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Molecular phylogeny of Pholadoidea Lamarck, 1809 supports a single origin for xylotrophy (wood feeding) and xylotrophic bacterial endosymbiosis in Bivalvia

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ABSTRACT

The ability to consume wood as food (xylotrophy) is unusual among animals. In terrestrial environments, termites and other xylotrophic insects are the principle wood consumers while in marine environments wood-boring bivalves fulfill this role. However, the evolutionary origin of wood feeding in bivalves has remained largely unexplored. Here we provide data indicating that xylotrophy has arisen just once in Bivalvia in a single wood-feeding bivalve lineage that subsequently diversified into distinct shallow- and deep-water branches, both of which have been broadly successful in colonizing the world's oceans. These data also suggest that the appearance of this remarkable life habit was approximately coincident with the acquisition of bacterial endosymbionts. Here we generate a robust phylogeny for xylotrophic bivalves and related species based on sequences of small and large subunit nuclear rRNA genes. We then trace the distribution among the modern taxa of morphological characters and character states associated with xylotrophy and xylotrepesis (wood-boring) and use a parsimony-based method to infer their ancestral states. Based on these ancestral state reconstructions we propose a set of plausible hypotheses describing the evolution of symbiotic xylotrophy in Bivalvia. Within this context, we reinterpret one of the most remarkable progressions in bivalve evolution, the transformation of the "typical" myoid body plan to create a unique lineage of worm-like, tube-forming, wood-feeding clams. The well-supported phylogeny presented here is inconsistent with most taxonomic treatments for xylotrophic bivalves, indicating that the bivalve family Pholadidae and the subfamilies Teredininae and Bankiinae of the family Teredinidae are non-monophyletic, and that the principle traits used for their taxonomic diagnosis are phylogenetically misleading.

1. Introduction

Wood-eating (xylotrophic) and wood-boring (xylotrepetic) bivalves have attracted considerable interest for their unusual biology and morphology, destructive economic impacts, problematic taxonomy, potential role in marine carbon cycles, capacity to degrade woody (lignocellulosic) plant materials, potential as a source of novel enzymes for industry, and extraordinary bacterial endosymbioses. These bivalves cause more than a billion dollars in damage to ships, fishing equipment, and wooden structures in marine environments annually, and may have influenced historical events ranging from the defeat of the Spanish Armada to the demise of the fourth expedition of Christopher Columbus. More recently, these bivalves and their symbionts have attracted interest as potential sources of novel enzymes for the cellulosic biofuel industry (Distel, 2003; Cobb, 2002).

Clams that eat and/or burrow in wood are found in two bivalve families, Teredinidae and Pholadidae. Teredinidae (commonly known as shipworms) are the principle degraders of wood in shallow, temperate and tropical marine waters. They are found in floating, sunken, or living wood at depths ranging from the inter-tidal zone to ~150 m. This diverse group contains more than 65 well-defined species and includes some of the most highly modified and most destructive marine bivalves (Turner, 1966). Their common name derives from the wormlike appearance of adult specimens, whose extremely elongate body plan, greatly reduced valves (shells), habit of burrowing in wood, ability to form shell-lined burrows (tubes), and possession of shell-like plates (pallets) that are used to seal the burrows, distinguish them from all other bivalve taxa (Fig. 1). With the possible exception of one species

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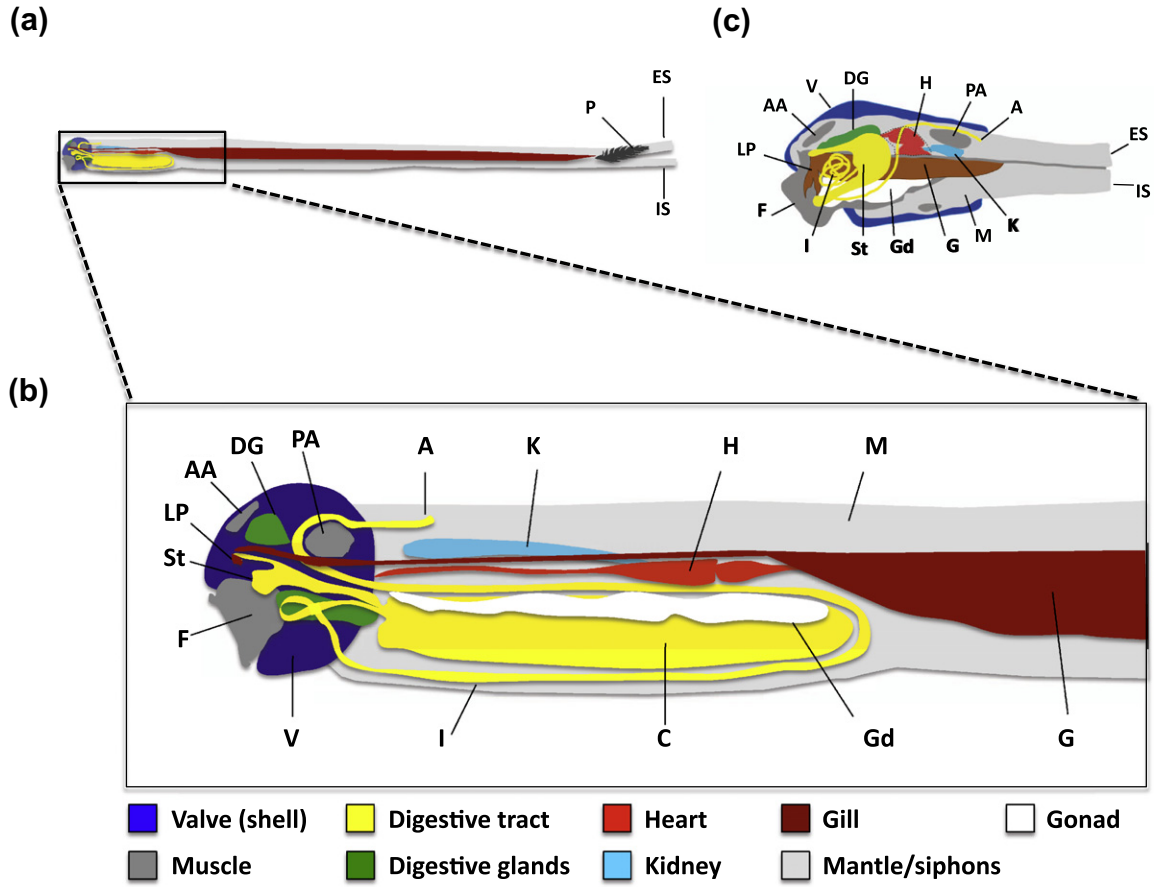

Fig. 1. Anatomical comparison of *Bankia setacea* (Teredinidae) and *Zirfea crispata* (Pholadidae) *Bankia setacea* (Teredinidae) a, b; *Zirfea crispata* (Pholadidae) c; anus, A; anterior adductor, AA; caecum, C; digestive gland, DG; excurrent siphon, ES; foot, F; gill, G; gonad, Gd; heart, H; intestine, I; incurrent siphon, IS; kidney, K; labial palps, LP; mantle, M; pallet, P; posterior adductor, PA; stomach, S; valve, V. Note that the major visceral organs of shipworms are posterior to and cannot be withdrawn within the confines of the valves as in other bivalves.

(*Kuphus polythalamia*), all Teredinidae burrow in and ingest wood or woody plant material. At least one species (*Lyrodus pedicellatus*) has been shown to grow and reproduce normally on a diet composed solely of wood (Gallager et al., 1981).

In contrast, most Pholadidae burrow in substrates other than wood. Exceptions are members of the subfamily Xylophagainae, which burrow in and ingest wood, and Martesianae, which burrow in wood and other substrates (Jenner et al., 2003; Scott, 1991; Springer and Beeman, 1960) but do not feed on wood particles (Turner, 1955). The distribution of Xylophagainae is limited largely to sunken wood deposits in deep marine waters (~100–7500 m) where these species are the most important consumers of deposited wood (Turner, 1973; Turner, 2002). Like other Pholadidae, Xylophagainae display none of the unusual vermiform characteristics of Teredinidae.

The mechanism of wood digestion in marine bivalves differs from that found in terrestrial wood consumers. Terrestrial organisms that consume wood as food contain within their digestive tracts communities of symbiotic microorganisms that are thought to aid in the digestion and metabolism of wood (Haigler and Weimer, 1991). Wood-boring bivalves appear to lack such highly developed microbial communities within their guts (Liu and Walden, 1970). Instead, the ability of both teredinid and xylophagainid clams to feed on wood is thought to depend on intracellular bacterial endosymbionts contained within specialized cells (bacteriocytes) of their gills. In Teredinidae, these bacterial endosymbionts are thought to produce cellulolytic enzymes that aid the host in digestion of wood (Distel, 2003), and that are known to fix nitrogen (Lechene et al., 2007; Waterbury et al., 1983) that may supplement the host's nitrogen deficient diet. These intracellular bacteria constitute a consortium of closely related species (Distel et al., 2002a; Luyten et al., 2006), only one of which has been grown in pure culture. This cultivated species, *Teredinibacter turnerae*, has been shown to degrade cellulose and to fix nitrogen in pure culture (Distel et al., 2002b; Waterbury et al., 1983). Members of Xylophagainae have also been shown to harbor bacterial endosymbionts within their gills (Distel and Roberts, 1997) although none have yet been cultivated.

Despite dramatic differences in body plan, Teredinidae and Xylophagainae share a number of traits that are unique or rare among bivalves, leading us to ask whether xylotrophy and xylotrophic symbiosis evolved independently in these taxa, as proposed previously (Turner, 1966; Turner, 2002) and as is implied by widely cited taxonomic treatments (e.g., (Newell, 1969)), or whether these properties evolved just once in a recent common ancestor of these nominally distinct lineages. The former would imply convergence, while the latter would suggest similarity due to homology.

Few phylogenetic treatments have been attempted for xylotrophic bivalves (Santos et al., 2005), in part because the highly modified body plans and highly specialized life habits of many xylotrophic bivalves create difficulties in identifying homologous traits and distinguishing them from convergent adaptations to the common challenges of wood-boring and wood-feeding habits (Hoagland and Turner, 1981). Indeed, the most comprehensive synthesis of the biology of wood-boring bivalves assembled to date

(Turner, 1966) describes the taxonomy of these bivalves as being "in a chaotic state."

Only five studies have specifically addressed evolutionary relationships among wood boring bivalves using biochemical or molecular methods; four of these studies were based on analysis of electrophoretic mobility of allozymes (Cole and Turner, 1978; Cole and Turner, 1977; Hoagland, 1986; Hoagland and Turner, 1981) and one was based on comparison of mitochondrial small subunit rRNA gene sequences (Santos et al., 2005). Although none of these studies have sampled wood-boring bivalves comprehensively, two raised significant questions with regard to widely accepted taxonomic treatments, both questioning the monophyly of the subfamilies of Teredinidae (Hoagland and Turner, 1981; Santos et al., 2005).

Analyses based on anatomical characters have also suggested alternative relationships among wood boring bivalves. For example, Purchon (1941) and Monari (2009) proposed that Xylophagainae share a more recent common ancestor with Teredinidae than with other Pholadidae, the latter conclusion resulting from a cladistic analysis (Monari, 2009; Purchon, 1941).

Here, to avoid the potentially confounding influences of convergent morphology, we infer a robust phylogeny for xylotrophic bivalves based on molecular characters that are independent of the xylotrophic habit. We then map the distribution of morphological characters and character states among modern taxa and use maximum parsimony to infer ancestral characteristics of xylotrophic bivalves.

2. Materials and methods

2.1. Taxon selection

Sixteen species of the family Teredinidae were selected, including representatives of all three teredinid subfamilies, Kuphinae, Bankiinae, and Teredininae, and five of the six anatomical groups (I, II, III, IV and VI) proposed by Turner (Turner, 1966). In addition, ten species of the family Pholadidae are considered. These include four species of the subfamily Xylophagainae that represent each of the three named genera (*Xylophaga*, *Xylopholas*, and *Xyloredo*). As of this writing (01/23/2011) the World Registry of Marine Species (http://www.marinespecies.org) lists 71 and 159 valid species names for Teredinidae and Pholadidae respectively. Reference taxa include representatives of the myoid families Myidae, Gastrochaenidae, and Hiatellidae, and additional selected species from Heterodonta, Paleoheterodonta, and Pteriomorphia. Taxonomic nomenclature is according to (Newell, 1969). A complete list of taxa used in this study appears in Table 1.

2.2. DNA extraction and sequencing

Tissue samples were stored frozen (−80 °C) or in 70–95% ethanol prior to analyses. DNA was extracted from mantle or gill tissue of 39 specimens representing 37 bivalve species (see Table 1) as in (Distel, 2000). Briefly, tissues were ground under liquid nitrogen and dissolved in a medium containing 5 M guanidinium thiocyanate. Insoluble material was sedimented by centrifugation and discarded. DNA was precipitated by addition of ethanol, redissolved in TE buffer, and extracted by the phenol chloroform method (Maniatis et al., 1982). Large and small subunit nuclear rRNA genes were amplified from the resultant DNA preparations by polymerase chain reaction (PCR) using high fidelity polymerase (Pfu; Stratagene or Phusion; Finnzymes) with the following amplification parameters (35 cycles, 1 min, 95 °C, 1 min 55 °C, 1 min 70 °C) and the primers listed in Table 2 (Lane, 1991; Medlin et al., 1988; Park and O' Foighil, 2000). Bidirectional sequencing was performed using an ABI 3100 capillary DNA sequencing platform with standard BigDye™ chemistry, thermal cycling conditions, and dye terminator removal, either directly on products pooled from three PCR amplifications, or on three independently sequenced clones from PCR products inserted into PCRblunt™vector (Invitrogen).

2.3. Phylogenetic analyses

DNA sequences were aligned using MacGDE v. 2.3 (Genetic Data Environment for Macintosh) taking into consideration secondary structural information inferred by comparison to *Placopecten magellanicus* large and small subunit rRNA secondary structure models (Comparative RNA Website and Project; http://www.rna.ccbb.utexas.edu). Nucleotide positions within structural features of variable length and other positions of uncertain alignment were removed from further consideration.

Phylogentic analyses were performed using PAUP* 4.0b10 (Swofford, 2003) and MrBayes v.3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) as implemented in MacGDE. Maximum parsimony (MP), minimum evolution (ME), maximum likelihood (ML), and Bayesian inference (BI) methods were evaluated. PAUP* was used for MP, ME, and ML analyses, while Mr. Bayes was used for BI. A character matrix consisting of 1686 bases for the small (18S) and 1148 bases for the large (28S) subunit rRNA genes, determined for each taxon in the study, was used for phylogenetic inference. Three data partitions were examined: 18S alone, 28S alone, and the combined [18S + 28S] data set. The partition homogeneity test (Cunningham, 1997) with 100 replicates was used to evaluate the validity of combining datasets. For analyses employing ML and BI, the best-fit model of evolution (GTR + I + G) and parameter value estimates were determined by the hierarchical log-likelihood ratio tests using algorithms from MODELTEST (Posada and Crandall, 1998) as implemented in MacGDE.

ME analyses were performed using a substitution model that allows approximation of variable substitution rates (HKY-85; (Hasegawa et al., 1985)), with rates assumed to follow a gamma distribution with shape parameter estimated from data, branch swapping algorithm = tree–bisection–reconnection (TBR), and minimum evolution optimality criterion. MP analyses were performed using the heuristic search option with random sequence addition (100 replicates) and TBR branch swapping. Characters were weighted equally (weight = 1) and gaps were treated as missing data. BI and ML analyses were performed using the general time reversible substitution model, assuming variable substitution rates with gamma shape parameter (GTR + I + G) with and without specifying parameter values estimated from data. Specified parameters, determined using (Posada and Crandall, 1998), were: Base = (0.2098 0.2743 0.3165), Nst = 6, Rmat = (0.8580 1.6452 0.9880 0.9840 3.4369), Rates = gamma, Shape = 0.4678, and Pinvar = 0.4588. Four chains in the MCMC analyses were used in each of four independent runs. For BI analyses, one million generations were performed with phylogenetic hypotheses sampled every 100 generations, resulting in 10,000 generations being saved. A burn-in of 20% (2000 generations) was used, resulting in a majority rule consensus of 8001 generations. Each of the independent runs converged on similar optimal log likelihood scores (as verified via the sump command in MrBayes) and identical tree topologies. Bootstrap analyses were performed with 1000 repetitions for MP and ME analyses. ML analyses were limited to 100 bootstrap repetitions due to the greater computational demands of this technique.

Where relationships (topologies) within the resultant trees were inconsistent with previously published taxonomic treatments or evolutionary hypothesis, the one-tailed Kishino–Hasegawa (KH) (Kishino and Hasegawa, 1989) and Shimodaira-Hasegawa (SH) (Shimodaira and Hasegawa, 1999) tests under ML criteria were per-

Table 1
Species examined in this investigation.

Species name	Voucher number*	GenBank accession 18S	GenBank accession 28S	Collection site
Abra nitida		DQ279940	DQ279965	
Angulus tenuis		AM774524	AM779698	
Arctica islandica		U93555	AM779737	
Astarte castanea	S00478	AF120551	AF120612	Falmouth, MA
Astarte sulcata		AM774480	AM779654	
Bankia australis	S00494	JF899202	JF899174	Manado Bay, Indonesia
Bankia carinata	S00492	JF899203	JF899175	Lac Bay, Bonaire, Netherlands Antilles
Bankia gouldi		JF899204	JF899176	Newport River, Beaufort, NC
Bankia setacea	S00489	JF899205	JF899177	Brown's Bay, WA
Bankia carinata	S00485	AF120625	AF120671	Tobago, driftwood
Barnea candida		AM774541	AM779715	
Barnea parva		AM774542	AM779716	
Barnea truncata	S00484	JF899206	JF899178	Wachapreague, VA
Codakia orbicularis	S00482	AM779674	AM774500	Guadeloupe
Corbicula fluminea		AF120557	AM779732	
Corbula sinensis		AM774545	AM779719	
Cyrtopleura costata	S00479	JF899207	JF899179	Wachapreague, VA
Dicyathifer manni	S00500	JF899208	JF899180	West coast of Minahasa Peninsula, Indonesia
Dreissena polymorpha		AM774543	AM779717	
Elliptio complanata	S00475	JF899209	JF899181	Fields Pond, Orrington ME
Ensis directus	S00477	AY553978	JF909603	Falmouth, MA
Gastrochaena dubia		AY192686	AF120623	
Hiatella arctica	S00471	AM774511	AM779685	La Jolla, CA, 15 m
Kuphus polythalamia	S00487	JF899210	JF899182	Zamboanga del Sur, Mindanao, Philippines
Lucinoma borealis	S00473	AM774501	AM779675	Mill Bay, Salcombe, UK
Lyrodus massa	S00495	JF899212	JF899184	Manado Bay, Indonesia
Lyrodus pedicellatus	S00502	JF899211	JF899183	Banana River, FL, mangrove wood
Lyrodus pedicellatus	S00469	AM774540	AM779714	Long Beach, CA
Macoma balthica		AM774526	AM779700	
Martesia striata	S00501	JF899213	JF899185	West coast of Minahasa Peninsula, Indonesia
Mercenaria mercenaria	S00235	AM774566	AM779740	
Mulinia lateralis		L11268	AF131003	
Mya arenaria	S00424	AF120560	AF120621	Hancock County, ME
Mya truncata	S00506	JF899214	JF899186	Cobscook Bay, ME
Nausitora dunlopei	S00499	JF899215	JF899187	West coast of Minahasa Peninsula, Indonesia
Nausitora fusticula	S00491	AY192697	JF899188	Praia Dura, Ubatuba, Brazil
Neoteredo reynei	S00490	JF899217	JF899189	Praia Dura, Ubatuba, Brazil
Panopea generosa	S00505	JF899218	JF899190	Samish Bay, WA
Petricola pholadiformis	S00480	JF899219	JF899191	Wachapreague, VA
Pholas dactylus	S00483	JF899220	JF899192	Rocky shore, Charmouth, Dorset, UK
Placopecten magellanicus		X53899	AF342798	
Spathoteredo obtusa	S00493	JF899221	JF899193	Manado Bay, Indonesia
Sphenia perversa		AM774544	AM779718	
Strigilla euronia		AM774525	AM779699	
Teredo navalis	S00486	JF899222	JF899194	Collection panels, Belfast pier, Belfast, ME
Teredora malleolus	S00497	JF899223	JF899195	Lagoen, Bonaire, NA, driftwood
Teredothyra dominicensis	S00496	JF899225	JF899197	Bachelor's Beach, Bonaire, NA, 3 m
Thyasira flexuosa	S00470	AJ581870	AJ581903	Port Alberni, BC, Canada
Thyasira gouldi	S00474	JF899224	JF899196	Mill Bay, Salcombe, UK
Venerupis philippinarum		EF426293	AM779742	
Xylophaga atlantica	S00472	AY070123	AY070132	12 miles east of Southwest Harbor, ME, 100 m
Xylophaga sp.	S00488	JF899226	JF899198	SE of Port Dunford (29°02.2′S, 32°19.6′E)800 m
Xylophaga washingtona	S00481	JF899227	JF899199	Dredged wood, Friday Harbor, WA
Xylopholas sp.	S00504	JF899228	JF899200	Gulf of Mexico (27°44.75′N, 91°13.31′W) 540 m
Xyloredo sp.	S00503	JF899229	JF899201	Gulf of Mexico (27°44.75′N, 91°13.31′W) 540 m

* Voucher specimens archived in the Ocean Genome Resource and can be accessed via the Ocean Genome Legacy, Ocean Genome Resource database. Published on the Web at: www.oglf.org/Catalog.htm; accessed 27 April 2011).

formed. Trees constrained to the optimal topology obtained in ML searches were compared to trees constrained to agree with published taxonomic or evolutionary hypotheses.

The states of 17 morphological characters were encoded (Table S1) and traced to the BI phylogeny presented in Fig. 2 using Mesquite v.2.74. Ancestral state reconstruction was performed using the parsimony method with discrete unordered character states.

3. Results

The resolving power of the examined data partitions was 18S < 28S < [18S + 28S], as evidenced by the fraction of resolved nodes and nodes with significant statistical support (i.e., nodes with bootstrap proportions (bp) >70% in ME, ML or MP analyses or posterior probabilities (pp) > 0.90 in BI analyses). The compatibility of 18S and 28S data sets was confirmed by the partition homogeneity test ($p = 0.01$) (Cunningham, 1997). Although resolving power varied, topologies of phylogenetic trees inferred independently for all data partitions, inference methods and substitution models were consistent with respect to all statistically supported nodes (bp > 70% and pp > 0.90). Optimal tree topologies inferred by ME, ML, MP and BI using the combined [18S + 28S] data partition were identical with respect to resolved nodes and so only one (BI) is shown here (Fig. 2).

The phylogenetic hypothesis presented here (Fig. 2 B) indicates that the subfamily Xylophagainae of the family Pholadidae and the

Table 2
Oligonucleotides used for sequencing and to prime polymerase chain reaction (PCR) amplification of nuclear 18S and 28S rRNA genes.

Target-Primer Name	Primer Sequence (5′ → 3′)	Sense	References
18S-EukF*	WAYCTGGTTGATCCTGCCAGT	Forward	(Medlin et al., 1988)
18S-EukR*	TGATCCTTCYGCAGGTTCACCTAC	Reverse	(Medlin et al., 1988)
18S-581F	CAAGTCTGGTGCCAGCAGCCGC	Forward	(Distel, 2000)
18S-560R	GCGGCTGCTGGCACCAGACTTG	Reverse	(Distel, 2000)
18S-926F	AAACTYAAAKGAATTGACGG	Forward	(Lane, 1991)
18S-907R	CCGTCAATTCMTTTRAGTTT	Reverse	(Distel, 2000)
28S-NLF184-21*	ACCCGCTGAAYTTAAGCATAT	Forward	www.psb.ugent.be/rRNA
28S-1600R*	AGCGCCATCCATTTTCAGG	Reverse	This study
28S-D23F	GAGAGTTCAAGAGTACGTG	Forward	(Park and O' Foighil, 2000)
28S-D24R	CACGTACTCTTGAACTCTC	Reverse	(Park and O' Foighil, 2000)
28S-D5CF	ACACGGACCAAGGAGTCT	Forward	(Park and O' Foighil, 2000)
28S-D4RB	TGTTAGACTCCTTGGTCCGTGT	Reverse	(Park and O' Foighil, 2000)
28S-D6R	CCAGCTATCCTGAGGGAAACTTCG	Reverse	(Park and O' Foighil, 2000)
28S-NLF105-22	CCGAAGTTTCCCTCAGGATAGC	Forward	www.psb.ugent.be/rRNA

* Denotes PCR primer pairs.

family Teredinidae are sister taxa and that the family Pholadidae is paraphyletic. Similarly, the subfamily Teredininae of the family Teredinidae is shown to be polyphyletic and the subfamily Bankiinae paraphyletic. Also, consistent with previous phylogenetic analyses based on molecular sequences e.g. (Dreyer et al., 2003; Giribet and Distel, 2003; Harper et al., 2006; Taylor et al., 2007), the tree topology presented here (Fig. 2A-B) does not support the monophyly of the order Myoida according to Newell (1969). Constraining the aforementioned assemblages to be monophyletic resulted in trees that are significantly less likely under the ML criteria ($p < 0.05$) as determined by the KH and SH tests (Table 3).

Cladograms displaying the results of ancestral state reconstructions for 17 characters related to xylotrepesis, xylotrophy and taxonomic diagnosis are displayed in supplemental data Figs. S1–S17. Character states inferred for critical nodes are summarized in Table 4.

4. Discussion

4.1. Anatomical features of xylotrophic bivalves and their proposed adaptive significance

The chaotic state of the taxonomy of xylotrophic bivalves no doubt reflects the difficulty of interpreting the unusual anatomy of Teredinidae. In adopting their modern worm-like shape, teredinids have undergone remarkable changes from the typical bivalve body plan (Fig. 1). During development, the visceral mass migrates so that most of the major organs are shifted ventral and posterior to the posterior adductor muscle. This places the bulk of the viscera, including the gills, heart, kidneys, gonads, and most of the digestive system, outside of the protective enclosure of valves (Fig. 1b). The heart and kidneys are inverted both in the dorsal–ventral and anterior-posterior axes and the rectum is separated from the pericardial cavity and does not traverse the heart as it does in other bivalves (Fig. 1c). Moreover, as the shipworm bores into wood, its burrow becomes lined with a calcareous secretion, forming a tube that is bounded by the excavation face of the burrow at the anterior end and open to the external environment at the posterior end. The shipworm can seal the burrow entrance using shell-like plates called pallets, which attach to musculature at the base of the siphons, and which in some species may be ornately sculpted. The sculpture and form of the pallets has been a primary source of characters for taxonomic diagnosis of the family Teredinidae and its subfamilies, genera, and species.

The described anatomical modifications have important consequences for teredinid biology and development. Together, the surrounding wood, calcified burrow lining, and pallets provide protection against predation, dehydration, and other environmental challenges. This frees the valves from their ancestral protective function, allowing them to become specialized as boring tools ornamented with microscopic rasp-like teeth. At the same time, the migration of the visceral organs outside the confines of the valves allows the viscera to become greatly elongated and increased in size relative to the valves as the animal grows to fill the expanding cavity of the burrow. The posterior migration of the gills and heart also allows increased volume for storage and degradation of wood in the caecum without impeding the function of these organs. These adaptations (along with many others detailed in Table 5) give teredinids the worm-like appearance and destructive habits that have earned them the common name of shipworms.

Because most published treatments of teredinid anatomy, taxonomy, and evolution preceded the discovery of the gill endosymbionts, the putative origins and adaptive significance of the features described above bear reconsideration. For example, it is now evident that enlargement and elongation of the gills increases the volume of intralamellar tissue available to accommodate bacteriocytes and symbiotic bacteria. Similarly, the transition to a single gill demibranch in Teredinidae and Xylophagainae and the fusion of the right and left gill lamellae in Teredinidae also allow for increased gill thickness and intralamellar volume. Therefore, although once considered evidence of greater reliance on filter feeding and decreased wood utilization (Turner, 1966), gill enlargement might now be reinterpreted to indicate a greater reliance on symbiotic xylotrophy.

In contrast to the wormlike Teredinidae, Xylophagainae display much more typical bivalve morphology. The gills and visceral mass are contained wholly between the valves, falling largely between the anterior and posterior adductor muscle attachments. The visceral organs have not undergone elongation or dramatic changes in orientation as observed in Teredinidae, and the intestine traverses the pericardial cavity and heart as in Pholadidae and other Bivalvia. Xylophagainae also lack pallets (common to Teredinidae) and apophyses (lever-like shell protrusions that serve as pedal retractor muscle attachments in Teredinidae and other Pholadidae). Finally, unlike Teredinidae, Xylophagainae and other Pholadidae possess accessory shell plates that protect the foot, hinge ligaments, and siphons during burrowing.

4.2. Morphological evidence for common ancestry of xylotrophic bivalves

Although inconsistent with most widely accepted taxonomic treatments, the recent common ancestry inferred here for

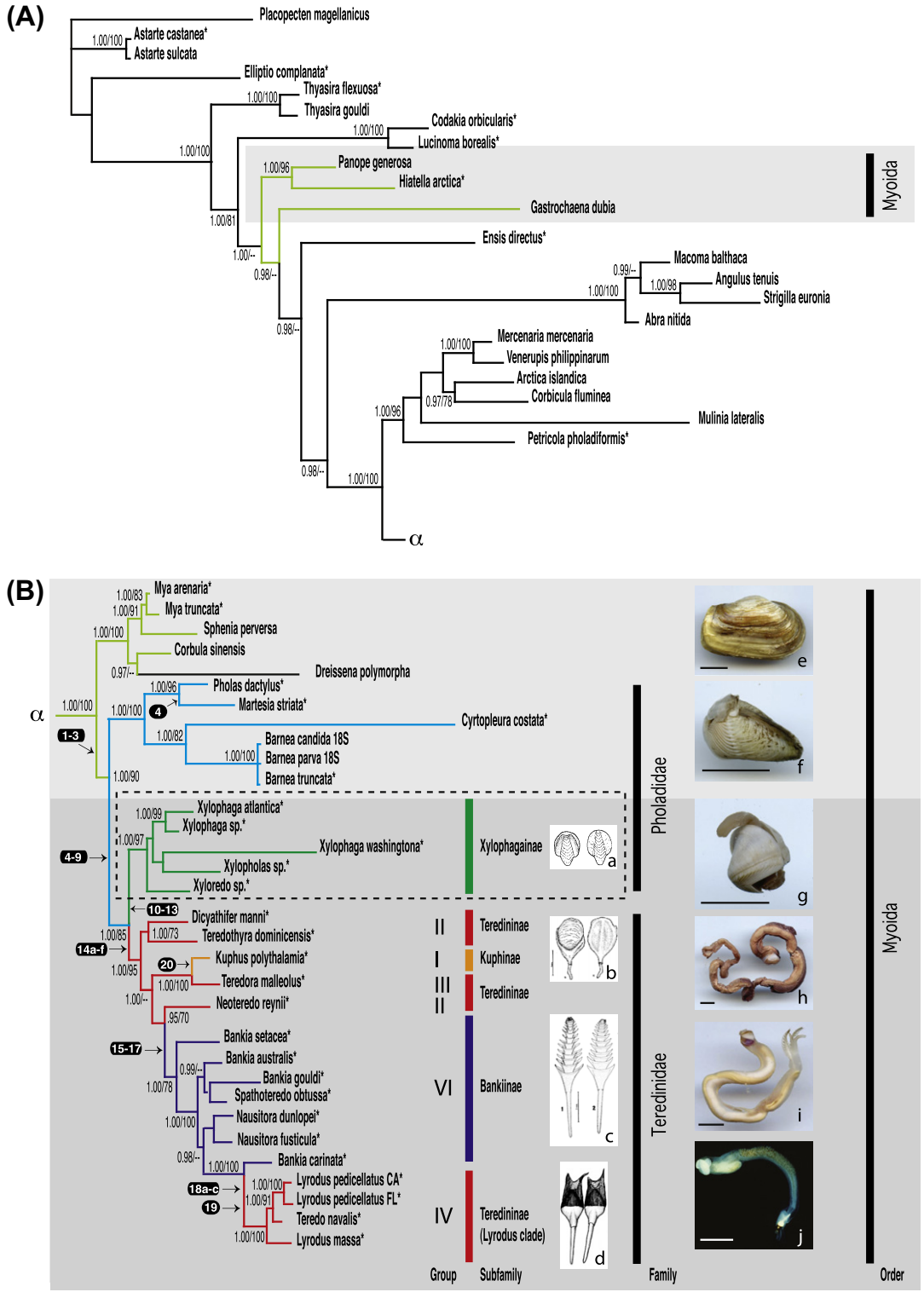

Fig. 2. Phylogenetic hypothesis for xylotrophic bivalves and related taxa. Phylogram inferred by Bayesian analysis (see methods) of concatenated partial sequences of small and large subunit nuclear rRNA genes. Posterior probabilities greater than 0.90 (BI) and bootstrap proportions greater than 70% (ML) are indicated at the associated nodes. (A) Root part of the tree. (B) Subtree rooted at node (α) in (A). Taxa displayed within the dark gray box are xylotrophic Myoida (with the exception of *K. polythalamia*). Dashed box denotes deep-water taxa (found primarily in depths >100 m). The light gray boxes circumscribe other Myoida. Asterisks indicate sequences determined in this study. Numbers within closed ovals correspond to traits described in Table 5. Text colors correspond to taxonomic designations as follows: subfamilies: Teredininae (red), Bankiinae (dark blue), Kuphinae (orange), Xylophagainae (green); other Pholadidae (light blue), other Myoida (light green). Insets: (a) siphonal plates of *Xylopholas altenae* (with permission from (Turner, 2002)); (b and d) pallets of *Teredora malleolus* (unsegmented), *Bankia carinata* (segmented) and *Lyrodus pedicellatus* (unsegmented) (with permission from (Turner, 1966)); (e) *Mya truncata*, (f) *Martesia striata*, (g) *Xylophaga washingtona*, (h) *Dicyathifer manni*, (i) *Bankia setacea*, (j) *Lyrodus pedicellatus*. Scale bars in (e–j) are approximately 1 cm. Roman numerals indicate major teredinid groups as defined by (Turner, 1966).

Table 3
Results of Kishino-Hasegawa (KH) and Shimodaira-Hasegawa (SH) tests under Maximum Likelihood for the sequence dataset using RELL bootstrap analysis and the one-tailed test.

Constraints[a]	Difference in		
	−ln L	−ln *L* values[b]	*p*-Value[c]
Unconstrained tree	30448.18730	Best	
(Teredininae)	30890.97722	442.78992	0.000*
(Bankiinae)	30628.58968	180.40238	0.000*
((Teredininae) (Bankiinae))	30890.97722	442.78992	0.000*
((Teredininae)(Bankiinae)(Pholadidae))	31256.53401	808.34671	0.000*
(Pholadidae)	30480.30983	32.12253	0.013*
((Teredinidae) (Pholadidae))	30480.30983	32.12253	0.013*
(Myoida)	30794.49036	346.30306	0.000*

[a] Monophyly is indicated by ().
[b] Difference between the unconstrained (=best ML, Fig. 2B) and constrained trees.
[c] Probability of getting a more extreme *T*-value under the null hypothesis of no difference between the two trees (one-tailed test) with significance at $p < 0.05$ (*).

Table 4
Summary of inferred ancestral character states.

		Inferred ancestral character state in the most recent common ancestor of the clade containing:						
		Pd + Xn + Td	Xn + Td	Xn	Td	Tn	Bn	Ly
1	Accessory shell plates	±	±	+	–	–	–	–
2	Apophysis	±	±	–	+	+	+	+
3	Burrows: calcareous lining	–	–	–	+	+	+	+
4	Deep water habitat	–	–	+	–	–	–	–
5	Gill endosymbionts	–	+	+	+	+	+	+
6	Gills: single demibranch	–	+	+	+	+	+	+
7	Intestine: loops forward	NA	NA	NA	+	+	–	–
8	Intestine: traversing heart	+	+	+	–	–	–	–
9	Larvipary	–	–	–	–	–	–	+
10	Pallets	–	–	–	+	+	+	+
11	Pallet segmentation	NA	NA	NA	–	–	+	–
12	Stomach: elongate	–	–	–	–	–	+	+
13	Valves: fine denticulation	–	+	+	+	+	+	+
14	Viscera: posterior to PA	–	–	–	+	+	+	+
15	Wood ingestion	–	+	+	+	+	+	+
16	Wood-storing caecum	–	+	+	+	+	+	+
17	Xylotrepesis: obligate	–	+	+	+	+	+	+

Pd; Pholadidae, Xn; Xylophagainae, Td; Teredinidae, Tn; Teredininae, Bn; Bankiinae, Ly; Lyrodus clade, PA; Posterior Adductor, +; present, –; absent, ±; equivocal, NA, not applicable.

Xylophagainae and Teredinidae is consistent with a number of unusual features shared by nearly all members of both groups but absent from other Pholadoidea. These include (1) obligate xylotrepesis (2) ingestion of wood particles (3) the presence of a caecum that becomes engorged with wood particles, (4) possession of a single gill demibranch, (5) presence of microscopic cutting teeth on the anterior slope of the valves, and (6) the presence of bacterial endosymbionts within specialized cells of a thickened and modified interlamellar tissue. Previously these similarities have been proposed to be the result of convergent adaptations to the common requirements of wood boring and wood feeding (Turner, 1966, 1967, 1973, 2002).

Additional similarities may also be due to common ancestry. For example, like Teredinidae, members of the most recently described xylophagainid genera (*Xyloredo* and *Xylopholas*) form lined burrows (Turner, 2002). Those of *Xyloredo* are partially calcified and closely resemble the tubes of Teredinidae while those of *Xylopholas* and some *Xylophaga* are composed of a proteinaceous membrane that does not become calcified. In addition, members of the genus *Xylopholas* have calcified structures (siphonal plates; Fig. 2a) at the base of the siphons (Turner, 2002) that closely resemble the pallets of Teredinidae in appearance, location, and muscle insertion.

Ancestral state reconstructions suggest that pallets and lined burrows are equivocal or absent in the ancestral xylotroph (Figs. S3, S10). However, in Teredinidae, pallets and lined burrows function together to prevent dehydration when the animals are exposed to the atmosphere in intertidal or floating wood. Given this function, it is unlikely that these features arose independently in selected members of Xylophagainae, a group of deep-sea organisms that have not been observed to occur in floating or intertidal wood (Turner, 2002). We suggest that pallets and lined burrows are more likely ancestral features that were retained in a few, but lost in most Xylophagainae species after the invasion of deep-water habitats by a shallow water ancestor.

4.3. Evidence for non-monophyly of Teredininae and Bankiinae

The principal diagnostic character that distinguishes the major subfamilies of Teredinidae is the presence of segmented pallets in Bankiinae and unsegmented pallets in Teredininae. The phylogeny proposed here, however, suggests (1) that unsegmented pallets are the ancestral condition in the family Teredinidae, (2) that segmented pallets first emerged with the subfamily Bankiinae, and (3) that this characteristic was subsequently lost in a recent clade, hereafter referred to as the Lyrodus clade, that emerged within Bankiinae (see Supplemental Fig. S11). The Lyrodus clade, which is assigned to the subfamily Teredininae because of its unsegmented pallets, forms a strongly supported nested clade within the subfamily Bankiinae (Fig. 2B), indicating that both of these nominal subfamilies are non-monophyletic. This conclusion

Table 5
Hypothesis for evolution of wood-boring and wood feeding in bivalvia*.

	Trait	Description	Putative functions/Notes
1	Accessory plates	Shell-like plates lying over the upper or lower margin, or attached to the internal ligament	May protect tissues (e.g., ligament and siphons) from abrasion caused by burrowing in hard substrates
2	Apophyses	Lever-like projections of the inner valve surface to which pedal muscles attach	May increase strength and leverage of muscle attachment required for burrowing in hard substrates
3	Denticulated valves	Valves with sculpted teeth on the outer surface	Facilitates burrowing in hard substrates e.g. wood
4	Xylotrepesis	Habit of burrowing preferentially in wood	Likely evolved independently in Martesianae and xylotrophic bivalves (Teredinidae + Xylophagainae)
5	Symbiosis	Acquisition of bacterial endosymbionts in in interlamellar tissue of the gills	Symbionts are known to fix nitrogen and may aid in lignocellulose degradation and utilization by the host
6	Unpaired gill demibranchs	Loss of outer gill demibranch, thickening of the inner demibranch	May increase volume of symbiont-bearing interlamellar tissue
7	Lined burrows	Calcareous tube lining the inner surface of the burrow	Along with 8, protects against dehydration and other environmental threats
8	Pallets	Paired calcareous plates that insert into the burrow entrance to form a watertight seal	Alleviates dehydration risk during exposure to air, facilitates colonization of floating or intertidal wood
9	Caecum (appendix)	Blind sac connected to the posterior end of the stomach	Functions in storage [1] and digestion [40] of wood particles excavated during burrowing
10	Invasion of the deep sea	Growth and reproduction largely restricted to depths >150 m	Utilization of deep-sea wood deposits, expansion into previously underexploited niche
11	Loss of lined burrows	Except in *Xyloredo* and *Xylopholas* where burrows are partially lined	Loss may reflect reduced dehydration risk and/or increased cost of maintaining calcareous structures in deep sea habitats
12	Loss of pallets	Except in *Xyloredo* (siphonal plates)	
13	Loss of apophyses	Pedal muscles attach directly to valve surface	May reflect adaptation to burrowing in softer waterlogged wood available on the sea floor
14	Vermiform body plan	(a) During development the major visceral organs migrate posterior and ventral to the posterior adductor muscle and the protective enclosure of the valves	Frees the valves from their ancestral protective function, may result in increased length and volume of symbiont-bearing tissue in gills and increased room for wood storage and digestion in the caecum
		(b) Lengthening and posterior migration of caecum, gonads, heart, kidney, and gills	Specialization of the valves for wood grinding, protective functions of valves and accessory plates assumed by burrow, tube, and pallets
		(c) Separation of the rectum and pericardium	
		(d) Reduction of valves relative to body mass	
		(e) Loss of accessory shell plates (except pallets, likely derived from the siphon plates)	
		(f) Fusion of left and right gill demibranchs	Increases volume of interlamellar tissues available to house endosymbiotic bacteria
15	Segmented pallets	Pallets composed of a series nested cups with flexible interdigitating sheaths of periostracum	May result in more effective closure of burrow after damage or wear to the tips of the tube and pallets
16	Elongate (Type III) stomach	Stomach long, not globular as in Pholadidae, Xylophagainae, and more basal Teredininae.	Proposed to facilitate digestion of wood particles [1]
17	Loss of anterior intestinal loop	Intestine proceeds from the midgut posteriorly, does not loop anteriorly over the style sac	
18	Larvipary	(a) Internal fertilization,	Capacity for rapid settlement and metamorphoses without prolonged planktonic phase, may facilitate rapid colonization of sparsely distributed marine wood deposits
		(b) development of internal brood pouchs, and	
		(c) retention of fertilized embryos to the veliger stage of larval development	
19	Loss of pallet segmentation	Pallets composed of a single cup-shaped unit with prominent perisotracal cap	May reduce risk of larvae becoming lodged between pallet segments in these larviparous species
20	Loss of xylotrepesis and xylotrophy	Burrowing in sediments, loss of caecum, shell denticulation, and other specializations for feeding on and burrowing in wood	

* Numbers at left refer to nodes depicted by closed ovals in Fig. 2.

is supported by the observation that trees inferred under the constraints of monophyly for Teredininae or Bankiinae are statistically less likely ($p < 0.05$, KH and SH test) than unconstrained optimal ML trees.

Although the proposed relationship between Bankiinae and the Lyrodus clade is not consistent with widely accepted taxonomy, it is notably consistent with reproductive strategies and anatomy of the stomach, intestines, and gills. For example, with the exception of the Lyrodus clade, members of Teredininae have globular (Type II) stomachs (Turner, 1966), resembling those of Xylophagainae and other Pholadidae, and the intestine loops forward over the style sac before proceeding anteriorly toward the caecum. However, in Bankiinae and the Lyrodus clade the stomach is elongate (Type III) (Turner, 1966) and the intestine proceeds immediately toward the posterior without looping forward over the style sac. In addition, members of the Lyrodus clade are larviparous. In these species, fertilization is internal and the larvae are retained within specialized brood pouches on the dorsal surface of the gills. In contrast, other Teredininae and all Bankiinae are broadcast spawners. Thus, the phylogeny presented here suggests that the Type II stomach is ancestral and that the emergence of the Type III stomach and loss of the anterior loop of the intestine occurred just once in Teredinidae, rather than having evolved independently in Teredininae and Bankiinae as previously proposed (Turner, 1966). Moreover, this phylogeny suggests that internal fertilization, larvipary, and internal brood pouches appear to have evolved recently within Bankiinae rather than in Teredininae.

In this regard, it is interesting to note that *Bankia carinata* is well supported as the most basal member of the clade containing the larviparous branch of Teredininae. This is significant because

juveniles of *B. carinata* have unsegmented pallets that become segmented only in mature adults (Turner, 1966). This observation suggests that the loss of pallet segmentation in larviparous Teredininae is an example of neoteny (retention of a juvenile characteristic into the adult stage). This secondary loss of pallet segmentation may be adaptive for these larviparous species because unsegmented pallets are less likely to trap newly released larvae that might otherwise become lodged between pallet segments, thereby damaging larvae and hindering the ability of the adult to seal its burrow.

4.4. Inferred characteristics of the ancestral xylotrophic bivalve

The phylogeny presented here contradicts the previously proposed hypothesis that the common ancestor of Teredinidae was a non-xylotrophic "worm-like mud-borer with a pholad-like stomach" most closely resembling the extant teredinid subfamily Kuphinae (Turner, 1966). Modern Kuphinae is represented by a single genus and species, *Kuphus polythalamia*. This species is extremely rare and has been described only from preserved specimens. Although no reliable description of its life habits has been published, adult specimens are reported to burrow in sediment rather than wood. Turner (Turner, 1966) considered this species to represent the most primitive branch of Teredinidae because it has a simple stomach (Type I) and lacks a caecum, shell denticulation, and other apparent adaptations for consumption of wood. The analysis presented here indicates that *K. polythalamia* falls clearly within the radiation of Teredinidae, suggesting that its unique characteristics are derived rather than ancestral. Thus, these results do not support the view that the worm-like body plan preceded xylotrepesis and xylotrophy in Teredinidae.

Based on the molecular phylogeny presented here and the distribution of traits associated with wood-boring and wood-feeding habits among extant species (Table 4, Figs. S1–S17), it is possible to infer the likely characteristics of the hypothetical ancestor of xylotrophic bivalves, and to propose a plausible ordered set of events leading to the evolution of the divergent characteristics observed in modern taxa (Fig. 2B and Table 5). These analyses suggest that the last common ancestor of Teredinidae and Xylophagainae burrowed in and fed on wood, and had a caecum for wood storage and digestion. It possessed unpaired gill demibranchs that contained xylotrophic symbionts in bacteriocytes housed within the interlamellar tissue. This ancestor likely displayed a mix of features resembling modern Teredinidae and Xylophagainae. It was not wormlike but instead had a typical bivalve body plan with the intestines traversing the heart and the visceral organs located between the anterior and posterior adductor muscles and largely enclosed by the valves. Like modern teredinids, this common ancestor may have possessed apophyses and formed lined burrows that were sealed by paired pallets. This hypothesis requires each of these unusual shared traits to have emerged just once in Bivalvia, rather than twice as is demanded by the currently accepted taxonomy.

5. Conclusions

The conclusion that Teredinidae and Xylophagainae share a recent common ancestor suggests that xylotrophy, and by extension, xylotrophic symbiosis, evolved just once in Bivalvia. This ancestral xylotrophic lineage then diverged into two morphologically and ecologically distinct lineages, respectively confined to shallow- and deep-water habitats. The observation that gill endosymbiosis appears roughly concomitantly with the onset of xylotrophy suggests that symbiont acquisition may have contributed to the success of this lineage in utilizing wood, a previously underexploited food source. This in turn facilitated the invasion of diverse habitats, ranging from brackish to marine salinities, intertidal to abyssal depths, and tropical to temperate latitudes, as well as substrates ranging from floating or sunken wood, plant fibers and nut hulls to living mangrove roots and sea grass rhizomes. Furthermore, it appears that only one extant taxon, represented by a single genus and species, has subsequently lost the xylotrophic habit. Thus, by most measures, the acquisition of xylotrophy and xylotrophic symbioses must be considered a formidable evolutionary success.

5.1. Taxonomic recommendations

The phylogenetic and ancestral state analysis presented here, as well as a recent cladistic analysis of morphological features of these taxa (Monari, 2009), combine to form a strong argument that the family Teredinidae and the subfamily Xylophagainae of the family Pholadidae are sister taxa that should be afforded equal taxanomic rank. This might be accomplished by elevating the subfamily Xylophagainae to the rank of family, as suggested by (Purchon, 1941), or by transferring the subfamily Xylophagainae from the family Pholadidae to the sister family Teredinidae. In either case, additional phylogenetic analyses will be required to resolve finer taxonomic divisions within the resulting taxonomic units.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.05.019.

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