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Research Article

Recognition and identification of *Ficopomatus* Southern 1921 (Polychaeta: Serpulidae) in Vietnam

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ABSTRACT

The genus *Ficopomatus* Southern, 1921 (subfamily Ficopomatinae Pillai, 1960) is extremely euryhaline, and is found in freshwater, brackish-water, marine, and hypersaline environments. They widely dispersed around the world and recently, in the buffer zone of Can Gio biosphere reserve and some southerly coastal provinces of Vietnam. The species have prevailed with a morphological appearance outside the genus *Ficopomatus* in whiteleg shrimp ponds. We collected samples and classified morphologic identifiers along with molecular biology. The results determined that the species *Ficopomatus* sp. appeared in the culture area from the Saigon estuary as *F. shenzhensis* was recorded by Wang & Deng, 2012.

Key words: Serpulidae, Taxonomy, *Ficopomatus,* shrimp ponds, Can Gio biosphere reserve, Vietnam

INTRODUCTION

Serpulidae is a family in the Sabellida, a group of sedentary polychaetes inhabiting calcareous tubes. The common name of a taxon is tube worms or flowers of the sea, because of their radiolar crown, and they separate the body into thoracic and abdominal regions (H. A. T. Hove & Kupriyanova 2009). The genus *Ficopomatus Southern, 1921 (subfamily Ficopomatinae Pillai, 1960) is extremely euryhaline, and is found in freshwater, brackish-water, marine, and hypersaline environments* (Southern, 1921; Fauvel, 1923; Treadwell, 1934; Pillai, 2008).

The tubeworm is seen inside shrimp ponds of Saigon river for some years and is currently present there inside in high density, but there have not been any documents recording the species there. In the local community, the serpulid tubes are called 'hao chi' which means a tube oster, because they have a hard shell like that of an oyster, and a long, thread-shaped tube. According to farmers, *Ficopomatus sp.* thrives in shrimp ponds, reducing the yield of the crop. The aim of this study was to identify the species name, thereby finding solutions to limit their growth in aquaculture ponds.

MATERIALS AND METHODS

Study area

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

Collection of samples

Specimens included (tubes and worms) were carefully scraped off hard substrates (such as the surface of the trunk, or other materials in a shrimp pond) with a scalpel. 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

The morphological characteristics of the serpulids were examined after 24h of starvation. Cross-sections of tubes were prepared by whetting the tube on a whetstone and polishing it on frosted glass. Chaetae and uncini were separated in a 50/50 solution of glycerin and ethyl alcohol, then stained with methylene blue. Images of live individuals were captured using Olympus SZ51 Stereo microscope and measured with a calibration slide. The uncini and chaetae were observed with a Leica Dmil Led microscope (Leica Microsystems, Wetzlar, Germany), and their images were captured with a Flexacam C1 digital camera.

For identification, we followed taxonomic keys and/or descriptions from H. ten Hove and Weerdenburg, 1978; Pillai, 2008; Li, Wang, and Deng 2012; Bastida-Zavala et al. 2017.

Genomic DNA extraction and PCR

Genomic DNA was extracted from 20 worm individuals using the standard extraction protocol. Briefly, the sample were incubated at 55◦C for 3 hours in 100 µl lysis

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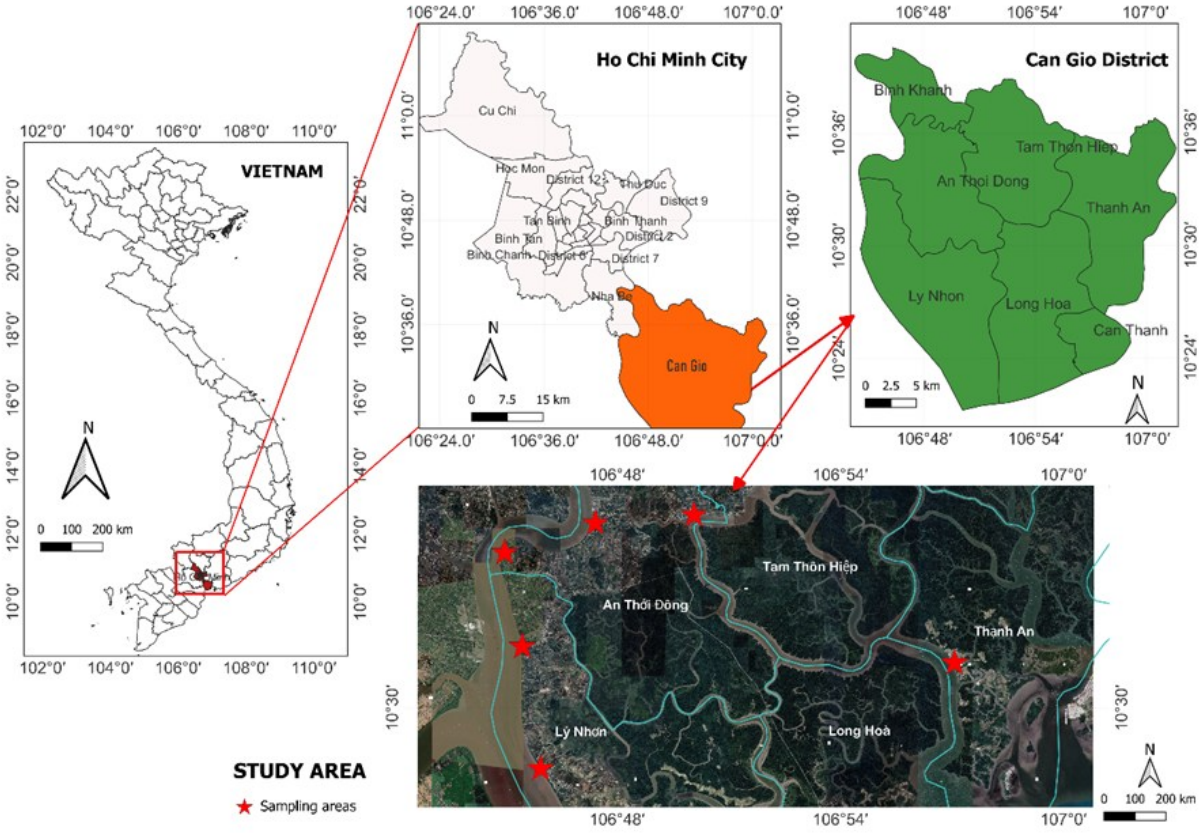

Figure 1. Sampling areas where specimens of *Ficopomatus sp.* were recollected

buffer solution (10 mM pH 8.0 Tris-HCl, 0.1 M EDTA, 0.5 % SDS) and 10 µl of 20 mg/ml proteinase K (Promega, Madison, WI). The genomic DNA was purified from lysate supernatant using phenol/chloroform extraction, then DNA pellets were obtained by alcohol precipitation.

Amplification reactions were performed in 20 µl containing 50 ng genomic DNA, 0.5 µM of forward primer ggc18f 5'-TAAGCCATGCACGTGTAAGT-3' and reverse primer ggc18r 5'-CAGTCTAGTTCGAACTTCTT-3', 400 µM dNTPs, 1X PCR buffer, and 0.5 U of OneTaq DNA polymerase (New England Biolabs, MA, USA) using a MyCycler™ thermal cycler (Bio-Rad, CA, USA). The thermal cycling profiles consisted of an initial denaturation at 95◦C for 5 min followed by 35 cycles of 1 min at 94◦C, 1 min at 58◦C, 1 min at 72◦C and a 5 min final extension at 72◦C. PCR products were checked by electrophoresis on 1.5 % agarose gel in 1X Tris-Acetate-EDTA (TAE) buffer. The gel was stained with Goldview dye (Solarbio, BJ, China) and visualized under ultraviolet (UV) light using transilluminator (UPV, CA, USA).

Direct sequencing

For the direct sequencing of PCR products, 5 µl of amplified products was cleaned up the residual primers and dNTPs by incubating for 30 min at 37◦C together with 1 U of exonuclease I, 0.8 U of shrimp alkaline phosphatase (New England Biolabs, MA, USA) in 1X reaction buffe. The reaction was stopped by incubation at 80°C for 15 min. The product was sent out to the commercial service (National Key Laboratory of Gene Technology, Hanoi, Vietnam) for sequencing of both directions using two PCR primer as sequencing primers.

Sequence comparison

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

RESULTS

Taxonomic description

Ficopomatus shenzhensis sp. nov. (Figs. 1, 2, 3)

Morphological description

The tube is shining white, forming aggregations (Fig. 1B), measuring approximately 9.20 ± 0.10mm (n = 10) in length. It is circular in cross-section; and lacking a longitudinal ridge (Fig. 1C). The body includes a branchial crown, thorax, and abdomen, 7.35 ± 1.70 mm (n = 10) in length (Fig. 1A).

The branchial crown corresponds to 1/4 or 1/5 of total body length, includes of 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. 1E).

The operculum and its peduncle occur in the position of the 1st branchial radiole on the left side (Fig. 1E). The operculum is 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 V-shaped furrow dorsally (Fig. 1F), and the hemispherical proximal part with black spots. The peduncle of the opercular is 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 towards the middle, and brown proximally.

The thorax is composed of 7 chaetigers, and fleshy thoracic membranes, which are white and not joined over the thorax. The collar is the entire margin, with well-developed lobes. Chaetae from the collar are serrated-shaped (Fig. 3B), consisting of 7-10 chaetae of 2 types on each side, those that end distally in simple blades (Fig. 3A). The collar chaetae bear 2 or 3 large teeth at the base, and 1 or 2 rows of sharp thin teeth distally (Fig. 3B-C). The chaetae of the thorax have simple blades shape (Figs. 3D-E), 0.50 ± 0.04 mm long (n = 10), and the thoracic unici is sawshaped with 9 teeth, 31 ± 15 µm long (n = 10) (Figs. 3H-I). Abdomens light greenish-yellow, 3.45 ± 0.22 mm long (n = 10); each segment bears 2-4 geniculate chaetae (Fig. 3F), half-proximal serrated-shaped (Fig. 3G). The abdominal chaetae measure in length 0,27 ± 0.025 mm (n = 10) of the end shaft and 0.05 ± 0.005 mm (n = 10) of the distal end. Abdominal uncini (Figs. 3K-L) rasp-shaped, bearing 2 rows of 10-12 teeth each.

Subsequently, the PCR product was submitted for direct Sanger sequencing using PCR primers as sequencing primers. Based on the successful sequencing results of both directions, we conduct sequence assembly, subsequently manual verification, resulting from high confident 1667-bp contig of 18S ribosomal RNA gene of the worm (Supp data). Besides, we also found that the new sequence was completely identical to the the reference, which is 18S rDNA sequences derived from *Ficopomatus shenzhensis (*Li, Wang & Deng, 2012). The comparison of the new contig sequence to NCBI data also shows the unique match (100% maximum identities) with Ficopomatus shenzhensis 18S DNA (No. accession HQ433336), the result of data mining by using BLAST search engine as shown in Table 1.

DISCUSSION

Previously, there was no record of the species *Ficopomatus shenzhensis* and other species within the genus Ficopomatus in the coastal area of Vietnam. Morphologically and genetically, the 18S ribosomal RNA gene, the worm in this study is completely identical to that of *Ficopomatus shenzhensis* Li, Wang & Deng, 2012. The MP and ML trees (Fig. 4, 5) based on 18S rDNA partial sequences of this study and 20 the most similar nucleotide sequences in GenBank with *Sabella spallanzanii* outgroup showed that *Ficopomatus* in this study and *Ficopomatus shenzhensis (HQ433336)* formed a clade with 100 bootstrap value probabilities. The phylogenetic analysis results showed that the Ficopomatus genus formed a well-supported clade compared to the other genera within the family Serpulidae. In addition, this result is in agreement with previous studies by Kupriyanova et al. (2009), Li, Wang & Deng (2012), Pillai (2008), Absolon & Hrabe (1930) that Ficopomatus and Marifugia likely have a possible close relationship.

Distribution

The presence of worm, resembling *Ficopomatus shenzhensis*, in the buffer zone of the Can Gio Biosphere Reserve was initially brought to the attention of the author's several years ago. However, it only attracted attention when there were complaints from shrimp farmers about the phenomenon of this species thriving causing damage to the crop. In contrast, the observations in the field show that *Ficopomatus shenzhensis* is widely distributed in the southern coastal provinces of Vietnam, suggestive that the invasion event of this species may have occurred for many years.

The origin of the species *Ficopomatus shenzhensis* in Can Gio Biosphere Reserve remains unclear,

Table 1. The 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

Figure 1. Photographs of *Ficopomatus shenzhensis* (A) Lateral view of a worm removed from its tube; (B) Tube; (C) Cross-section of tube; (D) Branchial radioles; (E-F) Dorsal view of operculum.

Table 2. Physical-chemical conditions were found in the shrimp pond, during the sampling period. Abbreviations: O_2 oxygen; T Temperature; TDS dissolved solids; Sal Salinity; Cond Conductivity

Locality	**Site**	**[O_2] (mg/L)**	**T (°C)**	**pH**	**TDS (ppt)**	**Sal (ppt)**	**Specific cond. (mS)**
Nha Be – HCM city	White shrimp pond	5.1±2.0	28.5±3.0	7.6±0.5	19.3±3.0	18.5±5.0	24.3±4.0

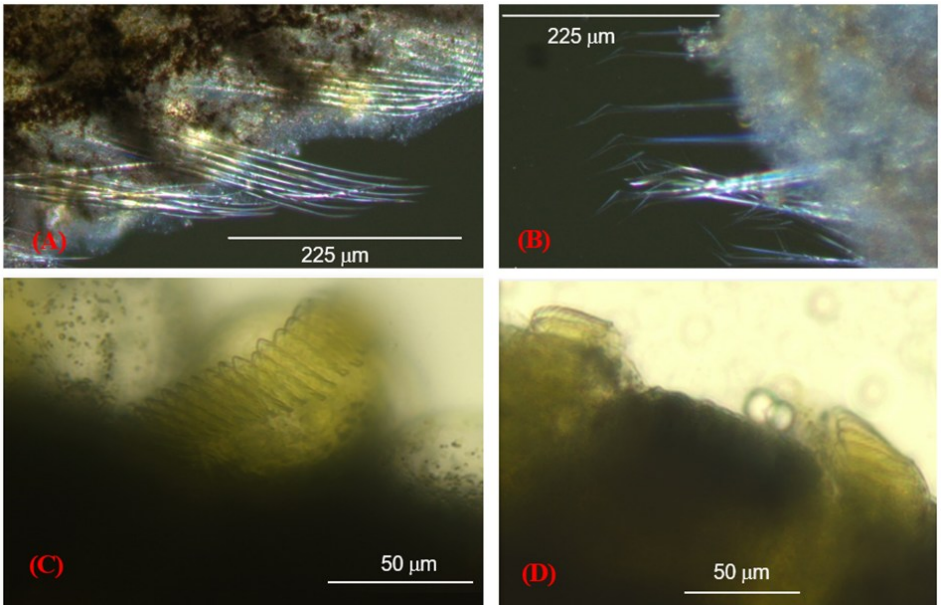

Figure 2. Photographs of chaetae and uncini of *Ficopomatus shenzhensis* in this study. (A) Thoracic chaetae; (B) Abdominal chaetae; (C) Thoracic uncini; (D) abdominal uncini.

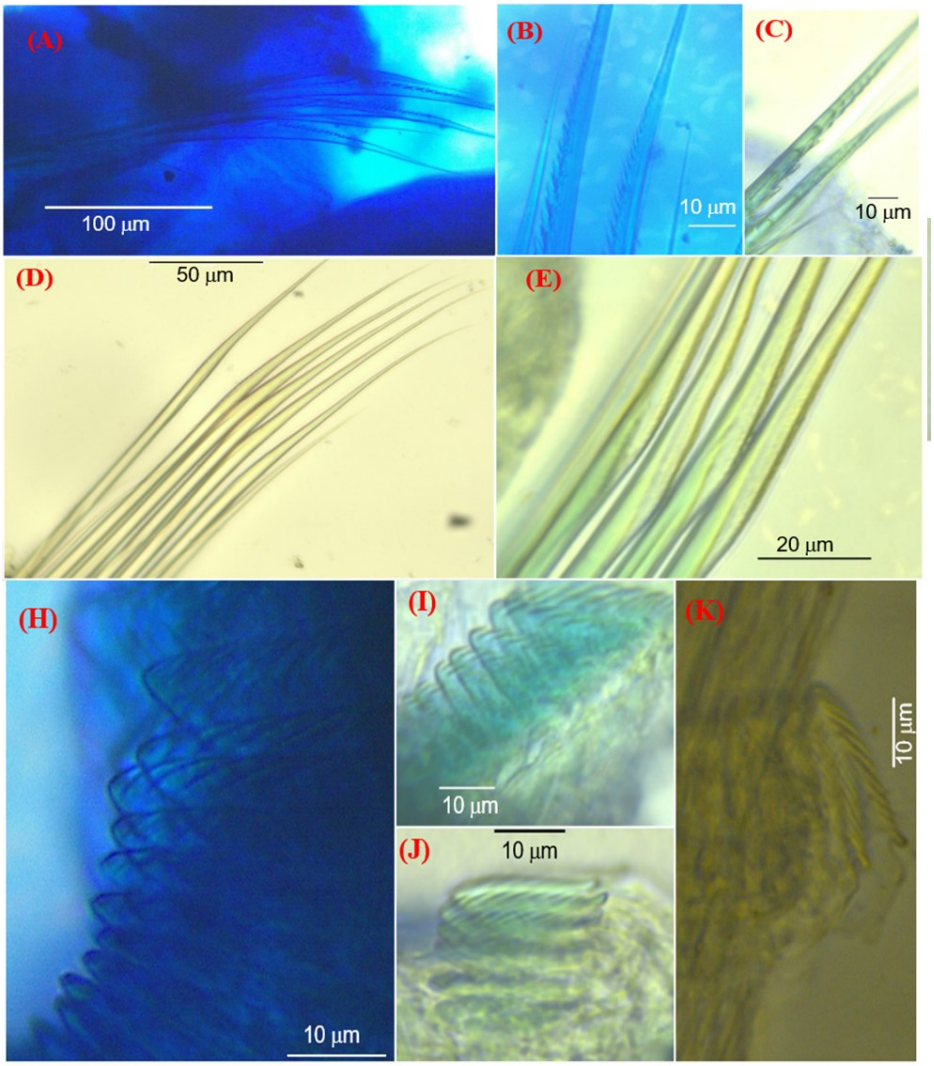

Figure 3. Details of chaetae and uncini of *Ficopomatus shenzensis*. (A-C) Toothed and limbate chaetae from collar (D-E) Thoracic chaetae; (F-G) Abdominal chaetae; (H-I) Thoracic uncini; (J-K) abdominal uncini.

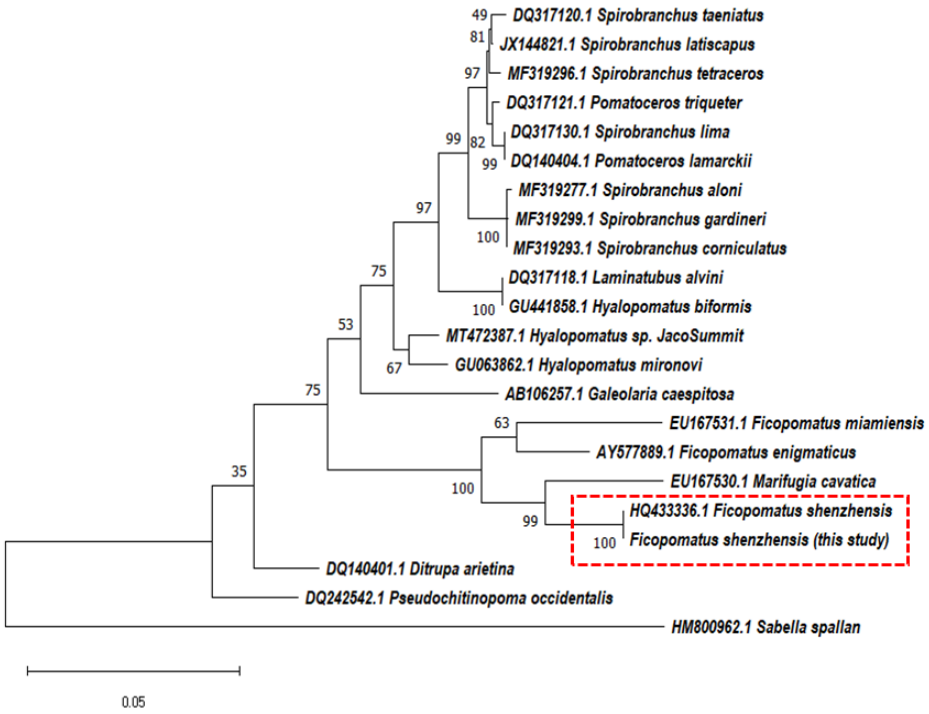

Figure. 4. Phylogenetic tree based on the Maximum Likelihood method and Kimura 2-parameter model with an outgroup *Sabella spallanzanii*. Numerals near the nodes indicate bootstrap values (%) based on 1000 replicates. The branch length indicator displays 0.05 substitutions per site.

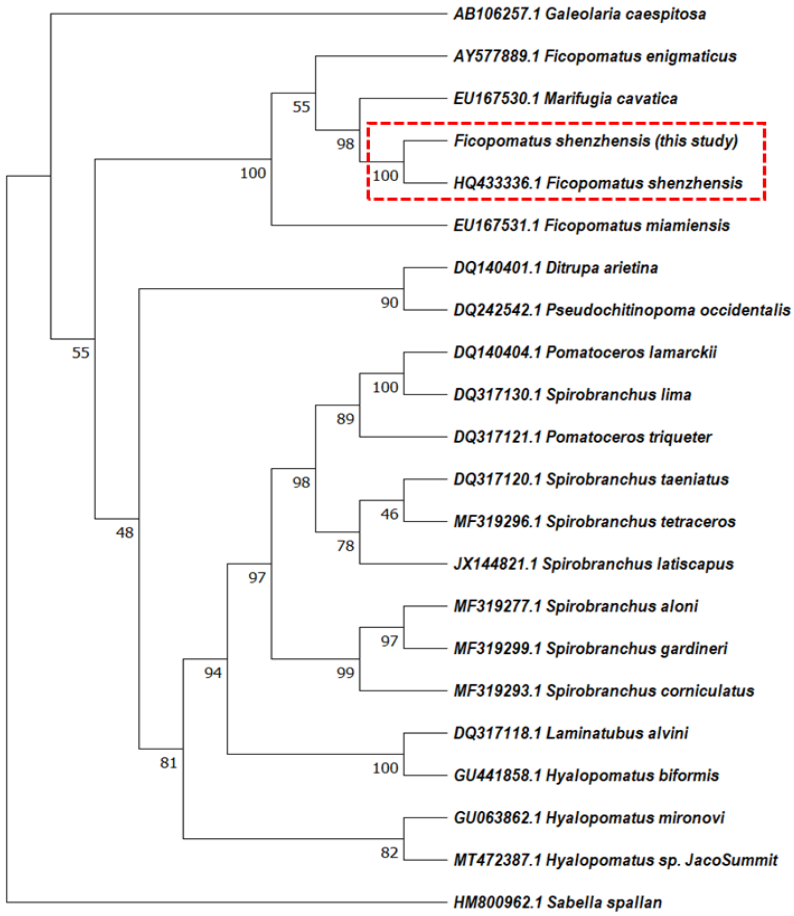

Figure 5. The phylogenetic analysis was inferred using the Maximum Parsimony method with numerals near the nodes indicate bootstrap values (%) based on 1000 replicates. The MP tree was obtained using the Tree-Bisection-Regrafting (TBR) algorithm and Sabella spallanzanii as a outgroup

but the Saigon River is an important waterway for Vietnam's trade with the worldwide port system. Ballast water, sediment transport and fouling are the main means of dispersal of aquatic exotic species (Carlton & Geller, 1993, Ruiz et al., 2000, Okolodkov et al., 2007). So most likely, ballast water was the vehicle that brought this species to Can Gio and surrounding areas. The beneficial effects of this species on the regional ecology have not been observed. Therefore, it is necessary to strengthen monitoring of their growth in the wild before they overgrow. It is also recommended that an exhaustive study in the coastal lagoons of Viet Nam be conducted to evaluate the real distribution, and the impact generated *by Ficopomatus shenzhensis.*

Ecology

The species *Ficopomatus shenzhensis* often clings to floating objects in shrimp pond systems. In abandoned ponds, they grow slowly, and the density is also lower than in cultured ponds. They can cling to most materials such as plastic, wood, iron, concrete, mangrove roots. It seems that the species *Ficopomatus shenzhensis* needs a high level of dissolved oxygen in water, as shown in Table 2.

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