

Observations on the Life History and Geographic Range of the Giant Chemosymbiotic Shipworm *Kuphus polythalamius* (Bivalvia: Teredinidae)

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Abstract. *Kuphus polythalamius* (Teredinidae) is one of the world's largest, most rarely observed, and least understood bivalves. *Kuphus polythalamius* is also among the few shallow-water marine species and the only teredinid species determined to harbor sulfur-oxidizing chemoautotrophic (thioautotrophic) symbionts. Until the recent discovery of living specimens in the Philippines, this species was known only from calcareous hard parts, fossils, and the preserved soft tissues of a single large specimen. As a result, the anatomy, biology, life history, and geographic range of *K. polythalamius* remain obscure. Here we report the collection and description of the smallest living specimens of *K. polythalamius* yet discovered and confirm the species identity of these individuals by using sequences of three genetic markers. Unlike previously collected specimens, all of which have been reported to occur in marine sediments, these specimens were observed burrowing in wood, the same substrate utilized by all other members of the family. These observations suggest that *K. polythalamius* initially settles on wood and subsequently

transitions into sediment, where this species may grow to enormous sizes. This discovery led us to search for and find previously unidentified and misidentified wood-boring specimens of this species within museum collections, and it allowed us to show that the recent geographic range (since 1933) of this species extends across a 3000-mile span from the Philippines to Papua New Guinea and the Solomon Islands.

Introduction

Since the initial misidentification of *Kuphus polythalamius* as a serpulid polychaete by Linnaeus in 1758, the biology of this species has remained enshrouded in mystery. This situation is at least partly explained by the fact that, until recently, this giant worm-like sediment-dwelling teredinid bivalve was known primarily from descriptions of calcareous hard parts (shells, tubes, and pallets) and isolated, sporadic, and often secondhand reports of the existence of living specimens (*e.g.*, Griffiths, 1806; Wright, 1866; Calman, 1927 quoted in Societies and Academies, 1927; Sivickis, 1928). In 1966, Turner provided the first and, to date, the only detailed anatomical description of this species, based on a single ethanol-preserved specimen collected in the Solomon Islands in 1933. In 2017, Distel *et al.* reported the collection of several large living individuals of *K. polythalamius* in Mindanao, Philippines, in marine sediments, the first described in peer-reviewed litera-

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Abbreviations: *mt-COI*, mitochondrial cytochrome oxidase I gene; PCR, polymerase chain reaction; rRNA, ribosomal RNA.

ture. These ranged in body length from 11 to 64 cm prior to preservation. To our knowledge, neither smaller living specimens of this species nor preserved soft tissues derived therefrom have been observed or described in peer-reviewed literature. As a result, little is known about the early life history and recent geographic range of this enigmatic species.

Kuphus polythalamius is the largest and among the most atypical members of the Teredinidae (Huber, 2015). Teredinid bivalves are commonly referred to as shipworms due to their worm-like appearance and habit of burrowing in and ingesting wood, including the timbers of wooden ships (Turner, 1966). A number of anatomical adaptations facilitate this unique habit, including shells that bear a dense array of microscopic teeth used for boring in wood; large posterior adductor muscles that enable the boring action of the shells; a long vermiform body that facilitates deep penetration into wood; secretion of a hard calcareous tube that lines the inner surface of the burrow and provides protection against predators and desiccation; a pair of calcareous paddle-like structures (pallets) that flank the siphons and serve to plug the entrance to the burrow; an organ, known as the cecum, that facilitates storage and digestion of excavated wood particles (Turner, 1966); and a unique relationship with intracellular cellulolytic bacterial symbionts that reside within the gills and provide enzymes that aid in the digestion of wood (Popham and Dickson, 1973; Waterbury *et al.*, 1983; Distel *et al.*, 1991; O'Connor *et al.*, 2014). This distinctive array of adaptations has allowed shipworms to become broadly distributed across the world's oceans, exploiting a wide range of woody plant materials in marine to brackish environments, from mangrove and seagrass rhizomes to naturally occurring driftwood and man-made structures such as piers, boats, fishing equipment, and sea defenses (Distel, 2003).

Although *K. polythalamius* is similar in general body plan to other more typical shipworms, the observed habitat, anatomy, and symbiotic bacteria differ substantially. In contrast to other teredinid species, which burrow in and ingest wood or woody plant materials (Distel, 2003), individuals of *K. polythalamius* described previously were found burrowing in marine sediments (Griffiths, 1806; Wright, 1866; Calman, 1927 quoted in Societies and Academies, 1927; Sivickis, 1928). *Kuphus polythalamius* also grows far larger than other shipworms, with some specimens exceeding 1.6 m in length and 7 cm in diameter (Wright, 1866). This unusual size places *K. polythalamius* among the largest of bivalve species (Huber, 2015). The burrow lining is far more stout and massive than that of other shipworm species, consistent with the need to protect and support the animal in the absence of rigid wood substrates. The anterior of the shell valves is smooth in appearance and is greatly reduced in size by comparison with the body. The posterior adductor muscle is reduced compared with typical wood-boring teredinids, suggesting a more limited ability to burrow in hard substrates. The digestive system is highly reduced, and the wood-storing caecum is not evident (Turner, 1966), sug-

gesting diminished capacity for wood digestion (Distel *et al.*, 2017). Finally, unlike other teredinid species, which harbor cellulolytic endosymbionts (Waterbury *et al.*, 1983; Distel *et al.*, 1991, 2017), the symbiont community of *K. polythalamius* is composed of sulphur-oxidizing chemoautotrophic bacteria, implying that reduced sulphur compounds, rather than wood, provide the major source of nutritional energy for large sediment-dwelling individuals of this species (Distel *et al.*, 2017).

However, whereas fully grown individuals of *K. polythalamius* are strongly differentiated from other shipworms in terms of habitat, anatomy, and trophic strategy, smaller specimens have not been observed; and so it is not clear whether the same is true at earlier points in their life history. Here we examine a recent collection of teredinid bivalves similar in appearance to *K. polythalamius*. Unlike previously described specimens of *K. polythalamius*, these were found burrowing in wood rather than sediment, and these included individuals smaller than any previously observed for this species. We use molecular and morphological evidence to determine the species identity of these specimens, to ask whether wooden substrates play a role in the life history of *K. polythalamius*, and to evaluate the current geographic range of this enigmatic and rarely observed species.

Materials and Methods

Collection

All specimen collection locations are shown in Figure 1. Sediment-dwelling individuals of *Kuphus polythalamius* (Linnaeus, 1767) were collected in Kalamansig, Sultan Kudarat, Mindanao, Philippines, in 2–3-m water depth. Wood-boring specimens were collected from a single large piece of partially decomposed wood (Fig. 2A), located in Mabini, Batangas, Philippines, at a water depth of about 2 m. Additional specimens from museum collections were examined, including a sediment-dwelling specimen collected in the Solomon Islands, currently housed at the Harvard Museum of Comparative Zoology (MCZ 229089) and the basis of the first and only published anatomical description of *K. polythalamius* (Turner, 1966); an incomplete (siphon, pallets, and tube) wood-boring specimen from Tingloy, Batangas, Philippines, housed at the California Academy of Sciences (CAS190664); and three whole wood-boring specimens collected in a lagoon near Bilbil Island, Madang, Papua New Guinea, at water depths of 2–3 m on fine coral-sand bottom during Biodiversity Expedition Papua Niugini 2012–2013, housed at the Museum National d'Histoire Naturelle (MNHN120508, MNHN120509, and MNHN80001). Details of specimens, collection locations, and habitat type are provided in Table 1.

Morphological examination

Intact individuals of *K. polythalamius* were carefully removed from their calcareous tubes (Fig. 3A), photographed,



Figure 1. Recent (since 1933) geographic occurrence of living specimens of *Kuphus polythalamius*. Red stars indicate the approximate locations of live-collected specimens examined in this study. Map created using <https://www.nauticalcharts.noaa.gov/ENConline/enconline.html>.

and measured prior to preservation. Calcareous structures (pallies and shell valves) were removed by dissection and imaged using a Nikon Eclipse E800 compound microscope (Tokyo, Japan).

Scanning electron microscopy

Shell valves (Fig. 4) were removed and dehydrated in absolute ethanol, critical-point dried using the SAMDRI-PVT-3D Critical Point Dryer (Tousimis, Rockville, MD), mounted on a standard aluminum scanning electron microscope stub, and coated with platinum to a thickness of 5 nm, using the Cressington 208 HR High Resolution Sputter Coater (Cressington Scientific Instruments, Watford, United Kingdom). Images were produced on the Hitachi S-4800 field emission scanning electron microscope (Krefeld, Germany).

DNA extraction and amplification

DNA was extracted from siphonal tissue and associated musculature. Total genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Concentration, yield, and purity of DNA were determined by UV spectrophotometry, estimating that 1 absorption unit at 260 nm was approximately equal to 50 ng μL^{-1} double-

stranded DNA. Genomic DNA was cryo-preserved at -80°C and archived at the Ocean Genome Legacy Center, Northeastern University, Nahant, Massachusetts.

Partial small (18S) and large (28S) subunit nuclear ribosomal RNA (rRNA) and mitochondrial cytochrome oxidase I (*mt-COI*) genes were amplified from the resulting DNA preparations by polymerase chain reaction (PCR). Amplification reactions were prepared using 12.5 μL of high-fidelity polymerase solution (OneTaq, New England Biolabs, Ipswich, MA), 0.5 μL of each primer (10 mmol L^{-1}), 1–2 μL of DNA template (10–20 ng μL^{-1}), brought to a total volume of 25 μL with ultra-pure water (18 mol L^{-1} $\Omega\text{-cm}$, 25°C). Fragments of the 18S and 28S subunit nuclear rRNA and *COI* subunit genes, of approximately 1686, 1416, and 591 bp, respectively, were amplified using the primer pairs shown in Table 2. All reactions were performed following the cycling parameters in Table 3 on a PTC-200 Thermal Cycler (MJ Research, Quebec, Canada).

Subsequently, PCR products were visualized and sized by electrophoresis in 1% agarose gels and purified using the Zymo Clean and Concentrate Kit (Irvine, CA), following the manufacturer's protocol. Resulting products were sequenced bi-directionally on a 3730xl DNA Analyzer (Life Technologies, Grand Island, NY), using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Life Technologies) at New England Biolabs (Ipswich, MA). The *mt-COI* sequences

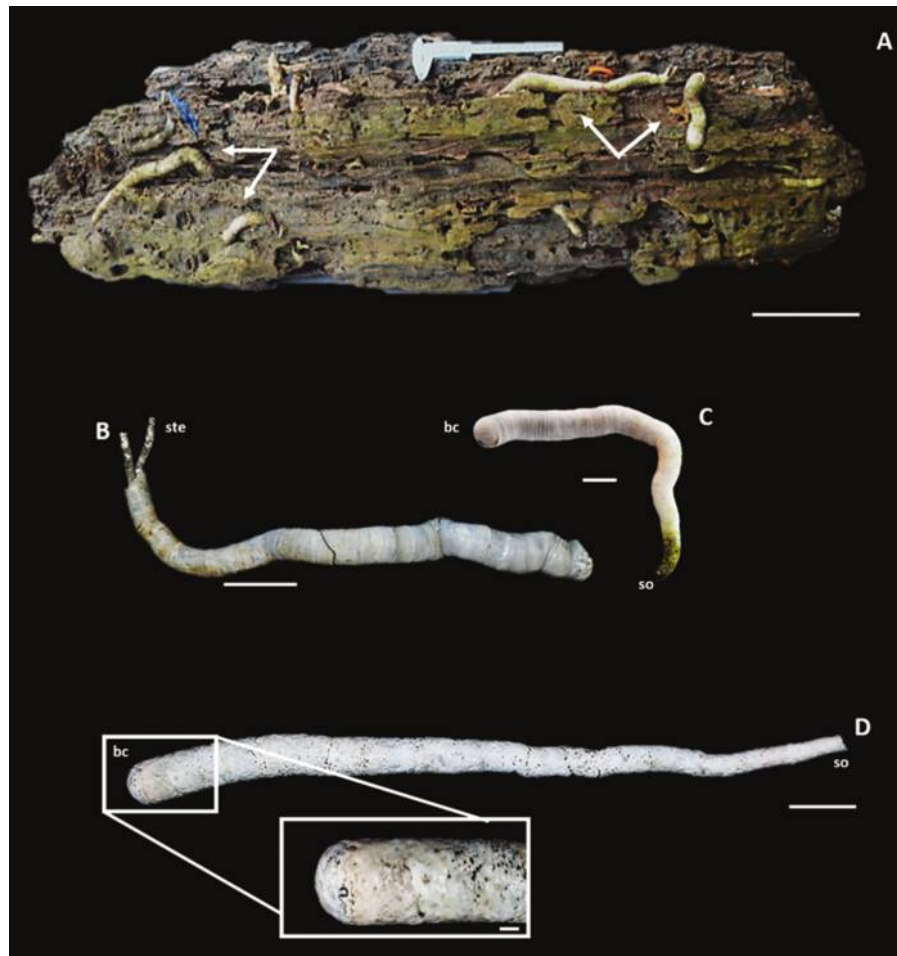


Figure 2. Specimens of *Kuphus polythalamius* from wood and mud. (A) Partially decomposed timber containing specimens of *K. polythalamius*, with arrows indicating calcareous tubes. (B–D) Capped calcareous tubes. (B, C) Tubes found in wood (specimens PMS-3495U and PMS-3405S, respectively). (D) Tubes found in sediment (specimen PMS-2132W). (Inset) Magnified region of the burrow cap. bc, burrow cap; so, siphonal opening; ste, siphonal tube extension. Scale bars = 10 cm (A), 5 cm (B), 1 cm (C), 10 cm (D), and 1 cm (inset).

for sediment-dwelling specimens were obtained from previously published gill metagenomic data (GenBank accession no. SAMN06338154).

Partial sequences for *18S* rRNA, *28S* rRNA, and *mt-COI* were aligned using MUSCLE (Edgar, 2004), with default parameters, trimmed to equal length; and pairwise distance matrices (Table 4) were constructed using the Kimura 2 Parameter (K2P) model as implemented in Geneious v8.0.5 (Biomatters Limited, Auckland, New Zealand). The phylogram in Figure 5 was constructed by neighbor joining, as implemented in the Geneious Tree Builder tool, Geneious v8.0.5 (Biomatters), using the Tamura-Nei genetic distance model and an alignment of 573 bp. The tree shown is a consensus of 10,000 bootstrap replicates. Sequences used in these analyses have been deposited in GenBank, and associated genomic DNA samples have been deposited at the Ocean

Genome Legacy Center, Northeastern University (all accession numbers in Table 5).

Results and Discussion

Often exceeding 1 m in body length, *Kuphus polythalamius* has been reported to occur in shallow coastal waters of the Indo-Pacific in proximity to mangrove forests and coral reefs and in substrates including mud, gravel, and sand (Hedley, 1895; Sivickis, 1928; Turner, 1966). Here we report the collection of several small living specimens matching the anatomical and morphological characteristics of this species (Figs. 2–4), found burrowing in partially decayed wooden substrates in Mabini, Batangas, Philippines, and in a decaying *Pandanus* trunk in Madang, Papua New Guinea, in 2012 at water depths of about 2–3 m (see Table 1; Figs. 1–4). In ad-

Table 1

Specimens used in this investigation

No. and specimen ID	Holding institution	Collection location	Collection date	Substrate	Water depth (m)	Tube length (cm)	Body length (cm)
1. PMS-1656S	UP	Kalamansig, Sultan Kudarat, Philippines	November 2010	Sediment	<3	92.4	61.7
2. PMS-1677P	UP	Kalamansig, Sultan Kudarat, Philippines	November 2010	Sediment	<3	66.3	47.8
3. PMS-1671X	UP	Kalamansig, Sultan Kudarat, Philippines	November 2010	Sediment	<3	94.0	51.7
4. PMS-2133X	UP	Kalamansig, Sultan Kudarat, Philippines	December 2011	Sediment	<3	106.7	63.5
5. PMS-2132W	UP	Kalamansig, Sultan Kudarat, Philippines	December 2011	Sediment	<3	115.1	...
6. PMS-2193M	UP	Kalamansig, Sultan Kudarat, Philippines	December 2011	Sediment	<3	27.9	10.8
7. PMS-3404P	UP	Mabini, Batangas, Philippines	May 2016	Wood	<2	9.3	...
8. PMS-3405S	UP	Mabini, Batangas, Philippines	May 2016	Wood	<2	10.5	4.2
9. PMS-3457L	UP	Mabini, Batangas, Philippines	May 2016	Wood	<2	24.4	14.1
10. PMS-3477H	UP	Mabini, Batangas, Philippines	May 2016	Wood	<2	10.6	5.1
11. PMS-3495U	UP	Mabini, Batangas, Philippines	May 2016	Wood	<2	35.0	19.9
12. PMS-3664H	UP	Mabini, Batangas, Philippines	May 2016	Wood	<2	...	5.8
13. PMS-3696Y	UP	Mabini, Batangas, Philippines	May 2016	Wood	<2	9.5	7.7
14. PMS-3715U	UP	Mabini, Batangas, Philippines	May 2016	Wood	<2	5.4	3.4
15. PMS-3720K	UP	Mabini, Batangas, Philippines	May 2016	Wood	<2	5.3	...
16. PMS-3721L	UP	Mabini, Batangas, Philippines	May 2016	Wood	<2	6.0	...
17. CAS190664	CAS	Tingloy, Batangas, Philippines	November 2012	Wood	...	...	...
18. MNHN120508	MNHN	Madang, Papua New Guinea	November 2012	Wood	<3	...	...
19. MNHN120509	MNHN	Madang, Papua New Guinea	November 2012	Wood	<3	...	...
20. MCZ 229089	MCZ	Solomon Islands	1933	Sediment	...	...	...

For holding institutions, UP, University of the Philippines, Marine Science Institute; CAS, California Academy of Science; MNHN, Mollusca Collection, Muséum National d'Histoire Naturelle; MCZ, Malacology Department, Museum of Comparative Zoology, Harvard University.

dition, we located and examined museum specimens of *K. polythalamius* that were collected live and for which preserved soft tissues were available. These include a single specimen collected in the Solomon Islands in 1933, upon which the sole published anatomical description of *K. polythalamius* was based (Turner, 1966). We also examined a single partial spec-

imen collected in soft decaying wood in Tingloy, Batangas, Philippines, in 2012 and previously identified as *Dicyathifer manni*.

The morphologically identified wood-dwelling individuals ranged in body length from 3.4 to 19.9 cm prior to preservation (Table 1). To our knowledge, this set of samples included



Figure 3. *Kuphus polythalamius*. (A) Specimen found in wood, tube removed. (B) Specimen found in sediment, tube removed. (C) Outer and inner face of a pallet from a wood-boring specimen. (D) Outer and inner face of a pallet from a sediment-dwelling specimen. Scale bars = 1 cm (A–B), 5 mm (C–D).

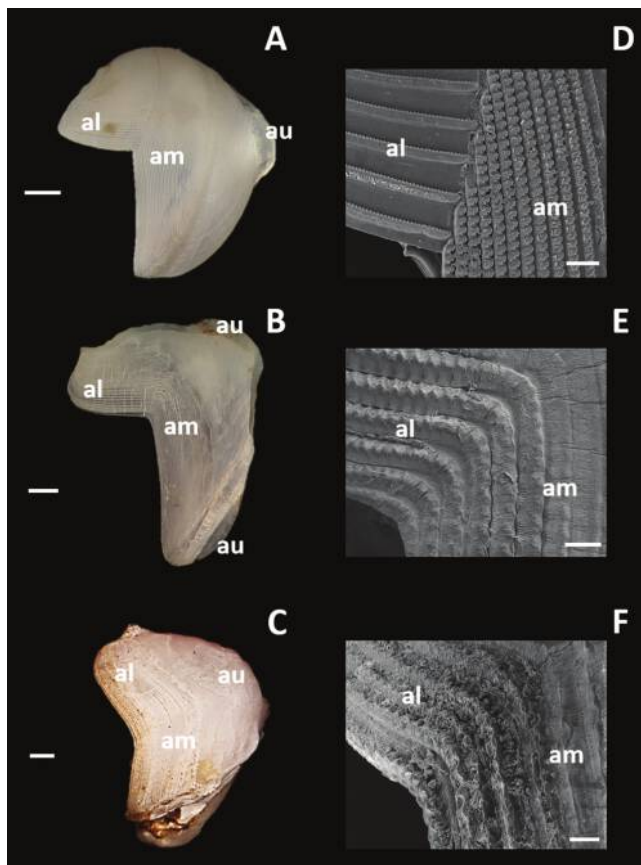


Figure 4. Shell valve architecture. The shell valves of (A) a typical wood-feeding teredinid bivalve, *Lyrodus pedicellatus*, compared with valves of (B) wood-dwelling and (C) sediment-dwelling specimens of *Kuphus polythalamius*. (D–F) Scanning electron micrographs showing shell denticles in shell regions indicated by the boxes in (A–C). al, anterior lobe; am, anterior median lobe; au, auricle. Scale bars = 1 mm (A–C), 100 μ m (D–F).

specimens smaller than any living *K. polythalamius* previously described in the literature, as well as specimens larger than some previously observed burrowing in sediments. To determine whether these small wood-boring specimens are of the same species as the large sediment-dwelling specimens described previously, we compared partial sequences of three genes frequently used in species diagnosis: the 18S and 28S

rRNA genes and *mt-COI*. We determined that all wood- and sediment-dwelling specimens examined were highly similar with respect to all three loci. Among two wood-boring specimens and one sediment-dwelling specimen examined, the 18S and 28S rRNA sequences were greater than 99.94% and 99.05% identical, respectively. The phylogram shown in Figure 5 illustrates that, based on *mt-COI* sequences, wood-boring and sediment-dwelling specimens examined in this study form a single well-supported clade that is well differentiated from other accepted teredinid species. Furthermore, evolutionary distances within this clade, and between this clade and members of other teredinid species, are comparable to distances observed within and between accepted teredinid species, respectively. For example, the lowest and highest pairwise divergences observed among *mt-COI* sequences were 0.09% and 1.22%, respectively (Table 4). This difference compares with previously observed intraspecies variations of 0.0%–1.7% among specimens within 4 shipworm species (*Bankia carinata*, *Lyrodus pedicellatus*, *Teredothyra dominicensis*, and *Neoterodo norvagica*). In contrast, interspecies sequence divergences ranged from 19.3% to 33.8% among the same species (Borges *et al.*, 2012; Shipway *et al.*, 2014). On this basis we conclude that both the wood-boring and sediment-dwelling specimens examined here are members of the single teredinid species *K. polythalamius*.

Historical reports, based primarily on observation of calcareous hard parts and dating back more than 300 years, suggest a broad distribution of *K. polythalamius* across the Indo-West Pacific region (*e.g.*, Rumphius, 1705; Griffiths, 1806; Calman, 1927 quoted in Societies and Academies, 1927; Sivicis, 1928; Haga, 2011). The data presented here confirm a recently (since 1933) observed geographic range for living *K. polythalamius* that extends at least 3000 miles from the northern Philippine island of Luzon to Papua New Guinea and the Solomon Islands (Fig. 1).

Observations on the anatomy, morphology, and behavior of the newly discovered specimens provide previously unknown or unconfirmed details regarding the reproductive strategy and life history of *K. polythalamius*. For example, one wood-boring specimen (4.2 cm in body length) was observed to release eggs upon removal from the wood, suggest-

Table 2

Amplification and sequencing primers used in this study

Primer name	Primer sequence (5'–3')	Target	Reference
18S EukF (forward)	WAYCTGGTTGATCCTGCCAGT	18S rRNA	Medlin <i>et al.</i> , 1988
18S EukR (reverse)	TGATCCTTCYGCAGGTTACCTAC	18S rRNA	Medlin <i>et al.</i> , 1988
28S-NLF184-21 (forward)	ACCCGCTGAAYTTAAGCATAT	28S rRNA	Distel <i>et al.</i> , 2011
28S-NLR1600 (reverse)	AGCGCCATCCATTTTCAGG	28S rRNA	Distel <i>et al.</i> , 2011
COI-1498-F (forward)	AAT-CAT-AAA-GAA-ATT-GGT-ACT-TTG-TAT-TT	<i>mt-COI</i>	This study
COI-854-R (reverse)	TCW-GGR-TGW-CCA-AAA-AAY-CAA-AA	<i>mt-COI</i>	This study

mt-COI, mitochondrial cytochrome oxidase I gene; rRNA, ribosomal RNA.

Table 3*Amplification cycling profiles*

Primer target	1 cycle	35 cycles	1 cycle
18S rRNA	94 °C, 3 min	94 °C for 20 s, 64 °C for 40 s, 68 °C for 60 s	68 °C, 3 min
28S rRNA	94 °C, 3 min	94 °C for 20 s, 63 °C for 30 s, 68 °C for 60 s	68 °C, 3 min
mt-COI	94 °C, 3 min	94 °C for 30 s, 51 °C for 90 s, 72 °C for 60 s	72 °C, 3 min

mt-COI, mitochondrial cytochrome oxidase I gene; rRNA, ribosomal RNA.

ing that this individual was a mature female and demonstrating that *K. polythalamius* reproduces by broadcast spawning, as do most Teredinidae. Although the viability of the gametes was not confirmed, the presence of an egg-bearing individual in wood suggests that this species may reach maturity and reproduce within wood, potentially completing the entire life cycle without entering sediments. We also suggest that the anatomy of the tubes likely rules out intromission, a form of reproduction that, although rare in Bivalvia, has been observed in the teredinid genera *Bankia* and *Nausitora* (Clapp, 1951 quoted in Turner, 1966; Hiroki *et al.*, 1994; Velásquez *et al.*, 2011). In these taxa, the male inserts the excurrent siphon into the incurrent siphon of the female before releasing sperm, resulting in internal fertilization. We observed that, even among small specimens of *K. polythalamius*, the movement of the siphons is strictly limited by a septum dividing the tube and by bifurcating calcareous tube extensions (see Fig. 2B), likely preventing intromission. These observations are consistent with the previous hypothesis that broadcast spawning is an ancestral condition in Teredinidae, whereas more complex reproductive strategies, including internal fertilization, intromission, larvipary, and male dwarfism, are derived traits confined to the lineage containing the genera *Bankia*, *Nausitora*, *Teredo*, *Lyrodus*, and *Zachisia* (Distel *et al.*, 2011; Shipway *et al.*, 2016).

Although *K. polythalamius* has long been thought to be a soft bottom dweller (Turner, 1966), the early life history and potential association of this species with wood has remained controversial. In 1927, Calman (quoted in Societies and Academies, 1927) postulated that *K. polythalamius* is, in fact, the mature form of the wood-boring species *Dicyathifer*

mannii, the pallets of which are very similar to those of *K. polythalamius*. Sivickis (1928) later argued against a wood-boring stage for *K. polythalamius*, citing a large colony of these animals found near Puerto Galera, Mindoro, in which “young” and “old” animals lived near each other, with all of them being buried in sand. Savazzi (1982), like Calman (1927 quoted in Societies and Academies, 1927), postulated, without evidence, that *K. polythalamius* initially settled on wood and subsequently transitioned to the sediment. However, to our knowledge, there are no verified observations of this species in wood in peer-reviewed literature prior to those presented in this investigation. Our discovery of small individuals of *K. polythalamius* burrowing in wood in four distant geographic locations confirms that the larvae of *K. polythalamius* can settle, metamorphose, and begin early development on wood. This pattern is consistent with the fact that all teredinid bivalves whose life histories have been described settle and metamorphose on wood (Turner, 1966, 1969).

We note, however, that the small size of specimens found on wood is not necessarily an indication that these specimens are younger than the larger specimens found in sediment. An alternate hypothesis is that individuals of *K. polythalamius* can settle and metamorphose on either wood or sediment, but that those settling on wood grow substantially more slowly and do not attain the very large sizes achieved by individuals settling on sediment. In other words, the small animals observed in wood may be stunted, rather than young.

Although direct evidence is lacking, several observations argue against this hypothesis. First, in Kalamansig, where large sediment-dwelling specimens are abundant, all specimens of *K. polythalamius* observed in sediment to date have

Table 4*Percent pairwise nucleotide identity matrix for mitochondrial cytochrome oxidase I (mt-COI) genes of specimens from sediments and wood*

No. and specimen ID	Location and substrate	1	2	3	4	5	6
1. PMS-2133X	Philippines, sediment	...	99.21	99.65	99.3	99.13	99.13
2. PMS-3664H	Philippines, wood	...	...	99.21	99.91	99.39	99.74
3. CAS190664	Philippines, wood	...	...	...	99.3	98.78	99.13
4. MNHN120508	Papua New Guinea, wood	...	...	...	...	99.48	99.83
5. MNHN120509	Papua New Guinea, wood	...	...	...	...	...	99.65
6. MNHN80001	Papua New Guinea, wood	...	...	...	...	...	...

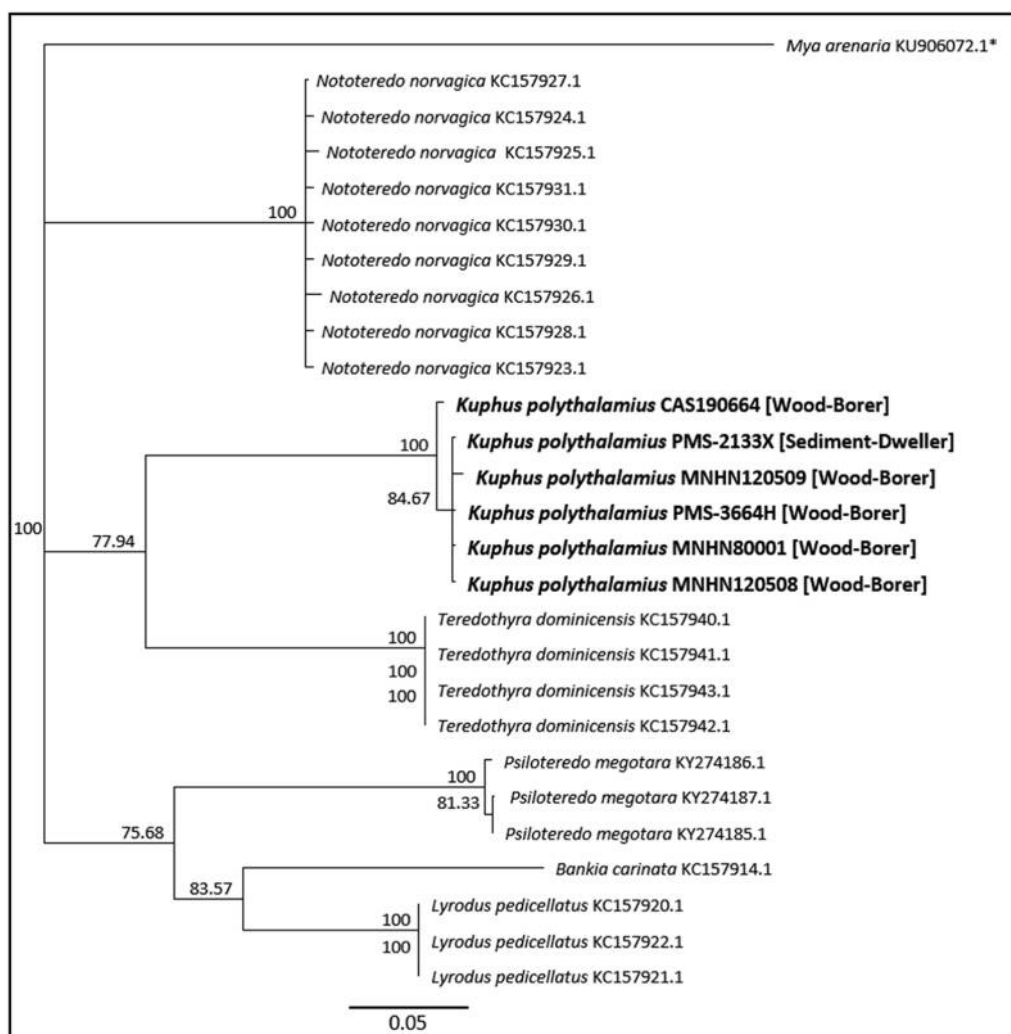


Figure 5. Phylogenetic relationships among selected wood-boring and sediment-dwelling Teredinidae. Partial sequences (600 bp) of the mitochondrial cytochrome oxidase I (*mt-COI*) gene of representative teredinid species were aligned using MUSCLE (Edgar, 2004), with default parameters, and phylogenetic relationships were inferred by neighbor joining (Tamura-Nei genetic distance model), using the Geneious Tree Builder tool as implemented in Geneious v8.0.5 (Biomatters Limited, Auckland, New Zealand). The tree portrayed is a consensus of 10,000 bootstrap replicates. Bootstrap proportions are indicated at each node. Accession numbers for sequences derived from GenBank are shown. Horizontal branch lengths are proportional to evolutionary distances expressed as nucleotide substitutions per nucleotide position.

been greater than 11 cm in length. However, in this same location, smaller wood-boring specimens and large sediment-dwelling specimens have been observed growing in close proximity, suggesting that recruitment in this location is to wood rather than to sediment. Additionally, the largest specimens of *K. polythalamius* found in wood are substantially larger than the smallest specimens found in sediments, indicating that individuals can achieve significant sizes in wood. Finally, in all observed locations, all individuals smaller than 11 cm in length have been found in wood, indicating that individuals on wood either are constrained by the size of the wooden substrate and cannot grow larger or may transition from wood to the sediment, where they may continue to grow

after the wood is decayed or consumed. Given these observations, and in the absence of evidence of radically different growth rates of wood-boring and sediment-dwelling individuals, it seems safest to assume that the small wood-boring specimens are juveniles or young adults and that the reproductive cycle of *K. polythalamius* resembles that of most teredinid species: beginning with broadcast spawning of eggs and larvae, followed by a period of planktonic development, settlement on a woody substrate, metamorphosis, and subsequent sexual maturation. We suggest that the very large size achieved by sediment-dwelling individuals of *K. polythalamius* more likely reflects the fact that these specimens can continue to grow unconstrained by the limited physical di-

Table 5

Genomic DNA and sequence accession numbers

Specimen ID	GenBank 18S	GenBank 28S	GenBank <i>mt-COI</i>	OGL no.
PMS-2132W	MH516332	MH516335	MH521028	...
PMS-3696Y	MH516333	MH516336	MH521029	A31831
PMS-3715U	MH516334	MH516337	...	A31836
MNHN120508	...	...	MH521030	A31832
MNHN120509	...	...	MH521031	A31833
MNHN80001	...	...	MH521032	A31834
MCZ 229089	...	...	MH521033	A31835

mt-COI, mitochondrial cytochrome oxidase I gene; OGL, Ocean Genome Legacy Center, Northeastern University.

mensions of wooden substrates, rather than a radical difference in their growth rates.

In a previous investigation, the gill symbiont community of large sediment-dwelling specimens of *K. polythalamius* was shown to be comprised nearly entirely of sulfur-oxidizing chemoautotrophic (thioautotrophic) bacteria (Distel *et al.*, 2017) rather than cellulolytic symbionts, as observed in other shipworm species (Distel *et al.*, 1991, 2002; Luyten *et al.*, 2006; O'Connor *et al.*, 2014). It was proposed that these thioautotrophic symbionts oxidized reduced sulfur compounds produced by microbial degradation of wood as an energy source and fixed inorganic carbon *via* the Calvin-Benson-Bassham cycle. The resulting fixed carbon was proposed to provide a source of carbon nutrition to the host, instead of, or in addition to, carbon derived from organic sources. It has been shown previously that sulfate reduction on decaying wood can produce sufficient quantities of reduced sulfur compounds to support thioautotrophic metabolism and thioautotrophic symbioses (Laurent *et al.*, 2009, 2013; Fagervold *et al.*, 2012; Bienhold *et al.*, 2013; Yücel *et al.*, 2013).

It remains an open question whether the small wood-dwelling specimens of *K. polythalamius* observed here also harbor thioautotrophic symbionts or whether these small wood-dwelling individuals harbor cellulolytic symbionts. In addition to thioautotrophic symbionts, which dominate the symbiont community of large sediment-dwelling specimens of *K. polythalamius* (Distel *et al.*, 2017), *Teredinibacter turnerae*, a widespread cellulolytic symbiont of Teredinidae, was also isolated from these tissues. It is therefore likely that cellulolytic symbionts are also present in the small wood-boring specimens, suggesting the possibility that cellulolytic symbiosis may play an important role in metabolism during wood-dwelling stages. If so, this species may undergo a shift from cellulolytic symbiosis to thioautotrophic symbiosis during later development.

Although we cannot rule out the possibility that *K. polythalamius* may be capable of wood feeding at earlier stages in development, as are other shipworms species (Mann and Gallagher, 1985), the specimens examined here did not appear to rely heavily on wood as a food source. First, as previously re-

ported (Distel *et al.*, 2017), a calcareous cap sealed the anterior end of the tubes of most of the wood-boring specimens examined (Fig. 2B, 2C). The same was true for most sediment-dwelling specimens (Fig. 2D). This cap forms a barrier between the valves and the excavation face of the burrow, thus precluding wood feeding by the wood-boring specimens, as well as ingestion of sediments by the large sediment dwellers, as long as the cap is in place. Although the tubes must be uncapped at least intermittently to facilitate burrowing and growth, it appears that the tubes are likely sealed during a significant portion of the life cycle. Second, the small wood-boring specimens, like their larger sediment-dwelling counterparts, have a greatly reduced digestive system. In both cases, a well-developed wood-storing cecum, thought to be critical to wood digestion in other Teredinidae, is not evident. Third, the posterior adductor muscles in the wood-dwelling specimens are reduced in size, as are those of the large sediment-dwelling specimens previously examined. This reduction has previously been interpreted as a lack of specialization for wood boring (Turner, 1966). Fourth, the shell valves of both wood- and sediment-dwelling specimens of *K. polythalamius* lack the small, sharp, and finely sculpted shell teeth that, in other teredinid species, are used to produce fine micron-scale particles that facilitate wood digestion (Fig. 4). Instead, the shell teeth of *K. polythalamius* are large, broad, blunt, triangular, or flattened protrusions. These may be suitable for burrowing in sediment or wood but would not be expected to produce fine particles similar to those made by wood-feeding species. Finally, we were unable to identify wood particles in the digestive system of the examined specimens. Taken together, the reduced digestive system, the reduced shell valves and musculature, the lack of fine shell dentition, the absence of a wood-storing organ, the intermittent capping of the burrow, and the absence of wood in the digestive tract suggest that wood likely played a limited role in the nutrition of both the wood-boring and sediment-dwelling specimens of *K. polythalamius* examined to date. Additional physiological, microbiological, genomic, and biochemical examination of these specimens, and of earlier life stages of this species, will be required to more fully address the extent to which reduced sulfur com-

pounds, wood, or other particulate food sources such as phytoplankton or sediments may contribute to the nutrition of this species.

Clearly, many aspects of the biology and life history of *K. polythalamius* remain to be uncovered. The description of small specimens of *K. polythalamius*, and the discovery that they burrow in wood, adds substantially to the sparse body of knowledge available for this rare and rarely observed species. These realizations point out the need to include woody substrates in the effort to uncover the geographic range, preferred habitats, life cycle, and early life history of this elusive species that has remained shrouded in mystery for more than 300 years.

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