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Short Communication

Recognition of *Ficopomatus southern* 1921 (polychaeta: serpulidae) in Can Gio, Vietnam

Quoc Phu Dao[1, 2], Thong Minh Le[1, 3], Anh Phuong Tran[1, 2], Ha Manh Bui[4]*

[1]Viet Nam National University, Thu Duc District, Ho Chi Minh City 70000, Vietnam
[2]Institute of Environment & Natural Resources, Thu Duc District, Ho Chi Minh City 70000, Vietnam
[3]International University, Ho Chi Minh City, Thu Duc District, Ho Chi Minh City 70000, Vietnam
[4]Faculty of Environment, Saigon University, 273 An Duong Vuong Street, District 5, Ho Chi Minh City 70000, Vietnam

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Abstract

This study focuses on the identification and characterization of *Ficopomatus shenzhensis*, a species of serpulid worm found in shrimp ponds of the Saigon River in Vietnam. The aim of the research is to understand the species' characteristics and find potential solutions to control its growth in aquaculture ponds. The methodology involved sample collection from shrimp ponds in the Can Gio biosphere reserve, morphological examination, and DNA-based species identification using the 18S ribosomal RNA gene. The results revealed the presence of *Ficopomatus shenzhensis* in the study area, and its morphological features were described. Additionally, DNA sequencing confirmed its identity, and phylogenetic analysis showed a close relationship with *Ficopomatus shenzhensis* from previous studies. The distribution of *Ficopomatus shenzhensis* was found to be widespread in the southern coastal provinces of Vietnam, suggesting a long-standing invasion. The species likely arrived through ballast water transportation. The ecological observations indicate that *Ficopomatus shenzhensis* attaches to various materials and requires high levels of dissolved oxygen. The study highlights the need for monitoring and further research on the distribution and impact of *Ficopomatus shenzhensis* in coastal lagoons of Vietnam.

Keywords: Serpulidae, Taxonomy, *Ficopomatus*, shrimp ponds, Can Gio biosphere reserve

Introduction

Shrimp farmers in the Can Gio region have recently faced a troubling issue: an unidentified worm species is proliferating in their farming ponds, causing a significant decline in shrimp productivity. This worm, despite its high population density, has remained unclassified, falling within the broad genus of *Ficopomatus* but without a specific species designation. Within the diverse family of polychaete worms, known as Serpulidae, and belonging to the Sabellida group, the *Ficopomatus* genus is of particular interest. Serpulidae worms are distinguished by their calcareous tubes, leading to their evocative nickname, "flowers of the sea". These organisms are further defined by their unique radiolar crowns and bipartite body division into thoracic and abdominal regions [1, 2]. The *Ficopomatus* genus, a member of the subfamily *Ficopomatinae*,

*e-mail: manhhakg@sgu.edu.vn

is remarkable for its euryhaline adaptability. This allows the species within this genus to flourish across various aquatic environments, including freshwater, brackish water, marine, and even hypersaline habitats [3-5].

However, the uncertainty surrounding the specific species within the *Ficopomatus* genus found in Can Gio's shrimp ponds necessitates further study. The central focus of our research, therefore, is to accurately identify the scientific name of this species, elucidate its environmental preferences, and map its regional distribution. Further, we aim to assess the potential risks that it poses to shrimp farming. This comprehensive understanding not only contributes to the broader knowledge of the *Ficopomatus* genus, but also offers pragmatic solutions for local shrimp farmers combating this pervasive issue. Thus, the novelty of our study lies in our systematic approach to both scientific identification and practical application.

Methodology

Study Site and Sample Collection

Specimens were collected in typical shrimp ponds in the buffer zone of the Can Gio biosphere reserve (Fig. 1). The survey of the habitat in shrimp ponds in this area lasted from March 2019 to February 2022.

Specimens (tubes and worms) were carefully scraped off hard substrates (such as the surface of the trunk or other materials in a shrimp pond) with scalpels. All specimens were kept in a 4% formaldehyde solution. For species identification using DNA information, the worms were kept in absolute ethanol (>90%) to preserve DNA for extraction.

Morphological Examination

After subjecting the serpulids to a 24-hour period of starvation, their morphological characteristics were examined. To obtain cross-sections of the tubes, they were wetted on a whetstone and then polished on frosted glass. Chaetae and uncini were separated and placed in a solution composed of 50% glycerin and ethyl alcohol, followed by staining with methylene blue. Live individuals were imaged using an Olympus SZ51 stereo microscope and measured using a calibration slide. The uncini and chaetae were further observed using a Leica Dmil Led microscope (Leica Microsystems, Wetzlar, Germany), with their images captured using a Flexacam C1 digital camera. The identification process was carried out by referencing taxonomic keys and/or descriptions outlined in previous studies [1, 3, 6].

Genomic DNA Extraction and PCR

Genomic DNA was extracted from individual specimens of 20 worms following a standard extraction protocol. In brief, each sample underwent incubation at

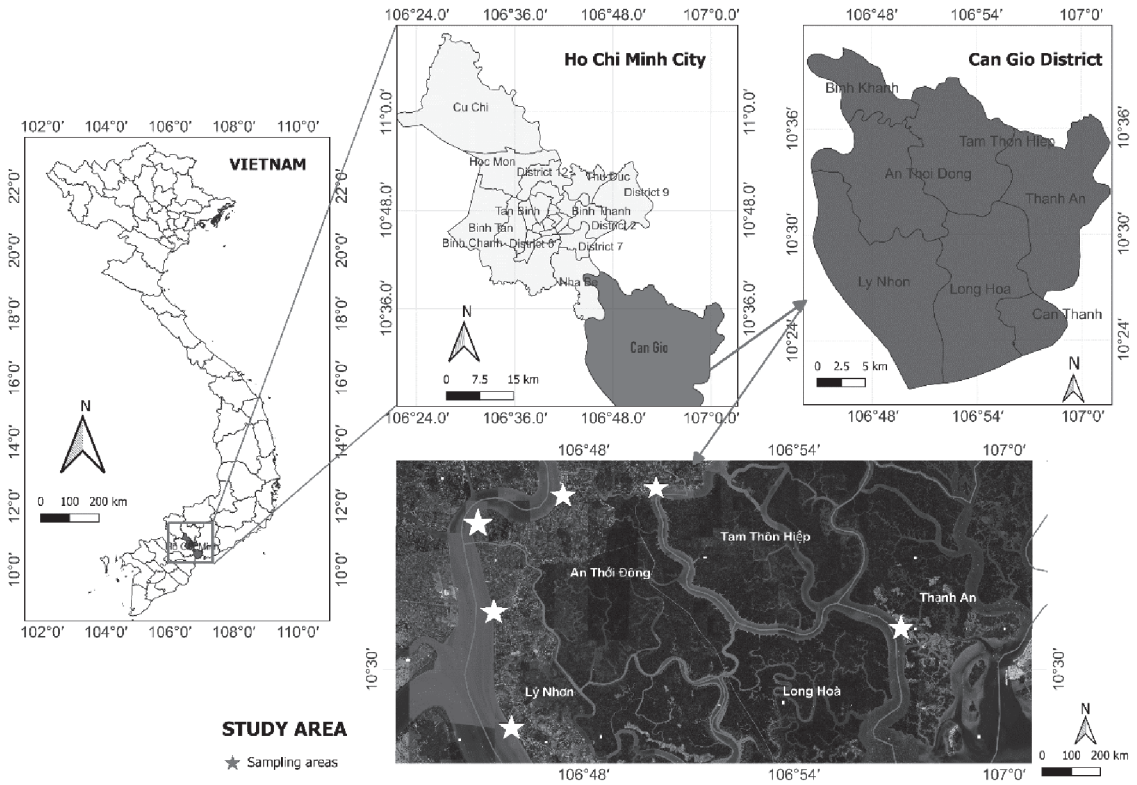

Fig. 1. Sampling locations of *Ficopomatus* sp. Specimens.

55° C for a period of 3 hours in a 100 µl solution of lysis buffer. This was succeeded by the introduction of 10 µl of proteinase K (20 mg/ml) from Promega, Madison, WI. Subsequently, the genomic DNA was purified via phenol/chloroform extraction and precipitated using alcohol. The Polymerase Chain Reaction (PCR) was employed to amplify the extracted DNA, utilizing forward and reverse primers. The sequence of the forward primer, ggc18f, was 5'-TAAGCCATGCACGTGTAAGT-3', while the reverse primer, ggc18r, was 5'-CAGTCTAGTTCGAACTTCTT-3' [6, 7]. The thermal cycling profiles for the PCR were established as follows: an initial denaturation phase at 95ºC for 5 minutes, followed by 35 cycles each consisting of 1 minute at 94ºC, 1 minute at 58ºC, and 1 minute at 72ºC. The process was concluded with a final extension phase

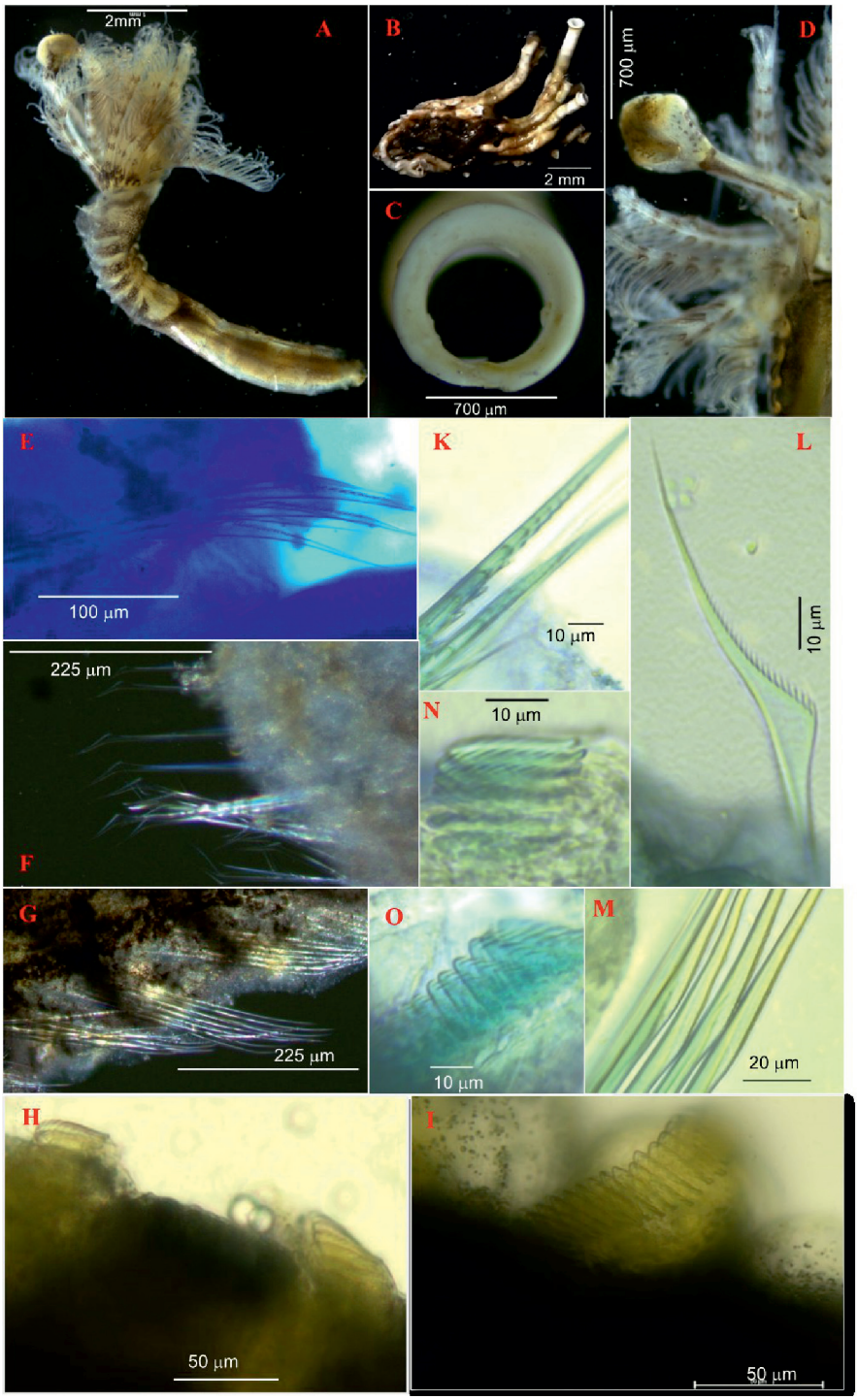

Fig. 2. Photographs of *Ficopomatus shenzhensis*: A) Lateral view of a worm removed from its tube; (B) Tube; (C) Cross-section of tube; (D) Dorsal view of the operculum; (E) Collar chaetae; (F) Abdominal chaetae; (G) Thoracic chaetae; (H) Abdominal uncini; (I) Thoracic uncini; (K) toothed and limbate chaetae from collar; (L) Details of abdominal chaetae; (M) Details of thoracic chaetae; (N) Details of abdominal uncini; (O) Details of thoracic uncini.

at 72ºC for 5 minutes, using both forward and reverse primers.

Sequencing and the Phylogenetic Analysis

The amplified products were purited and the resulting product was outsourced to a commercial service (National Key Laboratory of Gene Technology, Hanoi, Vietnam) for sequencing in both forward and reverse directions, utilizing the two PCR primers as sequencing primers.

Sequencing traces' files (ab1 raw) were assembled using CLC Main Workbench version 7.8.1 (Qiagen, Germany) with *Ficopomatus shenzhensis* 18S ribosomal RNA gene as a reference sequence (HQ433336). Sequencing quality and base calling accuracy were also manually verified on the alignment consisting of reference sequence, assembled sequence, and traced sequences in the CLC Main Workbench program. The verified assembly was compared to a non-redundant nucleotide database from NCBI using the Blastn algorithm embedded in CLC Main Workbench to infer phylogenetic relationships. ClustalW multiple alignment was carried out with BioEdit 7.0.1, and phylogenetic analyses used maximum-likelihood (ML) method by Mega 11.0, using the 18S rDNA sequence of *Sabella spallanzanii* (Sabellida, Sabellidae) (HM800962) as the outgroup.

Data Analysis

The environmental factors pH, DO, temperature, turbidity, TDS, Oxygen and conductivity were conducted by standard method and the samples were duplicated collected.

Results and Discussion

Prior to our research, there were no recorded instances of *Ficopomatus shenzhensis*, or any other species from the *Ficopomatus* genus, in Vietnam's coastal regions. However, our comprehensive examination, which included both morphological assessments and genetic analysis of the 18S ribosomal RNA gene, disclosed a perfect match between the worm studied in our investigation and *Ficopomatus shenzhensis* [7]. This newly identified *Ficopomatus shenzhensis* species, henceforth referred to as 'sp. nov,' is defined and depicted in the subsequent Fig. 2.

Morphological Description

The tube was shining white, forming aggregations (Fig. 2B), measuring approximately 9.20±0.10 mm (n = 10) in length. It is circular in cross-section and lacks a longitudinal ridge (Fig. 2C). The body included a branchial crown, thorax, and abdomen, 7.35±1.70 mm (n = 10) in length (Fig. 2A).

The branchial crown corresponds to 1/4 or 1/5 of the total body length, including the operculum and branchial radioles. Branchial radioles are yellow, with 6-7 dark bands on the radioles, bearing rows of ciliated filamentous pinnules, which have unequal size. Branchial radioles arising from the pair of lobes, having 9 radioles on each side, measuring on average 1.79±0.21 mm (n = 10) in length (Fig. 2A).

The operculum and its peduncle occur in the position of the 1st branchial radiole on the left side (Fig. 2D). The operculum was pear shaped as follows: 0.83±0.12 mm long (n = 10), 0.69±0.15 mm wide (n = 10), absent spines, with a convex horny plate. The horny plate has light brown spots with a V-shaped furrow dorsally (Fig. 2D), and the hemispherical proximal part has black spots. The peduncle of the opercular was smooth, 1.12±0.28 mm long (n = 10). It decreases in diameter from the base of the operculum to the base of the branchial crown, with brown distally, yellow toward the middle, and brown proximally.

The thorax of the organism consists of seven chaetigers, characterized by distinct fleshy thoracic membranes that are white and not interconnected across the thorax. The collar encompasses the entire margin and exhibits well-developed lobes. The collar chaetae are serrated in shape, comprising seven to ten chaetae of two types on each side. The distal ends of some chaetae in the collar terminate in simple blades (Fig. 2E). The collar chaetae possess two or three large teeth at the base and one or two rows of sharp, slender teeth towards the distal end (Fig. 2K). In the thoracic region, the chaetae exhibit a straightforward blade

Table 1. BLAST results of the worn collected from Vietnam by rDNA sequence analysis.

Sequence	Max score	Query coverage (%)	E-value	Per. Ident (%)	Accession
Ficopomatus shenzhensis 18S ribosomal RNA gene	3079	100	0.0	100.0	HQ433336
Marifugia cavatica 18S ribosomal RNA gene	2636	100	0.0	95.2	EU167530
Ficopomatus enigmaticus 18S ribosomal RNA gene	2595	99	0.0	94.8	AY577889
Ficopomatus miamiensis 18S ribosomal RNA gene	2490	99	0.0	93.6	EU167531

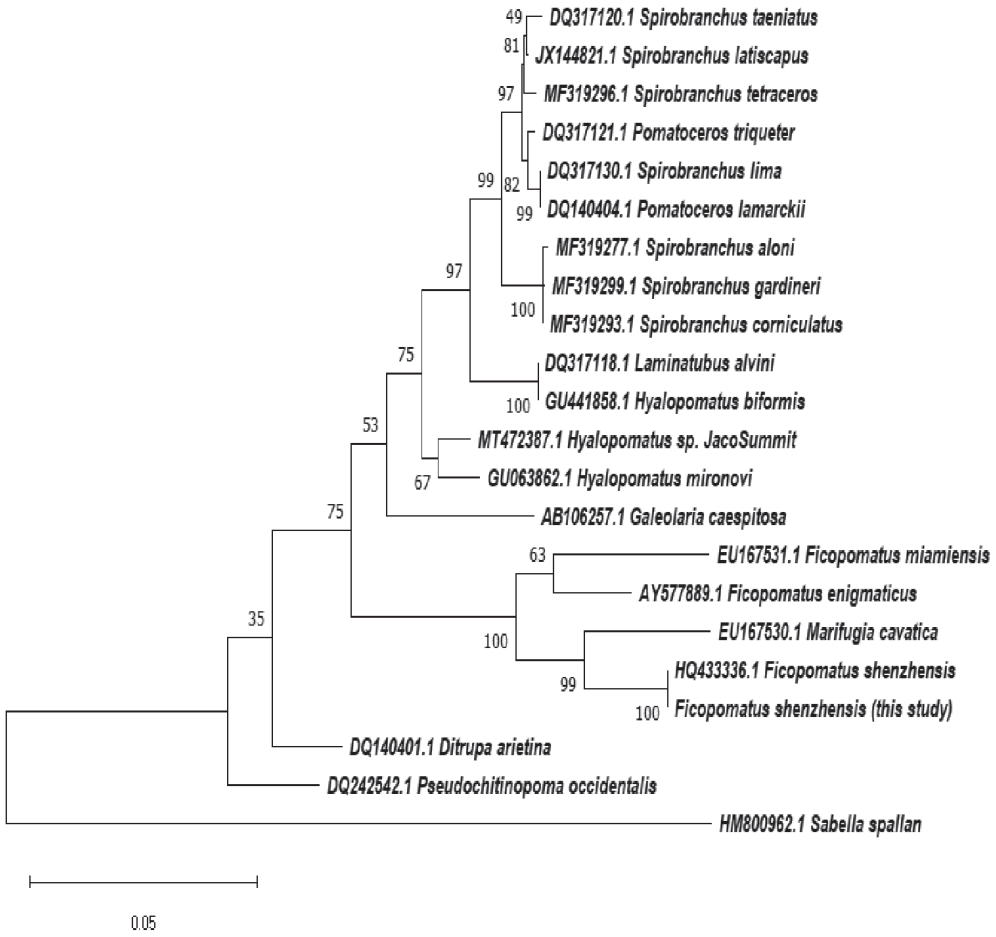

Fig. 3. Phylogenetic tree constructed via the maximum likelihood method and kimura 2-parameter model, with *sabella spallanzanii* as the outgroup. Bootstrap values (%) from 1000 replicates are indicated near nodes. branch lengths correspond to 0.05 substitutions per site.

shape (Figs 2G, M), measuring 0.50±0.04 mm in length (n = 10), while the thoracic unici display a saw-shaped structure with nine teeth, measuring 31±15 μm in length (n = 10) (Figs 2I, O).

The abdomen of the organism appears light greenish-yellow, with an average length of 3.45±0.22 mm (n = 10). Each abdominal segment bears 2-4 geniculate chaetae (Fig. 2F), with the proximal half displaying a serrated shape (Fig. 2L). The length of the abdominal chaetae measures 0.27±0.025 mm (n = 10) at the shaft end and 0.05±0.005 mm (n = 10) at the distal end. The abdominal uncini (Figs 2H, N) are rasp-shaped, featuring two rows of 10-12 teeth each.

DNA-Based Species Identification

To identify the species using genetic information, the genomic DNA of tube worms was utilized for the amplification of the 18S ribosomal RNA gene. Universal primers designed for 18S rDNA sequences of *F. enigmaticus*, *F. macrodon*, and *F. miamiensis* [1] were employed for this purpose. A 1765 bp amplicon was successfully generated, devoid of any nonspecific products.

Subsequently, the PCR product was subjected to direct Sanger sequencing, using the PCR primers as sequencing primers. Following successful sequencing in both directions, sequence assembly was performed, followed by manual verification, resulting in the generation of a 1667-bp contig of the 18S ribosomal RNA gene with high confidence (Supplemental data S1 and S2). Notably, we observed complete sequence identity between the new contig and the reference sequence derived from *Ficopomatus shenzhensis*, which corresponds to 18S rDNA sequences [7]. Furthermore, comparison of the newly generated contig sequence with data available on NCBI revealed a unique match with *Ficopomatus shenzhensis* 18S DNA (Accession No. HQ433336), exhibiting maximum identity of 100%. These findings were obtained through data mining utilizing the BLAST search engine, as depicted in Table 1.

The construction of maximum likelihood (ML) trees (Figure 3), utilizing partial 18S rDNA sequences from this study along with the 20 most closely related nucleotide sequences available in GenBank, employing *Sabella spallanzanii* as the outgroup, demonstrated the formation of a clade with a 1000 bootstrap value

probability for both the *Ficopomatus specimens* in this study and *Ficopomatus shenzhensis* (HQ433336). The outcomes of the phylogenetic analysis indicated the formation of a well-supported clade by the *Ficopomatus* genus, exhibiting clear distinction from other genera within the Serpulidae family. Furthermore, these findings are consistent with previous investigations conducted by [3, 5, 7-9], which propose a likely close relationship between *Ficopomatus* and *Marifugia*.

Distribution

The exact origins of *Ficopomatus shenzhensis* in the Can Gio Biosphere Reserve remains elusive; however, it is noteworthy that the Saigon River serves as a crucial waterway for Vietnam's international trade via the global port system. Ballast water, sediment transportation, and fouling represent primary mechanisms for the dispersal of aquatic invasive species [10]. Consequently, it is plausible that ballast water served as the vector for introducing this species to Can Gio and its surrounding areas. Despite extensive examination, no beneficial ecological effects stemming from the presence of this species have been observed. Therefore, it is imperative to enhance monitoring efforts to prevent uncontrolled proliferation in the wild. Additionally, conducting comprehensive studies in Vietnam's coastal lagoons is recommended to assess the actual distribution and impact imposed by *Ficopomatus shenzhensis*.

Ecology

The *Ficopomatus shenzhensis* species typically demonstrates a preference for adhering to a broad variety of substrates, including but not limited to plastic, wood, iron, concrete, and mangrove roots. In abandoned ponds, the species exhibits a slower growth rate and lower population density in comparison to its proliferation in cultivated ponds. Our findings suggest that *Ficopomatus shenzhensis* requires a substantial concentration of dissolved oxygen in its aquatic habitat. The environmental parameters measured during our study were as follows: dissolved oxygen registered at 5.1±2.0 mg/L, temperature was measured at 28.5±1.0°C, pH was noted at 7.6±0.5, total dissolved solids were quantified at 19.3±3.0 ppt, salinity was detected at 18.5±2.0 ppt, and specific conductivity was determined at 24.3±1.0 mS.

Conclusions

This investigation has successfully identified and characterized *Ficopomatus shenzhensis,* a serpulid worm species that has established a presence in Vietnamese shrimp ponds. Its existence was corroborated through meticulous morphological assessment coupled with DNA-based species identification. *Ficopomatus shenzhensis* is now found to have a pervasive distribution throughout the southern coastal provinces of Vietnam, a phenomenon potentially attributable to the influence of ballast water transportation. The species exhibits a propensity for attaching to an array of substrates, and demonstrates a marked preference for aquatic environments with high levels of dissolved oxygen. Given its prevalence and potential impact, we recommend ongoing surveillance and research into the distribution of *Ficopomatus shenzhensis*, as well as its ecological implications. These insights would be crucial in managing potential adverse effects on both aquaculture ponds and broader coastal ecosystems. We advocate the development of environmentally friendly control strategies in order to ensure the sustainability of aquaculture practices and the preservation of coastal ecosystems. These strategies would effectively balance the maintenance of productive aquaculture with the imperative of conserving our vital coastal habitats.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Data

Supp. Data 1. 18S ribosomal RNA gene sequence of *Serpulidae*. Spp. collected in Can Gio, Ho Chi Minh City, Vietnam (1667 bp).

TGGCTCATTAAATCATATGTGATTTCTTAGATCGTTGCCTCCCTCTTCGGGGAGGTGTGGATAACTG
TGGCAATTCTAGAGCTAATACATGCAATCAAGCTCAGACCTTCGGGGACGAGCGCACTTATTAGACCA
AGGCCAACCTGTGGGGCAACTCGCAGTTAGCCGTGGTGACTCTGGATAAGCCCAGTTGTTCGCACGA
CCTTGCGTCGGCGACGTATCTTACAAGCGTATGCCCTATCAGCTGTCGACGGTAGGGTAGCGGCCTAC
CGTGGCTGTTACGGGTAACGGGGGATCAGGGTCCGATCCCGGAGAGTATGCCTGAGAGACGGCTTACA
CATCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATGGGGAAACCCTGAGGTAGTGACAAGAAATA
ACAATACACGACTCTTTCGAGGCCGTGTAATTGGAATGAGTACATTCTAAATCCTTTAACGAGGATCA
ATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGC
TGCAGTTAAAAAGCTCGTAGTTGATAATCGGTGCTCGTCTCGGCGGTCCTGTTCACGCAGGCACTGCC
TTGGCGGCACTTACACATAGGCTGCTGGGTGCCAGTGGCAGGAAACTGTTGCTGGTAGCCAAAGCCC

CGGGTGCCCTTTAATGGGTGGCCGTGGGTGGCCTATGCGTTTACTTTGAAGAAATTAGAGTGTTCA
AAGCGGGCTCTTGTAGCTCGAATAGTCGTACATGGAATAATGGAAAAGGACTCTGGTCCATTTTCGTT
GGTCTATGGACATTGAGTAATGATTAAAAGGGACTGCCGGGGGCATTGGTATGGCGGGGGTAGAGGT
GAAATTCGGTGATCCTCGCCAGACCGACTACAGCGAAAGCATTTGCCAAGAATGTCTTCATTAATCAA
GAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTCGTAGTTCTGACCATAAACGATGCCAGCTT
GGAAGTGGCGAAGGTACTTTTTAGGACCTTGCCACGGCCCGTCGGGAAACCATAAGCGTTTGGGTTCC
GGGGGAAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGC
CTGCGGCTTAATTTGACTCAACACGGGAAAACTCACCCGGCCCGGACACTGTAAGGATTGACAGATT
GATAGCTCTTTCTCGATTCGGTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTG
GTTAATTCCGATAACGAACGAGACTCTAGCCTACTAATTAGTCAGGCGGCTACTTGTAGCCGTTCTAA
CTTCTTAGAGGGACAGCTGGCGCTTAGCCAGACGAGATGGAGCAATAACAGGTCTGTGATGCCCTTAG
ATGTCCGGGGCCGCACGCGCGCTACACTGAAGGAATCAACGTGTTTCCTAACCCGAAAGGGTGGGGC
AAACCGTTGAACCTCCTTCGTGCTTGGGATTGGGGCTTGTAATTCTTCCCCATGAACGAGGAATTCC
CAGTAAGCGTGAGTCATCAGCTCGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCG
ATTGGATGGCTTAGTGAGACCTTTGGACTGGACCGTCTAGGA

Supp. Data 2. Partial blast result of the 18S ribosomal RNA gene sequence of *Serpulidae*. spp. collected in Can Gio, Ho Chi Minh City, Vietnam (1667 bp) showed a complete identity with *Ficopomatus shenzhensis*.

>gb|HQ433336.1| Ficopomatus shenzhensis 18S ribosomal RNA gene, partial sequence
Length: 1765
Score = 3007.5 bits (3334), Expect = 0E00
Identities = 1667/1667 (100%), Gaps = 0/1667 (0%)
Strand = Plus/Plus

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Query  1    TGGCTCATTAAATCATATGTGATTTCTTAGATCGTTGCCTCCCTCTTCGGGGAGGTGTGG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  51   TGGCTCATTAAATCATATGTGATTTCTTAGATCGTTGCCTCCCTCTTCGGGGAGGTGTGG  110
Query  61   ATAACTGTGGCAATTCTAGAGCTAATACATGCAATCAAGCTCAGACCTTCGGGGACGAGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  111  ATAACTGTGGCAATTCTAGAGCTAATACATGCAATCAAGCTCAGACCTTCGGGGACGAGC  170
Query  121  GCACTTATTAGACCAAGGCCAACCTGTGGGGCAACTCGCAGTTAGCCGTGGTGACTCTGG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  171  GCACTTATTAGACCAAGGCCAACCTGTGGGGCAACTCGCAGTTAGCCGTGGTGACTCTGG  230
Query  181  ATAAGCCCAGTTGTTCGCACGACCTTGCGTCGGCGACGTATCTTACAAGCGTATGCCCTA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  231  ATAAGCCCAGTTGTTCGCACGACCTTGCGTCGGCGACGTATCTTACAAGCGTATGCCCTA  290
Query  241  TCAGCTGTCGACGGTAGGGTAGCGGCCTACCGTGGCTGTTACGGGTAACGGGGGATCAGG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  291  TCAGCTGTCGACGGTAGGGTAGCGGCCTACCGTGGCTGTTACGGGTAACGGGGGATCAGG  350
Query  301  GTCCGATCCCGGAGAGTATGCCTGAGAGACGGCTTACACATCTAAGGAAGGCAGCAGGCG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  351  GTCCGATCCCGGAGAGTATGCCTGAGAGACGGCTTACACATCTAAGGAAGGCAGCAGGCG  410
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