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Research Article

Report on the invasive American brackish-water mussel *Mytella strigata* (Hanley, 1843) (Mollusca: Mytilidae) in Beibu Gulf

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Abstract

This study reports the first record of the biofouling mussel *Mytella strigata* (Hanley, 1843) in Beibu Gulf, China. Phylogenetic analysis based on the mitochondrial DNA cytochrome *c* oxidase gene sequences demonstrated a close relationship between mussels in Chinese seas and those in Singapore, Venezuela, India, the USA, the Philippines, Brazil, Colombia, Ecuador, and Trinidad. The results of multiple species delimitations showed that the mussel was *Mytella strigata*. Analysis of the haplotype network indicated that the American brackish water mussel may have invaded China from Colombia or other Asian waters through ships docked in ports or biofouling on the hulls. Mussels attach to oyster shells in large quantities. The minimum shell length of mussels collected in Guangxi Province was 23.9 mm, which exceeded the minimum length for sexual reproduction. The identification of *Mytella strigata* in Beibu Gulf represents the sixth record of this species from the Indo-Pacific region. In addition, the mussel was also found have invaded Fujian Province. Alien marine species may cause many negative effects and have the potential to harm the invaded ecosystems. Therefore, the distribution of this species within Chinese waters and potential damage caused by this mussel to the ecosystem and economy must be determined. The findings of this study will also provide a reference for formulating management policies for invasive species.

Key words: invasive species, biofouling mussel, Chinese waters, haplotype network, species delimitation

Introduction

Alien species are biological species that have never been distributed within a local area but were subsequently directly or indirectly introduced by humans. A suitable environment includes the absence of natural enemies, and invasive organisms compete fiercely with local organisms through rapid reproduction, thereby depriving endemic species of living space and food resources and eventually excluding and eliminating competing local species, which destroys the local ecological balance (Roy et al. 2014). With

the acceleration of globalization, an increase in shipping, aquaculture, and other activities has introduced species that were previously isolated for hundreds of millions of years to various seas worldwide, thus increasing the frequency of biological invasions (Walters et al. 2010). At present, more than 84% of marine ecological regions (ecoregions) are threatened by invasive species, and sharp declines in the biodiversity of various marine ecosystems have been caused by biological invasions (Bax et al. 2003; Molnar et al. 2008). China has a long coastline, numerous ports, a high population density, and frequent human activities, which provide favorable conditions for the introduction, colonization, and naturalization of many invasive organisms (Song et al. 2015).

In marine, brackish, and freshwater ecosystems, sessile suspended feeders, namely, bivalve mollusks, such as dreissenids and mytilids, are considered the most important invasive organisms (Aldridge et al. 2008), such as in Chinese Seas. For example, the notorious *Mytilopsis sallei* (Récluz, 1849), which was previously native to the tropical waters of Central America, invaded Fiji Island through the Panama Canal and then successively invaded Japan, Taiwan, and Fujian Province. It has now spread to most coastal areas of the South China Sea and has become the main invasive species in offshore waters (Cai et al. 2014; Gaonkar et al. 2010; Tan and Morton 2006; Wong et al. 2011). *Mytilus galloprovincialis* Lamarck, 1819 was successfully introduced into China, Japan, Australia, North America, and South Africa from the Mediterranean Sea in the early 20th century through shipping or farming (Anderson et al. 2002; Branch and Steffani 2004; Lee and Morton 1985; McDonald et al. 1991). Currently, *Mytilus galloprovincialis* is distributed along the coast of China, mainly in the Yellow Sea and Bohai Sea.

Mytella strigata (Hanley, 1843) is another notorious invasive biofouling mussel, and it was reported to have invaded Taiwan (Huang et al. 2021). The native distribution of these mussels includes the Atlantic coast of South America and the Pacific coast of Central and South America from Mexico to Ecuador (Boehs et al. 2004; Boudreaux and Walters 2006; Keen 1971; Szefer et al. 1998). It has been recorded as an invasive species in Florida (Boudreaux and Walters 2006). In the Indo-Pacific, it has invaded Luzon Island, the Philippines (Rice et al. 2016; Vallejo et al. 2017a), Johor Strait, Singapore (Lim et al. 2018), the inner Gulf of Thailand (Sanpanich and Wells 2019), brackish water in Cochin, India (Jayachandran et al. 2019) and the southwestern coast of Taiwan Island, China (Huang et al. 2021). Researchers have pointed out that *Mytella strigata* may harm local ecological environments and economic shellfish farming. Due to the frequent occurrence of biological invasions, alien species that have invaded or may invade Chinese seas must be identified.

During a sampling trip to Guangxi Province in 2021, an unknown mussel species attached to oysters was found, and it was also found in

mangrove forests and mud flats near mangroves. Based on morphological and molecular evidence, this mussel was identified as *Mytella strigata* (Hanley, 1843), which was previously reported to have invaded Taiwan (Huang et al. 2021). The finding of this species on the coast of Guangxi Province represents its sixth record from the Indo-Pacific region. The present study aimed to report the invasion of *Mytella strigata* into the Beibu Gulf, South China. We preliminarily analyzed the origin of the invasive population based on the haplotype network. The findings of this study provide a reference for formulating management policies for invasive species.

Materials and methods

Samples were collected from a seafood market in Guangxi Province, China, as well as from the wild in October 2021. All specimens were stored in 75% alcohol for morphological examination and molecular analysis. The shells were observed under a Zeiss SteREO Discovery.V12 stereo microscope (Zeiss, Wetzlar, Germany) and photos were taken using a Canon EOS6D camera. Since *Mytella strigata* has double uniparental inheritance (Alves et al. 2012; de Souza et al. 2015), we did not extract DNA from the gonad tissue to avoid confusion. Genomic DNA was extracted from the foot of the mussels using the DNeasy Blood & Tissue kit (QIAGEN, Germany) according to the manufacturer's instructions and frozen at −20 °C.

The mitochondrial DNA (mtDNA) cytochrome *c* oxidase subunit I (COI) region was amplified using universal primers LCO1490 and HCO2198 (Folmer et al. 1994). The PCR mix consisted of 12.5 μL of 2× Es TaqMasterMix (Dye) (Cwbio, China), 1.0 μL of each primer (5.0 μM), and 2 μL crude DNA, and then water was added to a volume of 25 μL. The amplification conditions consisted of an initial denaturation for 5 min at 95 °C, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 46 °C for 30 s, and extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. Amplification products were detected using agarose gel electrophoresis and sequenced by Sangon Biotech. The sequences were uploaded and compared with the available sequences in GenBank using the National Center for Biotechnology Information Basic Local Alignment Search Tool (BLAST). All available *Mytella strigata* COI sequences were retrieved from GenBank (Table 1, Figure 1). Two to five sequences from each locality were randomly selected for the phylogenetic analysis. The sequences of each region were aligned independently using MAFFT v.7 (Katoh and Standley 2013) with the G-INS-i and Q-INS-i algorithms for the protein-coding and ribosomal regions. The best-fit evolutionary models were selected using the Akaike information criterion, as implemented in jModeltest2 (Darriba et al. 2012). Phylogenetic trees were constructed based on the maximum likelihood (ML) and neighbor-joining (NJ) methods using MEGA X with bootstrap values for 2000 replicates (Kumar et al. 2018).

Table 1. *Mytella strigata* (= *Mytella charruana*) mt-COI sequences used to construct the TCS haplotype network.

GenBank Accession No.	Location	Abbreviation	Reference
KX776480, KX499630	Philippines	Phi	Rice et al. (2016)
KP013759–KP013804, JQ685156–JQ685157, MF075049–MF075069, MF075071–MF075072	Brazil	Bra	Alves et al. (2012); Calazans et al. (2017); de Souza et al. (2015)
MF075035–MF075048	Uruguay	Uru	Calazans et al. (2017)
EU917165–EU917175, MF075008–MF075034	Colombia	Col	Calazans et al. (2017); Gillis et al. (2009)
EU917142–EU917163	Ecuador	Ecu	Gillis et al. (2009)
MF074990–MF074963, EU917172–EU917180, FJ940721	USA	USA	Calazans et al. (2017); Gillis et al. (2009)
MN165292–MN165293, MN165295–MN165296, MN531546–MN531553, MN603972	India	Ind	Jayachandran et al. (2019)
MF075073–MF075107	Trinidad	Trin	Calazans et al. (2017)
MG736074–MG736082	Singapore	Sin	Lim et al. (2018)
OM194200–OM194202, OM194195–OM194198, OM194191	Venezuela	Ven	Lodeiros et al. (2021)
MW020358, MW020361, MW020364	Taiwan	TW	Huang et al. (2021)
OQ430715–OQ430719	Guangxi Prov.	GX	This study

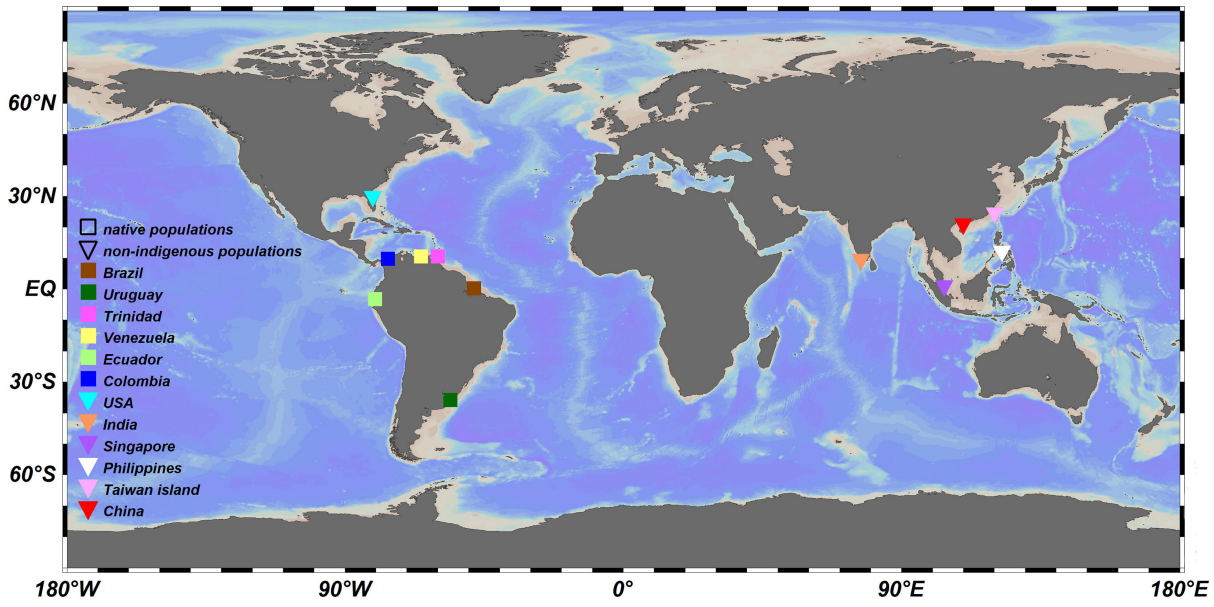

Figure 1. Distribution of *Mytella strigata* populations analyzed in this study.

Bayesian inference (BI) analysis was performed using MrBayes v.3.2.7, with three parallel runs of five million generations each, sampling every 1000 generations, and burn-in set to 25% (Huelsenbeck et al. 2001).

Multiple species delimitation methods were performed to examine whether this mussel is the same species as *Mytella strigata* from other regions. COI data were analyzed using the program Automated Barcode Gap Discovery (ABGD; Puillandre et al. 2012). Single gene trees were analyzed by applying the Bayesian implementation of the Poisson Tree Processes model (bPTP; Zhang et al. 2013) at the web server of the Heidelberg Institute for Theoretical Studies, Germany (http://species.h-its.org/). The general mixed Yule coalescent model (GMYC; Pons et al. 2006) was used to determine the species of analyzed individuals according to ultrametric time trees derived from single-locus data.

To infer the invasive path of mussels off the coast of China, a TCS haplotype network was constructed based on the mtCOI sequences listed

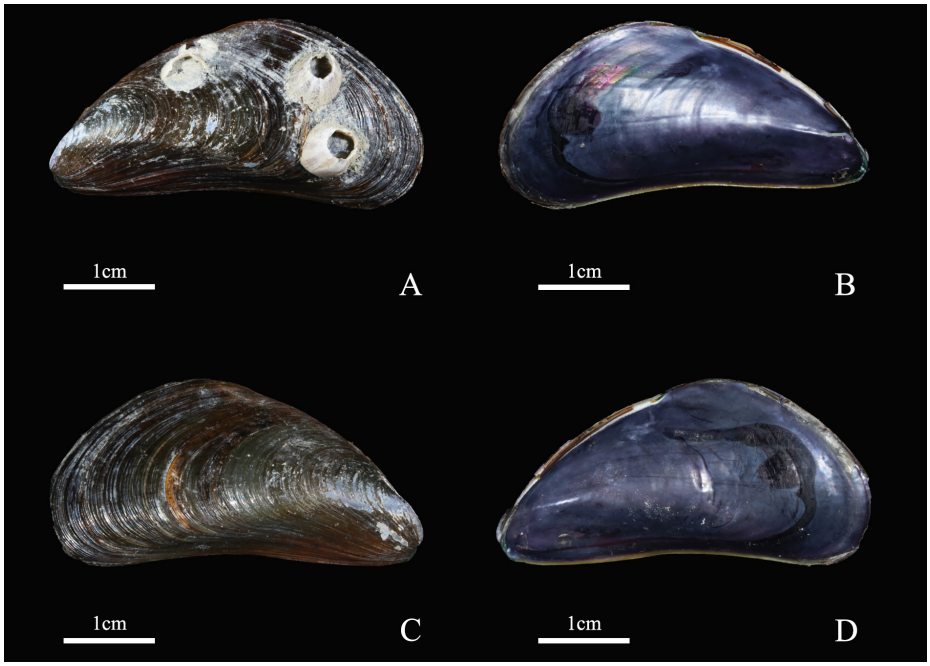

Figure 2. Shells of *Mytella strigata* collected along the coast of Guangxi, China, in 2021. Photos by Yanan Yu.

in Table 1 (Clement et al. 2000). Identification of haplotypes, grouping of individuals by population, and the generation of the file to be run in Arlequin v.3.5.2.2 (Excoffier and Lischer 2010) were carried out using DNASp v.6 (Rozas et al. 2003). Networks were constructed using PopART v.1.7 (Leigh and Bryant 2015).

Results

Identification and description of the shell

The maximum shell length of mussels collected in Guangxi Province was 40.9 mm (Figures 2, 3A, B). The shells of this species are cuneiform, equivalve, inequilateral, nutbrown, or black, and small individuals have bluish-brown shells. The shell surfaces are coarse with conspicuous commarginal lines that are coarser near to umbo. The dorsal margin is straight and slightly curved, with a depressed umbo, whereas the ventral margin is slightly concave but longer than the dorsal margin. The interior of the shell is dark purple and glazed with a pitted resilial ridge below the ligamental channel in the dorsal margin. Two strong lateral teeth are located near the umbo area in the ventral margin. The anterior adductor scars are small and inconspicuous, situated near the anteroventral margin, and the posterior adductor scars are round, large, and continuous, with elongated pedal retractor scars. The pallial sinus is a curved line towards the adductor scar, which is a unique characteristic of *Mytella strigata* compared to other mussels. Based on this morphological identification, we confirmed that this mussel was *Mytella strigata*, which was previously reported to have invaded Taiwan (Huang et al. 2021).

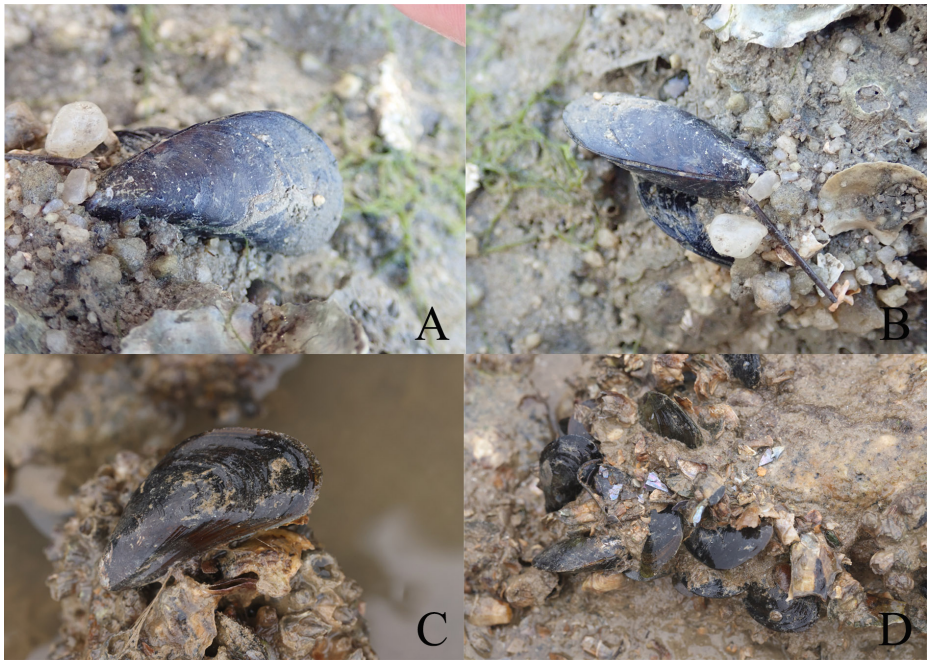

Figure 3. Invasive mussel *Mytella strigata* observed in Jinhai Bay, Beihai, Guangxi Province (A and B), and Tong'an Beach, Xiamen, Fujian Province (C and D). Photos by Mengling Liu and Yi Liu.

According to the photos provided by Dr. Yi Liu, the mussel also occurred in large quantities along the coast of Fujian Province (Figure 3C, D), at the Yunxiao National Mangrove Reserve, and along the beaches of Xiamen.

Phylogenetic analysis of the COI sequences

The nucleotide BLAST results showed 99–100% similarity with the *Mytella strigata* COI sequences recently reported from Singapore, India, and Taiwan. Sequences from *Perna viridis* (Linnaeus, 1758) belonging to the family Mytilidae were selected as an outgroup. The best-fitted evolutionary models were selected as HKY+I using the Akaike information criterion implemented in jModeltest2. The evolutionary relationship of the mussels was depicted on a phylogenetic tree constructed using mtDNA COI sequences based on the ML, NJ, and BI methods. All these trees showed very similar topologies (Figure 4). All species delimitation results indicated that the mussel was *Mytella strigata*. Phylogenetic analysis demonstrated that all sequences of *Mytella strigata* clustered into two clades (Clade A and Clade B). Clade A revealed a close relationship between mussels in Chinese seas, Singapore, Venezuela, India, the USA, the Philippines, Brazil, Colombia, Ecuador, and Trinidad. The largest genetic distance between all sequences of *Mytella strigata* was 0.0757. The mean distance between clades A and B was 0.0695.

Haplotype patterns and network

A haplotype analysis of *Mytella strigata* was performed based on 279 COI sequences from the USA, Brazil (Bra), Colombia (Col), Uruguay (Uru),

Figure 4. Phylogenic tree based on the mt-COI gene showing the phylogenetic relationship between *Mytella strigata* samples and the outgroup *Perna viridis*. Bayesian posterior probability, maximum likelihood, and neighbor joining bootstrap scores (left, middle, and right, respectively) are shown above the branch. The results of three species delimitation methods are shown on the right of figure (each gray rectangle represents one species).

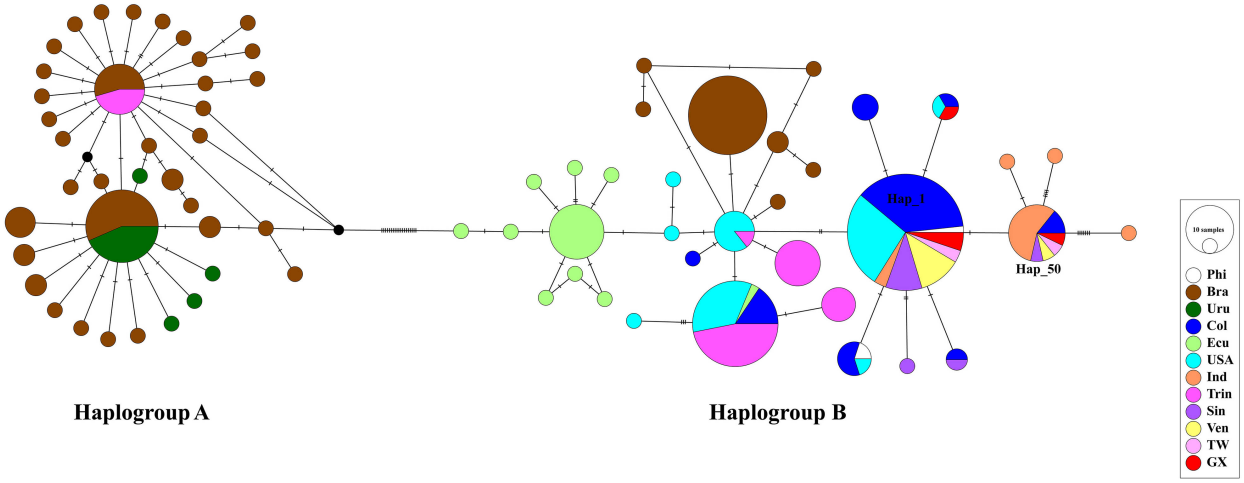

Figure 5. TCS haplotype network constructed using mt-COI sequences shows the relationship between natural and introduced *Mytella strigata* populations in the world. The lengths of the lines connecting the circles are not drawn to scale. The overall frequency of each unique haplotype used in the analysis is indicated by the size of the circle. The colors of the circles indicate the areas in which the haplotypes were present (consistent with the colors in Figure 3), while the black circles indicate inferred or missing haplotypes.

Ecuador (Ecu), Trinidad (Trin), Venezuela (Ven), the Philippines (Phi), India (Ind), Singapore (Sin), Taiwan (TW) and Guangxi Province (GX) (Table 1, Figure 1). All sequences were divided into 12 groups according to their geographical distribution (Table 1). A total of 72 distinct mt-COI haplotypes (Hap_1-Hap_72, N = 74) were identified. The TCS haplotype network indicated that all haplotypes clustered into two haplogroups (Figure 5). The grouping of haplotypes was consistent with the phylogenetic tree clades. Haplogroup A contained Brazilian, Trinidadian, and Uruguayan individuals, and haplogroup B contained all geographic regions, except for

Uruguay. Haplogroup A was separated from haplogroup B by the presence of 19 mutations. In addition, the Brazilian population had high genetic diversity, with 41 haplotypes. The GX haplotypes occurred in Hap_1, Hap_48, and Hap_50. Among them, GX shared three haplotypes with Col (Hap_1, Hap_48, Hap_50), two haplotypes with TW, Sin, Ind, Ven (Hap_1, Hap_50), and USA (Hap_1, Hap_48), and one haplotype with Phi (Hap_1). Each of the three shared haplotypes was separated by a single mutation.

Discussion

The curved pallial line can be used as a unique feature for distinguishing *Mytella strigata* from other mussel species (Mediodia et al. 2017). After morphological identification and species delimitation, we confirmed that this mussel was the invasive species *Mytella strigata*. Stenyakina et al. (2010) performed analyses of gonad morphology, gametogenesis, and sex ratios and found that *Mytella strigata* could be reproductively mature even at 12.5 mm in length. The minimum shell length of mussels collected in Beibu Gulf was 23.9 mm, which exceeded the minimum length of sexually reproductive individuals. This suggests that the population of *Mytella strigata* is fully established and already reproducing in China. This is not surprising because the regions around Chinese seas have a long coastline, numerous ports, high population density, and frequent human activities, thus providing favorable conditions for the introduction, colonization, and naturalization of invasive organisms (Song et al. 2015).

Mussels are native to both the Pacific and Atlantic coasts of tropical America (Keen 1971). The invasive nature of this species outside its native range was first reported in 1986 when a large population of *Mytella strigata* was observed inside seawater intake pipes at a power plant in Florida (Harry 1987). Since then, *Mytella strigata* has been reported to have gradually invaded the United States, the Philippines, Singapore, India, Thailand, and Taiwan (Fabiosa et al. 2021; Huang et al. 2021; Jayachandran et al. 2019; Lim et al. 2018; Sanpanich and Wells 2019; Vallejo et al. 2017b). Calazans et al. (2017) showed that all matrilinear haplotypes from invasive populations in the southeastern USA were attributable to Colombian haplotypes. In addition, a haplotype network analysis between populations suggested that mussels in the Philippines and Singapore originated from the Caribbean coast of South America (Lim et al. 2018; Rice et al. 2016). In this study, GX shared haplotypes with all Asian populations (Figure 5), thus revealing frequent genetic exchange between populations in the Indo-Pacific region. The haplotype network showed that all GX haplotypes were shared with Col. According to the TCS haplotype network, we inferred that *Mytella strigata* might have invaded China from Colombian or other Asian waters. In addition, the phylogenetic tree and haplotype network showed that *Mytella strigata* could be clustered into two groups, which may be due to genetic divergence caused by distance isolation.

Why does *Mytella strigata* invade other seas so frequently and rapidly? Oliveira et al. (2005) suggested that *Mytella strigata* has a planktonic larval stage that remains in the water column for 10–15 days before settlement, thus allowing for a high level of dispersal ability. Mussels can tolerate a wide range of salinities between 2 and 40 psu and have the capacity to invade a wide variety of saline environments with significant freshwater or marine input (Rice et al. 2016). In addition, this species can withstand long-distance travel in ballast water (Yip et al. 2021). Undoubtedly, these traits have enabled this mussel to become an important invasive species in several regions of the world. Lim et al. (2018) suggested that the invasion of *Mytella strigata* into Singapore was mediated by ballast water discharge from ships or biofouling on the hulls of shipping boats. These characteristics allow *Mytella strigata* to invade other areas frequently and rapidly. In addition, Beibu Bay is well developed for maritime applications, which provide convenient conditions for species invasion. Thus, we inferred that *Mytella strigata* invaded the Chinese coast through ships docked in ports or biofouling on the hulls. Unfortunately, direct identification of the pathways and frequencies of invasion would require detailed and frequent examinations of ballast water and ship hulls, which are difficult to sample adequately, even under optimal circumstances (Wong et al. 2011).

In most cases, marine alien species may cause many negative effects and have the potential to harm local ecosystems because these alien species compete with native organisms and disrupt the ecological balance of the associated water bodies. Moreover, invasive organisms interbreed with indigenous organisms, resulting in genetic contamination, their mass reproduction affects fishery production and causes biofouling or corrosion, and they may introduce pathogenic microorganisms (Molnar et al. 2008; Xu et al. 2006; Zhang et al. 2020). Although *Mytella strigata* is sold in Thailand and has been trialed as a potential food source for rock lobsters (Sanpanich and Wells 2019), it currently has no economic benefit in most countries. During sampling, we found that mussels were attached to oyster shells in large quantities. This indicated that *Mytella strigata* could exert a negative impact on oyster farming. In addition, mussels were also found to occur in large quantities along the coast of Fujian Province (Figure 2), at the Yunxiao National Mangrove Reserve, and on the beaches of Xiamen. Therefore, the distribution of this species within Chinese waters must be determined and whether it has caused damage to the ecosystem and economy should be studied. The correct identification of invasive species is the first step for mitigating the associated ecological and economic damage (Huang et al. 2021). The findings of this study provide a reference for formulating management policies for invasive species. Furthermore, the frequent occurrence of biological invasions necessitates the formulation of monitoring, surveillance, and impact mitigation measures.

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Author's contribution

Junlong Zhang: research conceptualization, funding provision, writing – review and editing; Yanan Yu: sample design and methodology, data analysis and interpretation, writing – original draft, review, and editing; Qi Gao: morphological study, writing – original draft; Mengling Liu: sample collection, morphological study, writing – original draft; Jingqi Li: molecular experiments; Shuo Wang: writing – review and editing. All authors contributed critically to the drafts and gave final approval for publication.

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