading South American countries. Hence, and considering that several Asian ships have been shown to arrive at the port of Chimbote [37], we hypothesize that *M. japonicus* was likely introduced from ships coming from Asia rather than those coming from the Mediterranean or the USA, regions where *M. japonicus* has also successfully settled.

We should also mention that species imported for aquaculture are known vectors for non-indigenous species [72,73]. Nevertheless, the only imported species native to Asia is the Japanese oyster *Crassostrea gigas* Thunberg, a species imported to Peru not from Japan but Chile, a country that has not reported the presence of *M. japonicus* within their biogeographic limits [74]. Hence, imported species for aquaculture can be excluded as an introduction vector for *M. japonicus*. In light of these observations, ballast waters and hull fouling from Asian ships are the most apparent sources of *M. japonicus*. It is very likely that after a first introduction event, the presence of favorable environmental conditions in Chimbote's Port (a water temperature between 17.2 and 22.8 °C, a temperature range in which *M. japonicus* can grow rapidly [60,75], and the minimal water current activity) contributed to the rapid adaptation and successful settlement of *M. japonicus* in this port.

Although several studies have shown the introduction and successful establishment of *M. japonicus* in different coastal regions [18,22], it is still unknown if its introduction will negatively impact native communities. By simulating the global spreading dynamics of marine non-indigenous species and comparing the predicted and observed species ranges, Seebens et al. [76] observed that *M. harveyi* (Bailey) Díaz-Tapia & Maggs, a species morphologically indistinguishable from *M. japonicus*, was placed within the top ten highly invasive species. Even though not all species from the same genus will necessarily demonstrate invasiveness [77], it has been shown that *M. japonicus* has rapidly spread through the regions into which it was introduced. For instance, Rindi et al. [20] and Sfriso et al. [21] reported that *M. japonicus* is nowadays one of the most common and widespread macroalgae in the Conero Riviera and the Venice Lagoon, respectively, indicating the high invasive potential of *M. japonicus*. Another important consequence of the establishment of *M. japonicus* in Peru is DNA introgression from the introduced species into a native species, a phenomenon already shown between *M. japonicus* and *M. harveyi* [22]. If this occurs, it could give rise to a more vigorous individual with a higher spreading ability, which could displace the parental species [78]. Future studies are still required to determine whether hybridization between *M. japonicus* and native Peruvian species is possible.

Additionally, Dijkstra et al. [7] indicated that the introduction of non-indigenous algal species leads to positive ecological effects, such as the generation of a more biogenetically complex habitat that is able to support twice or three times the richness and abundance of species from lower trophic levels as compared with native seaweed assemblages. Since the introduction of non-indigenous species can have different consequences, Katsanevakis et al. [79] stated that the "native good, alien bad" view is a misconception and that the role of most non-indigenous species in marine ecosystems is rather complex. Because species introductions can result in either good or bad consequences for the native community, it is necessary to establish preventive and mitigating measures to avoid any potential negative

impacts. On a global scale, Ficetola et al. [80] showed that climatic and land use information are useful tools for creating environmental models that can delineate areas with the highest risk of invasions. Considering that the databases containing the global distribution of algal species, such as Algaebase [81] and the Global Biodiversity Information Facility [82], are publicly available, and that from there, the climate tolerance range for each species can be inferred, an approach similar to the one implemented by Ficetola et al. could be conducted to identify probable introduction points for invasive algal species. If this were possible, such data could accelerate the implementation of preventive methods in areas with the highest risk of invasion.

In Peru, as a preventive measure, the law requires that all ships renew their ballast waters outside 12 nautical miles from the coast before entering the ports (Supreme Decree N° 009-2014-MINAM). However, this measure was not sufficient to stop the introduction of *M. japonicus* to Peru, which may have been introduced through hull fouling. Thus, it is also necessary to establish mitigating measures that take into account present introductions. This will require both studies focusing on detecting non-indigenous species and studies on the ecology and genetics of current introductions being prioritized. For the Peruvian government, these actions should be of utmost importance considering that in November 2014, the National Peruvian Strategy for Biological Diversity established as one of its goals to increase the regulatory mechanisms of invasive non-indigenous species by 2021 [83]. Considering that there is no previous information related to the study of marine non-indigenous species in Peru, studies concerning the presence of these species are not only needed but are urgent for the effective management of non-indigenous species introductions.

In conclusion, our phylogenetic analyses using the *rbcL*-5P gene dataset revealed the introduction of the Asian species *M. japonicus* into a South American country. Future research needs to focus on revealing the geographic extension of *M. japonicus* and determining the principal risks of its presence on the coasts of Peru, which until 2008 were considered pristine in terms of non-indigenous marine species [84]. Because our study showed the successful settlement of a non-native species likely transported into Chimbote's Port aboard international ships, our data could promote studies prioritizing the biomonitoring of *M. japonicus* along the Pacific coasts of Peru and other South American countries where this species might also have been introduced. Importantly, because the source region and possible vectors for the introduced species can be identified by comparing the introduced genotypes' distribution in the native range [61], the studies focused on biomonitoring exotic species should consider using markers able to detect genetic variation within species. Supplementary molecular data generated from mitochondrial DNA-derived markers provide an ideal approach for this purpose. Their high mutation rate in red algae, approximately four times that of the plastid and nuclear DNA [63,85], makes them the best markers to infer intraspecific genetic variation [50,64,86]. Finally, this study could serve as the starting point to implement preventive and mitigating measures against invasive species, especially in South American countries that adhere to the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM) [87], whose primary goal is to eliminate the risk of modifying the oceans' environmental conditions through the transfer of non-native species. This is strongly encouraged since although our survey did not constitute a comprehensive barcoding library of the Peruvian Florideophyceae diversity, an exotic species was revealed, suggesting there are more to be discovered.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/d13050176/s1>, Table S1: Sample information including locality, collectors name, collection codes, and GenBank accession number. GenBank accession numbers of the sequences generated during the present study are shown in bold.

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