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Identification of *Tambelo* (*Bactronophorus thoracites*) in Wamesa Waters, Manokwari, West Papua, Indonesia

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Abstract. *Akabessy JL, Yahya, Fadjar M, Suprayitno E. 2022. Identification of Tambelo (*Bactronophorus thoracites*) in Wamesa Waters, Manokwari, West Papua, Indonesia. Biodiversitas 23: 6021-6030. Tambelo*, otherwise known as shipworm, is a marine bivalve mollusk comprising 16 genera in the Teridinidae family, of which Teredo is the most frequently mentioned. Furthermore, the only species in the genus *Bactronophorus* is the wood borer, *Bactronophorus thoracites* (Teredinidae), which has a wide distribution in the Indo-West Pacific biogeographical region. Identifying shipworm species is difficult due to their high diversity and the limited information about their taxonomic characteristics, which often leads to identification errors by collectors. Papuan people consume *tambelo* as a substitute for side dishes because they are believed to be able to cure various diseases. Therefore, this study aims to establish by morphology and molecular analysis that *tambelo* belongs to the *B. thoracites* of the Teredinidae family. *Tambelo* was identified using the morphological method of observing the physical characterization of the palette, which is unique between genera. It was collected from a mangrove forest near Wamesa Beach, Manokwari District, Indonesia. Meanwhile, this study adopted PCR, an identification method that uses DNA from a small number of samples and can provide a DNA sample sequence. Morphological analysis based on pallet characterization showed that the characteristics of the *tambelo* pallet were similar to those of the *B. thoracites* based on the identification book. In addition, the molecular phylogenetic analysis using two mitochondrial protein-coding genes, cytochrome c oxidase subunit I (COI), showed *tambelo* was 91% similar to *B. thoracites* from mangroves environment on the west coast of Peninsular Malaysia, Malaysia.

Keywords: *Bactronophorus thoracites*, identification of *tambelo*, Manokwari, Wamesa waters

INTRODUCTION

The *tambelo* or shipworm is a marine mollusk in the family Teredinidae, which consists of a group of long, soft, naked saltwater clams found in almost all aquatic ecosystems from tropical to cold waters. They are notorious for drilling into wood, such as wooden dock structures and ships submerged in seawater, through a pair of shell valves so tiny at one end (Turner 1966) that they are called pests because they destroy wood faster than marine fungi or bacteria. (Borges et al. 2012). But they have an important role in the ecosystem, namely playing a role in the carbon cycle and degrading lignocellulose in mangrove forests (Voight 2015). Marimuthu et al. (2015) stated that mangrove forests have muddy sediments that are rich in organic matter, so they become a supporting factor as a source of habitat for the sea wood borer worm. Their presence has attracted a lot of attention recently, as evidenced by the availability of many publications (Distel et al. 2017; Treneman et al. 2018).

Several recent studies on the identification of wood borer characteristics, such as Velásquez and Shipway (2018), reported that they found a new species of shipworm, namely *Nivanteredo coronatan*, found in wood panels laid at depths of 240-773 m off the coasts of Vanuatu, the Solomon Islands, and Papua New Guinea. They identified and found several characters of *Nivanteredo coronatan* that differ from those of other teredinids in that they have an elongated and segmented palette consisting of a golden brown periostracum blade, a mantle collar that extends about half the length of the pallet, and a siphon and *Nivanteredo* has an open anal canal. Lee et al. (2019) characterize *Bactronophorus thoracites* in a mangrove environment on the west coast of Peninsular Malaysia. In their study, they identified morphologically, based on Turner's (1966) identification key, that this species is distinguished from a non-segmented palette consisting of basal cups with dagger-like extensions. Meanwhile, molecular phylogenetic analysis using 16S gene sequences from six different species (family Teredinidae) and a combination of 18S and 28S rRNA gene sequence data from 12 different species (family Teredinidae) showed a monophyletic and paraphyletic relationship, and *B. thoracites* had a kinship relationship with *Neoteredo reynei*. However, when using COI DNA barcodes, *B. thoracites* clearly separated from other teredinids.

Loo et al. (2022) identified shipworm specimens by observing the physical characteristics of the pallet, with a focus on the re-description of *B. thoracites* and *Bankia gracilis* from Sabah Waters, Malaysia. They said that the characteristic palette of *B. thoracites* is dagger-like with a sheath, whereas *B. gracilis* is characterized by a dark periostacum covering the 3-lobbed upper margin of the calcareous portion of the conical interior. However, researchers have had difficulty identifying shipworm species due to their diversity and limited information on their taxonomic characteristics, which often leads to misidentification by collectors due to limited literature and precise taxonomic metrics on wood borer worm species (Fofonoff et al. 2019; Loo et al. 2019). Recently, several attempts have been made to use morphological and molecular approaches. This approach has been used to establish systematic classification and phylogenetic relationships between shipworm species (Borges et al. 2012). Furthermore, several studies have identified *B. thoracites*, such as Turner (1966) categorized Teredinidae into three different subfamilies, mainly based on the development of the shipworm cecum and line segments on its pallet. However, further classification has grouped *Bactronophorus* with *Dicyantifer*, *Neoteredo*, and *Teredothyra*, which were identified based on the highly specialized closed anal canal structure.

Wamesa Village is one of the villages located in the South Manokwari Sub-district, Manokwari District, West Papua Province, Indonesia has a mangrove area of 22 hectares. However, the area of mangrove forests has decreased. Several problems cause the decline of mangrove forests, namely the occurrence of poor exploitation and changing roles; limited efforts to rehabilitate mangrove forests that have not been integrated between economic interests and conservation of mangrove areas or ecosystems, lack of coordination among stakeholders related to management; and a lack of public awareness of mangrove forests and mangrove ecosystems so that some people around the mangrove forest area still throw garbage and cut down mangrove trees illegally. Some of the mangrove trees that have been cut down by the community are taken to be used as firewood, and some are left submerged in seawater or brackish water for a month to harvest shipworms. Papuan people consume shipworms derived from the dead wood of *Rhizophora* sp. as a substitute for side dishes because it tastes sweet and has a very high protein content when compared to other types of mangrove forests. They are believed to be able to treat several diseases, whether consumed raw or cooked (Leiwakabessy 2015; Maldonado and Skinner 2016). Local people refer to this shipworm as *tambelo* (Leiwakabessy 2015). Leiwakabessy (2011) reported that *tambelo*, which is thought to be a species of *B. thoracites* measuring up to 83 cm, was found in dead wood of *Rhizophora* sp. in Andai waters, South Manokwari Sub-district, Manokwari District. Coastal shipworms are known to inhabit the low intertidal zone of mangrove and brackish water ecosystems in the Indo-West Pacific. According to Lee et al. (2019), most of the distributions are in several countries, including Indonesia, especially in Papua. Its presence was reported in Merauke District, Papua Province, while in Manokwari District, West Papua Province, only the shipworm *Bankia bipalmulata* was found. Research on the presence of *B. thoracites* in Manokwari waters has never been done, so it is necessary to research the presence of *B. thoracites* in Manokwari based on morphological and molecular analysis. Therefore, this study aims to identify the presence of *B. thoracites* species from the Teredinidae family in the dead mangrove wood of *Rhizophora* sp. in Wamesa waters, Manokwari District, Indonesia.

MATERIALS AND METHODS

Specimen collection

Specimens were collected in May 2019 from a mangrove forest near Wamesa waters, South Manokwari Sub-district, Manokwari District, West Papua Province, Indonesia (Figure 1). *Tambelo* inhabits dead logs of *Rhizophora* sp., a species of mangrove tree in the area. *Tambelo* specimens were carefully separated in complete body condition from wood using manual tools such as axes and machetes. Then the samples were stored in plastic bags, frozen at 4ºC, and returned to the Laboratory of Aquatic Resources, Faculty of Fisheries and Marine Sciences, University of Papua, Manokwari, and then the samples were taken to the Laboratory of the Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, East Java, Indonesia for the analysis.

Morphological characterization

The morphology of *tambelo* was identified using eye observations assisted by the key Turner (1966). Furthermore, the complete specimen was placed in 95% alcohol and stored at the Laboratory of Molecular Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, East Java, Indonesia.

DNA extraction

DNA isolation using the PromegaÔ kit wizard and following the procedure implemented by Abinawanto et al. (2019). 1 gram of *tambelo* was washed using distilled water until clean, put in 70% ethanol, and incubated for one day. Furthermore, the isolation process was initiated by inserting 500 µL of Nuclei Lysis solution and 120 µL of EDTA (0.5 M) pH 8 into a 1.5 mL microtube. The solution in the tube was cooled for ±10 minutes. 0.2 grams of *tambelo* worm mantle tissue were placed in a sterile mortal, ground until smooth to form a homogenate, placed in a sterile tube, and added 600 µL of a mixture of EDTA-Nuclei lysis and 17.5 µL of proteinase K. The mixture was incubated overnight at 55ºC. The results of incubation were added 200 µL of protein precipitation solution and vortexed for 20 seconds, then the mixture was centrifuged for 4 minutes at a speed of 13,000-16,000 rpm. A pellet will appear at the bottom of the tube. The supernatant was transferred to a tube containing 600 µL of isopropanol and centrifuged for 1 minute at a speed of 13,000-16,000 rpm.

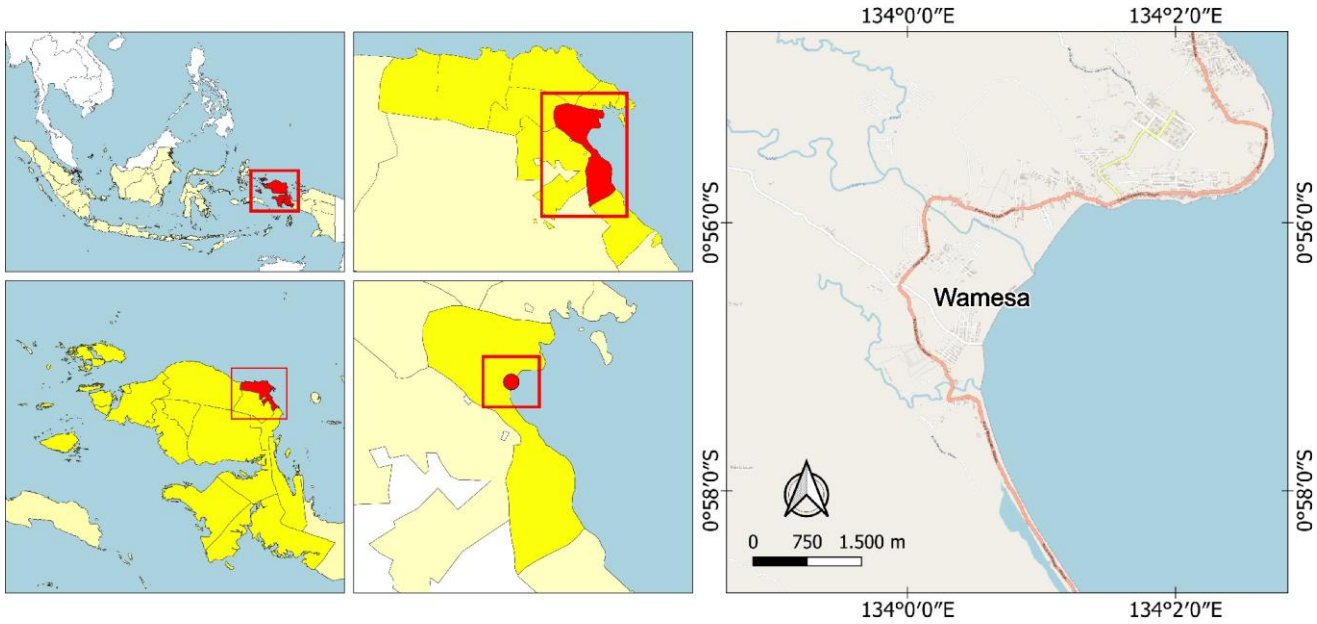

Figure 1. Location of mangrove forest, Wamesa Waters, Manokwari District, West Papua Province, sampling of *tambelo* (*Bactronophorus thoracites*)

The DNA pellet will appear white, and the supernatant was removed, then 600 µL of 70% ethanol was added to the pellets and centrifuged for 1 minute at a speed of 13,000-16,000 rpm. The ethanol was removed, and the pellets were aerated for 10 minutes. The pellets were added with 100 µL of DNA rehydration solution and incubated at 65°C for 1 hour. Furthermore, DNA can be stored at a temperature of ± 4°C.

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DNA isolation using the PromegaÔ kit wizard and following the procedure implemented by Abinawanto et al. (2019). 1 gram of tambelo was washed using distilled water until clean, put in 70% ethanol, and incubated for one day. Furthermore, the isolation process was initiated by inserting 500 µL of Nuclei Lysis solution and 120 µL of EDTA (0.5 M) pH 8 into a 1.5 mL microtube. The solution in the tube was cooled for ± 10 minutes. 0.2 grams of tambelo worm mantle tissue were placed in a sterile mortal, ground until smooth to form a homogenate, placed in a sterile tube, and added 600 µL of mixture of EDTA-Nuclei lysis and 17.5 µL of proteinase K. The mixture was incubated overnight at 55oC. The results of incubation were added 200 µL of protein precipitation solution and vortexed for 20 seconds, then the mixture was centrifuged for 4 minutes at a speed of 13,000-16,000 rpm. A pellet will appear at the bottom of the tube. The supernatant was transferred to a tube containing 600 µL of isopropanol and centrifuged for 1 minute at a speed of 13,000-16,000 rpm. The DNA pellet will appear white, and the supernatant was removed, then 600 µL of 70% ethanol was added to the pellets and centrifuged for 1 minute at a speed of 13,000-16,000 rpm. The ethanol was removed, and the pellets were aerated for 10 minutes. The pellets were added with 100 µL of DNA rehydration solution and incubated at 65°C for 1 hour. Furthermore, DNA can be stored at a temperature of ± 4°C.

PCR and sequencing

The purified tambelo worm DNA was then amplified using primers Cytochrome c oxidase subunit I (COI) gene fragment was amplified using a pair of primers, namely using primer forward LCO-1490 (5'- GGT CAA CAA ATC ATA AAG ATA TTG G-3') and reverse HCO 2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA- 3') (Folmer et al.1994) with the following composition: 3 µl DNA, 3 µl primer LCO1490 (10 µM), 3 µl primer HCO2198 (10 µM), 6 µl ddh2O and 15 µl Go Taq green (Promega). Furthermore, each of these solutions was placed in a PCR tube and amplified with a predenaturation temperature of 95ºC for 5 minutes, followed by 35 denaturation cycles at 95ºC for 30 seconds, annealing at 50ºC for 30 seconds, and extension at 72ºC for a second. The cycle was terminated with a final extension at 72ºC for 10 min. After completion of the PCR, the amplification results were analyzed using 1% agarose gel. If the target band has appeared. The PCR results were then sent to First Base Laboratories, Malaysia, for sequencing. The obtained sequences were then analyzed using Geneious v5.6. software following the procedure of Tallei and Kolondam (2015) and BLAST in the gene database (GenBank) within

NCBI. The chromatogram data from the sequencing was then edited and looked for similarities using BLAST.

Data analysis

The results of the sequencing were then read out using the sequencer scanner V1 (ABI). Sequence readings using both forward and reverse primers were contiguous using Bioedit software. From the software, the contig sequences that will be blasted are obtained. The nucleotide contig results were copied and blasted on the sites https://blast.ncbi.nlm.nih.gov/Blast.cgi and http://blast.ddbj.nig.ac.jp/ to check the similarity of the samples with the database. From the database, several sequence data were taken as a comparison with the sample sequence data to be tested, which are listed in Table 1 of Bankea setacea as an outgroup. DNA sequences were entered on a notepad and saved in fasta format. The results of phylogenetically aligned DNA sequences were analyzed by MEGA 11 (Kumar et al. 2018). The phylogenetic tree was constructed using the Neighbor-Joining method (Saitou and Nei 1987) to obtain a phylogenetic tree and genetic distance and similarity values using 1000 bootstrap. Next, the tree is drawn to scale, with branch lengths in units equal to the evolutionary distance used to deduce the phylogenetic tree. Use the Maximum Composite Use Method (Tamura et al. 2004) to calculate evolutionary distance and total unit substitution per site. This analysis involved 190 nucleotide sequences with codon positions 1 + 2 + 3 + Noncoding. All positions have been removed from each pair sequence (delete pair option). For paired distance data from Mega software, then copy and click the xls format for data in Microsoft Excel. The data equation is obtained by the formula =(1-genetic distance)*100.

RESULTS AND DISCUSSION

Fresh *tambelo* specimens were collected randomly from the dead mangrove trunk of *Rhizophora* sp. in the Wamesa mangrove forest. There were 8 specimens collected. Shells and pallets were separated from the *tambelo* body for morphological identification. The purpose of measuring specimens is to provide a more accurate understanding and insight for future species identification. The average of them, collected from the remnants of the fallen *Rhizophora* sp mangrove trees around the Mangrove Forest near Wamesa Waters, Manokwari District, can live in salinities between 7 and 30. The waters are slightly brackish and muddy around the research site because, in general, shipworms can live in seawater and brackish water (Borges et al. 2012; Roszaini and Salmiah 2014; Rajapakse 2016). Based on the analysis and morphological characteristics of *tambelo* scattered on dead mangrove trunks, the body is vermiform in shape and protected by a calcium tube (Chan and Lau 2021), which can be seen in Figure 2B. The body size of *tambelo* was obtained in three types, namely: long, medium, and short. These three body sizes were categorized as 30 cm long, 15 cm medium, and 9 cm short and had a siphon length of 1.0-2.0 cm (Figure 2A). Meanwhile, Lee et al. (2019) showed that the length of *B. thoracites* is between 16.0 to 47.0 cm, measured from mouth to siphon, and the diameter is 1.0 -2.0 cm. Chan and Lau (2021) reported that the *B. thoracites* worm, found in rotting mangrove wood around a muddy substrate on the island of Singapore, Api-Api river, has a body length of about 40 cm. Loo et al. (2022) reported that pallets of *B. thoracites* collected from the debris of fallen trees in mangroves in Kota Kinabalu, Malaysia had a total length of 31 cm and a siphon length of 0.6 cm.

Table 1. Specimen DNA sequences as a comparison used in phylogenetic using the COI gene

Species	Specimen source	COI gene length (bp)	GenBank accession number	Reference
Nototeredo norvagica NNS3	Japanese Tsunami Marine Debris	618	KY274184.1	Treneman et al. (2018)
Nototeredo norvagica NNS1	Japanese Tsunami Marine Debris	616	KY274182.1	Treneman et al. (2018)
Nototeredo norvegica	Morbihan, Toulindac and Berder, France; Mersin Bay, Turkey	658	KC157924.1	Borges et al. (2012)
Teredothyra matocotana	North Atlantic and Japan	658	MW362066	Borges et al. (2021)
Teredothyra dominicensis	Morbihan, Toulindac and Berder, France; Mersin Bay, Turkey	658	KC157941.1	Borges et al. (2012)
Bactronophorus thoracites	Peninsular, Malaysia	878	MK039837.1	Lee et al. (2019)
Bactronophorus thoracites	Peninsular, Malaysia	878	MK039838.1	Lee et al. (2019)
Bactronophorus thoracites	Wamesa Waters, Manokwari*	612	MK039837; MK039838	Leiwakabessy et al. (2022)
Bankia bipennata	North Atlantic; Japan	658	MW362054	Borges et al. (2021)
Bankia carinata	Morbihan, Toulindac and Berder, France	657	KC157914.1	Borges,and Merckelbach. (2012)
Teredo navalis	Central Europe marine waters	675	MF071125.1	Weigelt and Bastrop (2017)
Teredo navalis	all over Europe and North America	675	KU201186.1	Weigelt and Bastrop (2016)
Lyrodus pedicellatus	Mersin Bay, Turkey	658	KC157937.1	Borges et al. (2012)
Bankia setacea	North Atlantic and Japan	332	MZ427482.1	Borges et al. (2021)

Note: *Samples from this research

Specimens from valve shells and pallets were characterized morphologically. The inside and outside view of the valve shell (Figure 3BC), and in Figure 4B, the outside (a) and inside (b) surface view of the pallet. The anterior portion of the *tambelo* contains a smooth-surfaced shell valve and dorsal denticle with brown and fine lines (shown by black arrows) (Figure 3A), with a mean size ranging from 11 to 20 mm. Shell valves are used to drill dead wood (Velásquez and Shipway 2018) (shown by black arrows) (Figure 2AB). The activities carried out by *tambelo* begin at the larval stage. When the teredinid larva settles in untreated wood, it will use tooth-like valves to drill into the wood, which is barely visible on the surface, but the inside of the wood will be filled, affecting the structural integrity of the wood (Paalvast and Velde 2013; Fojutowski et al. 2014; Shipway et al. 2018). Teredinid damage is most severe in the mud line but can also attack wood at high tide (Sivrikaya 2019). One of *tambelo*'s identifiers is the shape of the pallet used to distinguish shipworm types, such as daggers and sheaths, highly asymmetrical and unsegmented, with blades extending from the basal cup (Figure 4). The palette shows considerable interspecific variation and is a major diagnostic character. A Teredinidae pallet is located at the end of the body, which is used to cover their burrows at low tide (shown by yellow arrows) (Figure 2C). The siphon functions as a breathing apparatus, sucking plankton and waste (Swain et al. 2017).

The results of measurements using an SZX stereo microscope showed that the total length of the pallets ranged from 15.6 to 35.5 mm. The blades are 5 to 15.0 mm long and 2.8 to 3.7 mm wide; stalk length and width range from 6.8 to 20.3 mm and 0.5 to 0.9 mm, respectively. Basalt cups are 2 to 6.3 mm long and 2 to 8.7 mm wide. A clear dark brown periostracum was seen in the basal cup area (shown by red arrows) (Figure 4AB) and along the outer surface of the blade, which was covered by small protrusions (shown by blue arrows) (Figure 4A). Furthermore, the mound coverage may extend from the base of the blade to the tip (shown by black arrows) (Figure 4B). Chan and Lau (2021) found a pallet length of about 19 mm. Loo et al. (2022) reported that the length of the pallet was 26 cm, with a rod length and width of 12 mm and 2 mm, respectively. Based on the results, Figure 5A shows that the pallet has many interspecific variations and is the main diagnostic character of Teredinidae (Velásquez and Shipway 2018). However, this structure changes when extracting animals from wood and can become damaged or deformed due to ecological conditions (shown by white arrows) (Velásquez and López 2016). Thus, other anatomical characteristics, such as the anatomy of the digestive system, siphon structure, pigmentation, shell valves, etc., should be considered for classifying their characteristics in addition to the palette (MacIntosh 2012; Huber 2015; Shipway et al. 2016; Borges and Merckelbach 2018). Based on the results of morphology identification on the pallet from this study, the *Tambelo* sample was similar to *B. thoracites* in Malaysian waters (Lee et al. 2019) (Figure 5B). The characteristics of the examined specimens matched the description of *B. thoracites* by Turner (1966) in particular, the shape and length of the pallet.

Figure 2. *Tambelo* from mangrove forest, Wamesa waters, Manokwari District, Indonesia. Specimens were extracted from dead *Rhizophora* sp. (A,B). The anterior portion of *tambelo* with a pair of shell valves (shown by black arrows); (B). Calcium tube (shown by blue arrows); (C). A pair of *tambelo* pallet located at the rear end of the body (shown by yellow arrows)

Figure 3. Shells valves of *tambelo.* (A) Shellst with labels to the parts measured; (B) outer; and (C) inner views

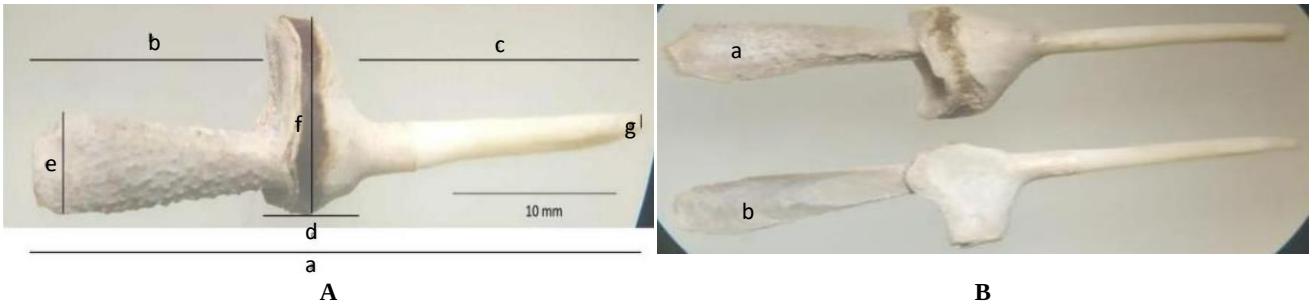

Figure 4. Pallets of *tambelo*. A. Pallet with labels to the parts measured: a. total length (TL), b. blade length (BL), c. stalk length (SL), d. cup length (CL), e. blade width (BW), f. cup width, g. stalk width (SW); B. (a) outer, and (b) inner faces

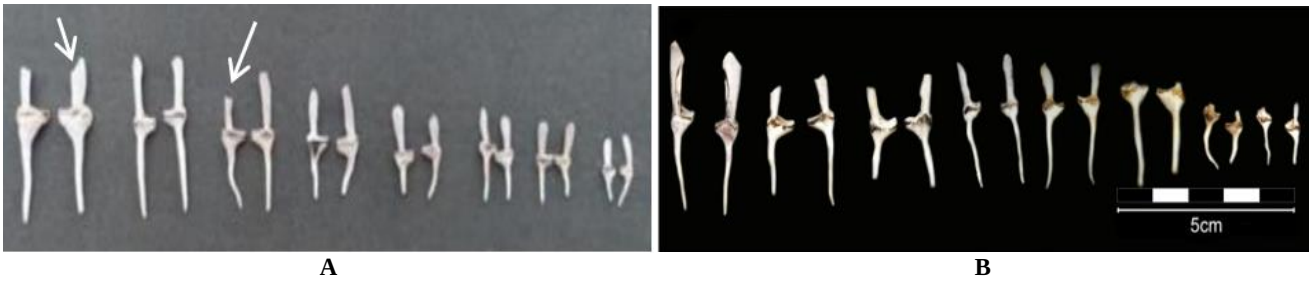

Figure 5. Based on the shape of the palette. A. From mangrove forests in the Manokwari District have similarities with; B. *Bactronophorus thoracites* from Malaysian waters (Lee et al. 2019)

Furthermore, to determine the level of similarity of the tambelo sample with *B. thoracites* and 12 other shipworm species, we took the data from GenBank (NCBI) and performed sequence alignment. Sequence alignment is one way to determine the level of similarity by matching DNA, RNA, or protein sequences, where the compilation process is carried out on two or more sequences so that the equations of the sequences appear real. This sequence alignment method is used to search for similar sequences in the sequence database. Mismatches in alignment are associated with the mutation process, while gaps are associated with insertions or deletions (Singh 2015).

The COI gene fragment from *tambelo* was successfully amplified using primers LCO-1490 and HCO-2198, resulting in 612 bp. Based on the alignment between *tambelo* DNA sequences and *B. thoracites* (NCBI), the resulting sequence length was read from 612 to 878 bp. The process of editing this DNA fragment sequence begins with the deletion of the DNA sequence at the end. The goal is to get the core region and the appropriate accuracy in data processing, which will facilitate alignment and reduce the risk of misreading when constructing a phylogenetic tree. Based on the results of the alignment, it shows that in sequence numbers 1-247 and 860-881, there is no *tambelo* nucleotide base to pair with the nucleotide base *B. thoracites* from the GenBank data (NCBI). The *tambelo* nucleotide bases began to appear in sequence numbers 247-849. The nucleotide compositions of T, G, A, and C from specimens of *tambelo* in the waters of Wamesa village were 40.1%, 27.9%, 17.2%, and 14.8%, respectively. The percentage of G+C content in the *tambelo* CO1 sequence is 42.7%. These percentage figures indicate that the universal primer for the CO1 gene in this study worked very well on shipworm specimens in general.

The results of the juxtaposition of the sample with its close relative *B. thoracites* showed intraspecies variation; namely, there were 94 points of nucleotide differences in the COI gene (Table 2). However, when viewed in more detail, the sample has similarities with *B. thoracites*, which is 84.6%, with 518 identical sites. Variations in nucleotide bases that occur at 94 points of nucleotide differences in the COI gene are probably due to transition substitutions that occur more often than transversions, causing mutations. Keller et al. (2007) said that mutations in animals tend to go through a transition mechanism more often than transversion. Mutations are the main cause of nucleotide base differences in the COI gene. Small differences in nucleotide bases can affect the identity of a species and can even affect the amino acids that code for proteins (Almeida et al. 2014). The difference in nucleotide bases may be due to the process of dispersal and gene flow of *tambelo* larvae that live in floating wood or live in wooden boats and are carried by currents (Borges et al. 2014), so that they travel quite a long distance from Peninsular Malaysia waters to Manokwari waters in West Papua Province, Indonesia, because based on its geographical location, Manokwari is located between the Pacific Ocean and the Seram sea on the head of the Papuan birds, so they have to adapt to different environments. Environmental changes are thought to cause this low genetic similarity as the second reason for variations in nucleotide bases. Lee et al. (2019) state that the North and

South Atlantic provide access for the ancestors of the *Bactronophorus-Neoteredo* clade to enter the Atlantic Ocean, which is evident from the presence of *Neoteredo reynei* on the western Atlantic coast. They speculate that other species may have migrated eastward, which explains the presence of *B. thoracites* in the Indo-West Pacific Ocean.

Dogan and Dogan (2016) describe genetic distance as the ratio of genetic differences between species or populations. Tallei et al. (2016) stated that the lower the value of the genetic distance between two organisms, the closer the kinship or similarity. Nei (1972) categorizes genetic distances based on their values, namely low (0.01-0.099), medium (0.1-0.99), and high (1.00-2.00). In summary, the genetic distance between *tambelo* specimens and other shipworm specimens is presented in Table 3. The genetic distance between *tambelo* and *B. thoracites* from GenBank (NCBI) obtained a medium category with a value of 0.173, meaning the overall genetic distance average is 17.3%, indicating that from a sequence length of 612 to 878 bp there are 94 different nucleotides. The genetic distance value indicates the presence of intraspecies variation in the sample caused by mutations. Genetic distance indicates the possible influence of geographic isolation on a population (Schmidt 2003). The greater the value of the genetic distance (p-distance) between a population or individual, the more isolated they are from one another. Based on genetic distance, it was shown that the *tambelo* sample in this study had mutations in its gene, but based on the phylogenetic tree analysis, the *tambelo* still had a kinship with *B. thoracites* from the waters of Peninsular Malaysia.

Reconstruction of the phylogenetic tree shows the grouping of species based on taxonomic proximity. In addition, species were grouped according to genetic distance, which indicates that the species can be differentiated effectively (Roesma et al. 2022). The COI gene is one of the optimal DNA barcode regions for the marine wood borer. This has been useful for identifying the geographic origins of specimens of *L. pedicellatus*, *Teredo navalis*, and *B. thoracites* (Borges et al. 2012; Weigelt et al. 2016; Lee et al. 2019), and for distinguishing close species within the genus (Borges and Merckelbach 2018). A phylogenetic tree was constructed using the Neighbor-Joining method to determine the relationship between the *tambelo* sample and other shipworm samples. Based on the COI gene sequencing results from *tambelo* DNA with other shipworms shown in the phylogenetic tree, it showed that *tambelo* samples from Wamesa waters, Manokwari, West Papua, had similarities with *B. thoracites* from Peninsular Malaysia (NCBI) than other shipworm species, with tree robustness of 91% obtained from 1000x bootstrap, so they are grouped into one clade. It can be seen in Figure 7. The bootstrap value of 91% is high because the higher the bootstrap value, the higher the level of tree confidence (Nikmah et al. 2016). According to Hall (2001), a clade can be trusted with 90% and not with 25%.

Identifying shipworm species (Teredinidae) is difficult and always challenging. According to Lee et al. (2019), molecular phylogeny can be applied to complement the morphological taxonomy of the Teredinidae family. Phylogenetics is carried out to understand the diversity between species and build kinship relationships. Although shipworms of the same species may exhibit limited morphological differences between sampling sites (Weigelt et al. 2016), it is plausible that geographic location determines evolutionary influences in terms of environment and behavior. Furthermore, morphological characters can influence the evolution of the underlying morphology in phylogenetic analysis. The same characters indicate close kinship and the possibility of having a common ancestor.

Table 2. Multiple alignments of *tambelo* DNA with *Bactronophorus thoracites* from GenBank data

	251	**254**	**255**	**257**	**263**	**278**	**290**	**296**	**305**	**311**	**314**	**315**	**320**	**326**	**329**	**338**	**347**	**353**	**368**
Consensus	K	R	Y	R	W	R	R	K	R	K	R	Y	S	K	Y	Y	S	Y	R
B. thoracites	T	A	C	A	T	A	A	T	G	T	G	C	G	G	C	T	C	T	G
Tambelo	G	G	T	G	A	G	G	G	A	G	A	T	C	T	T	C	G	C	A
	374	**377**	**380**	**386**	**389**	**392**	**410**	**413**	**431**	**443**	**446**	**451**	**462**	**471**	**472**	**486**	**489**	**494**	**495**
Consensus	Y	Y	Y	R	W	R	K	Y	R	S	Y	N	K	W	Y	R	R	N	S
B. thoracites	T	C	C	G	A	A	T	C	A	G	T	.	T	A	T	A	A	.	G
Tambelo	C	T	T	A	T	G	G	T	G	C	C	A	G	T	C	G	G	T	C
	499	**502**	**506**	**508**	**509**	**516**	**517**	**520**	**525**	**535**	**538**	**544**	**545**	**547**	**557**	**560**	**578**	**596**	**599**
Consensus	K	R	Y	R	Y	K	R	W	K	R	W	K	S	K	N	W	W	K	W
B. thoracites	G	G	T	A	T	T	A	A	T	G	T	T	G	T	.	A	T	G	A
Tambelo	T	A	C	G	C	G	G	T	G	A	A	G	C	G	C	T	A	T	T
	602	**608**	**611**	**614**	**620**	**623**	**624**	**626**	**644**	**647**	**656**	**659**	**671**	**674**	**686**	**692**	**704**	**708**	**716**
Consensus	K	Y	Y	W	W	K	Y	W	W	K	R	M	R	S	W	K	Y	Y	R
B. thoracites	G	C	T	T	T	T	C	T	T	G	G	C	G	G	T	T	C	T	G
Tambelo	T	T	C	A	A	G	T	A	A	T	A	A	A	C	A	G	T	C	A
	731	**738**	**740**	**743**	**752**	**756**	**758**	**759**	**770**	**773**	**779**	**791**	**794**	**815**	**833**	**848**	**851**	**854**	
Consensus	Y	Y	W	R	S	Y	K	Y	K	R	Y	K	W	Y	Y	K	R	W	
B. thoracites	T	T	A	G	G	T	G	T	G	G	T	T	T	T	T	T	G	A	
Tambelo	C	C	T	A	C	C	T	C	T	A	C	G	A	C	C	G	A	T	

Table 3. Genetic distance of *tambelo* with shipworms from GenBank data

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
KC157914.1													
KC157924.1	0.266												
KC157937.1	0.207	0.256											
KC157941.1	0.297	0.266	0.286										
KU201186.1	27.811	27.817	27.800	27.848									
KY274182.1	0.269	0.006	0.259	0.269	27.820								
KY274184.1	0.271	0.008	0.260	0.271	27.822	0.005							
MF071125.1	49.095	49.101	49.084	49.132	21.284	49.104	49.106						
MK039837.1	0.242	0.248	0.231	0.278	27.793	0.251	0.253	49.077					
MK039838.1	0.242	0.248	0.231	0.278	27.793	0.251	0.253	49.077	0				
MW362054.1	0.259	0.266	0.248	0.296	27.810	0.269	0.270	49.094	0.241	0.241			
MW362066.1	0.296	0.227	0.285	0.295	27.846	0.230	0.232	49.130	0.277	0.277	0.295		
MZ427482.1	210.240	210.247	210.230	210.277	182.430	210.250	210.252	161.146	210.222	210.222	210.240	210.276	
Tambelo	0.264	0.270	0.253	0.301	.27.815	0.273	0.275	49.099	0.173	0.173	0.263	0.300	210.245

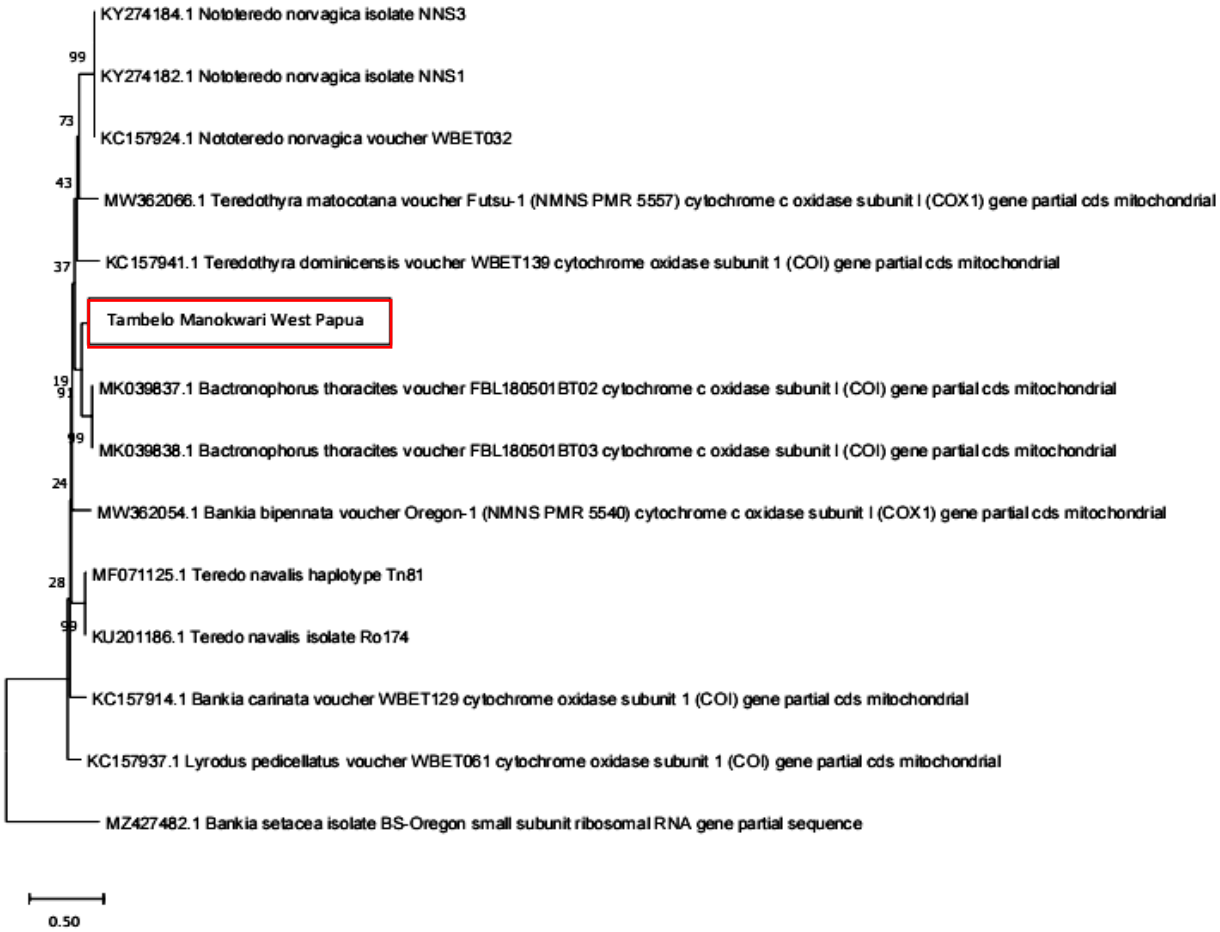

Figure 7. Neighbor-Joining Tree of 9 species of the family Teredinidae based on partial sequences of the cytochrome c subunit I (COI) gene, corresponding to the COI DNA bar code region. Trees are drawn to scale, with branch lengths in units equal to the evolutionary distances used to conclude the phylogenetic tree. The distance is calculated using the Maximum Composite Likelihood method and is in units of the number of basic substitutions per location. This analysis involved 190 nucleotide sequences. The codon positions included are 1 + 2 + 3 + Noncoding. All ambiguous positions have been removed for each sequence pair (pair option). There were a total of 1180 positions in the final dataset. The *tambelo* from this study is in the red-lined box. *Bankia setacea* is included as an outer group. The bootstrap support value is indicated at the appropriate node

In conclusion, this study concludes that the results of morphology and PCR sequencing of *tambelo* samples extracted from mangrove stems of *Rhizophora* sp., which had died in Wamesa waters, Manokwari District, Indonesia, showed that *tambelo* that had undergone evolutionary variation has similarity with *B. thoracites* from Peninsular Malaysia than other shipworm species, with a bootstrap value of 91% and a genetic distance of 0.173.

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