# Species Richness of Deep-sea Wood-boring Clams (Subfamily Xylophagainae) from the Northeast Pacific

by

Mathis Stoeckle
Diploma, University of Basel, Switzerland, 2003

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

MASTER OF SCIENCE

in the Department of Biology

University of Victoria

# Supervisory Committee

Species Richness of Deep-sea Wood-boring Clams (Subfamily Xylophagainae)
from the Northeast Pacific

by

Mathis Stoeckle

Diploma, University of Basel, Switzerland, 2003

Dr. Verena Tunnicliffe (Department of Biology, School of Earth and Ocean Sciences)

Supervisor

Dr. Réal Roy (Department of Biology)

Departmental Member

Dr. John Taylor (Department of Biology)

Departmental Member

Dr. Edward Ishiguro (Department of Biochemistry and Microbiology)

External Examiner

## Supervisory Committee

Dr. Verena Tunnicliffe (Department of Biology, SEOS) - Supervisor
Dr. Réal Roy (Department of Biology) - Departmental Member
Dr. John Taylor (Department of Biology) - Departmental Member
Dr. Edward Ishiguro (Department of Biochemistry and Microbiology) - External Examiner

## Abstract

The deep sea, for a long time, had been perceived as a homogenous and stable environment, with little diversity. The discovery of island habitats such as hot vents, cold seeps and whale carcasses revealed the presence of high biomasses in the deep sea. Species richness on island habitats is low however, compared to the species richness on abyssal plains (Tunnicliffe 1991; Stecher et al. 2003). A study addressing species richness on abyssal plains concludes there to be tens of millions of new species (Grassle and Maciolek 1992). Other authors dispute these numbers and suggest a total deep-sea species richness of approximately half a million (May 1993). The discrepancy in these numbers by multiple orders of magnitude emphasize how under-sampled the deep sea is.

The Northeast Pacific is recognized as a biogeographic species province both in the intertidal, shallow subtidal (Valentine 1966; Roy et al. 1994) as well as in hydrothermally active areas (Van Dover et al. 2002). The Northeast Pacific hydrothermally active areas are unique in that they appear to foster single dominant species within ecosystems: e.g. *Ridgeia piscesae* (Vestimentifera, Annelida) (Southward et al. 1995); *Lepetodrilus fucensis* (Gastropoda, Mollusca) (McLean 1993).

*Xylophaga washingtona* BARTSCH (Bivalvia, Mollusca) is the only described species of deep-sea wood-boring clams in the Northeast Pacific (Bartsch 1921).

We investigated the species richness of deep-sea wood-boring clams (Family Pholadidae, Subfamily Xylophagainae) in the Northeast Pacific by means of strategically placed, experimental wood blocks. The objective of the study was to a) assess the morphological diversity and separate the specimens into distinct morphological taxa; b) to use genetic markers (CO1, 18S) to discern the genetic diversity within the morphological taxa and c) to elucidate the phylogenetic relationships among the new species.

We discovered eight morphological taxa (B, F, G, M, R, T, U, X), defined by a set of characters, previously used to describe new pholad species. The most distinguishing characters were the morphologies of the siphon and mesoplax (accessory plates).

The CO1 gene sequences showed little diversity within the morphological Taxa B, F, G, U and X. The 18S gene data split Taxa B and T into B1, B2 and T1, T2, respectively. There was no genetic diversity within 18S sequences of Taxon R. Thus, we discovered at least six new species: *Xylophaga* species B1, F, G, R, U and *Xylopholas* species X. I cannot confirm the statuses of Taxa M and T due to missing morphological characters as well as the unavailability of their CO1 sequences.

The newly discovered species increase the number of deep-sea, wood-boring species from one to seven, in the Northeast Pacific. Globally, a total of 41 species have been described. Given the discoveries in this study, this number is likely an underestimate of the global species richness of the Xylophagainae.

The phylogenetic relationships of the genus level remain inconclusive due to too few samples. Species relationships emerged, placing Species G and R as most closely related, as well as B1 and *X. washingtona*. Additional samples from all ocean basins are necessary to further investigate the relationships and origins of the deep-sea woodborers.

Future avenues that need to be explored are threefold: a) Ancestry: Has there been one or multiple invasions into the Northeast Pacific, with subsequent adaptive radiations? Where do this/these ancestor species come from? b) Support for Diversity: What mechanisms allow the deep-sea wood-boring clams to co-occur? What are the deep-sea wood-boring clams' micro-niches? c) Effects of Diversity: What is the role of the wood-boring clams in species communities inhabiting deep-sea wood?

# Table of Contents

## List of Tables

## List of Figures

# Acknowledgments

I would like to thank Dr. Verena Tunnicliffe for taking me on as an international graduate student. My initial contact with Verena consisted of an email from the Republic of the Maledives, where I was working as a public education instructor at the time. I recognize the risks involved with hiring people over email and thank you very much for taking the plunge. I would also like to extend a very special thank you to Jonathan Rose (Tunnicliffe lab manager), Amanda Bates, Ian Beveridge and Jennifer Tyler for their support. Always good times.

Real Roy, thank you for introducing me to the wonderful world of genetics and your help with initiating the project as well as providing lab space. On that note, Melissa and Charmaine, thanks for putting up with a complete newbie in your space and for your instructions. John Taylor, thank you for the expertise you shared with me and for letting me use your equipment.

Many thanks to the Centre for Biomedical Research (Roderick Haesevoets) and Janet Voight from The Field Museum in Chicago, IL, for our collaborative efforts, specimens and information exchanges. Furthermore I would like to thank Craig Smith (U of Hawaii) for his donation of the samples from Santa Cruz and Dan Distel for helpful information and data. I would also like to thank Erin Pemberton for her help during the summer 2005.

Monetary support was provided by NSERC Canada, the University of Victoria, the Theodor Engelmann Stiftung, Janggen-Poehn Stiftung, Basellandschaftliche Kantonalbank, Switzerland and the families of Maureen de Burgh, Randy Baker and Gordon Fields.

# Chapter 1: Introduction

Edward Forbes published the concept of an 'azoic zone' below 300 fathoms (~550 m) in 1844, despite published data on animals recovered from depths as deep as 1600 m by British explorer John Ross, in 1818. This concept, derived from Forbes' dredging work in the Aegean, an area that later proved to be particularly sparse in deep-sea life, inspired deep-sea research, aiming to disprove the azoic zone. Reports of animals recovered from depths greater than 300 fathoms were plentiful soon thereafter. Inspired by these deep-sea findings, Thomson and Carpenter initiated the world's first deep-sea biological research cruises aboard the *LIGHTNING* (1868), followed by the *PORCUPINE* (1869) and the *VALOROUS* (1875). Samples collected during those cruises disproved Forbes' theory beyond any doubt and stimulated the *CHALLENGER* (1872) expedition that studied the physical and biological properties of the world's oceans, in a ~70000 mile roundtrip around the globe. The Danish *GALATHEA* (1950) and the Russian *VITIAZ* (1949) research cruises subsequently demonstrated the existence of life in the deepest parts of the oceans, namely the deep-sea trenches of the Pacific.

The average depth of the oceans is approximately 3800 m whereas the average height of the continents is only 840 m. Consequently, 99% of the living space on the planet is contained within the seas, with 85% of the area and 90% of the volume of the oceans constituting the dark, cold environment that we call the deep sea, an area of approximately 270 mio km$^2$ (Gage 1996). Despite ongoing deep-sea research for the past ~200 years, we still know very little about this vast expanse. A generous estimate of the total deep-sea surface area, sampled with quantitative cores, to date, is ~500 m$^2$ (Gage 1996). According to Gage (1996), in coastal areas, ecologists attempt to sample approximately one millionth of the habitat in order to characterize a benthic community. Aiming to do even a hundredth of this effort in the deep sea, would take daily sampling of 500 m$^2$ for the next 14 years. These numbers highlight the vastness of the deep-sea environment and emphasize how little we actually know about it to date.

The uniform characteristics of physical properties such as temperature, salinity and dissolved oxygen (Menzies 1965), were considered a dominant feature of the deep-sea. This uniformity led early oceanographers to a view of constancy in time of the physical characteristics, with concurrent low levels of biomass and biodiversity. Emerging evidence has led to the formulation of a new concept of distinct niches in the deep sea. Today we assume that an estimated 80% of all eukaryotic life on earth is found in the ocean. There are many more phyla and major sub-phyletic groups found in the oceans than on land. Of the ~35 metazoan phyla found in the oceans, 22 are exclusively marine, with 11 endemic to the benthos and only one to the pelagos. By contrast, only 12 phyla are found on land (including fresh-water phyla) with only a single phylum, the Onychophora, being exclusively land-based (Gage 1996).

A recent study that assessed deep-sea species richness through quantitative bottom grab samples suggests that the common deep-sea floor is extensively colonized by millions of species (Grassle and Maciolek 1992). Their projections are three orders of magnitude higher than was previously estimated and subsequently heavily criticized by May (1992). May uses Grassle and Maciolek's results to emphasize his belief that the total number of species of benthic macrofauna is about twice the number currently known and unlikely higher than half-a-million. Poore and Wilson (1993) counter by presenting data on isopods, corroborating Grassle and Maciolek's estimates, even stating they were too low in certain cases. They contest May's factor of two to extrapolate from known to total biodiversity and suggest a factor exceeding 20 as more reasonable for the oceans as a whole.

Regardless of who produces the correct overall deep-sea species richness estimates, it is important to note the discrepancy among the various estimates. This debate serves as a reminder of how little we know about the deep-sea environment.

### 1.1 The Discovery of Island Habitats

A discrete insular habitat surrounded by an environment that presents barriers to dispersal can be considered an island habitat (Schoener 1988; Rosenzweig 1995). In the deep sea, this 'surrounding' environment constitutes the vast extent of soft sediment (Beaulieu 2001). The 'islands' in the deep sea include large features such as seamounts (Mullineaux 1994), hydrothermal vents (e.g. Lutz 1988) and smaller features like rock outcrops (Lissner et al. 1991), waterlogged driftwood (Turner 1973; 1981) and manganese nodules (Mullineaux 1987). In recent years, discoveries of such deep-sea habitats have increased (e.g. Hashimoto et al. 2001; Klinkhammer et al. 2001; de Ronde et al. 2002; Chen et al. 2006). These ecosystems (reviewed in Tunnicliffe et al. 2003) house specialized groups of animals that thrive in the deep, displaying high biomasses (e.g. Stecher et al. 2003).

**Hot vents** are places where sub-seafloor water becomes superheated and emerges from the sea floor through high-velocity water jets, containing dissolved metals and minerals. Upon hitting the cold, ambient water, the dissolved metals and minerals precipitate, often as complexes of sulphur, to form chimneys. The temperature difference between the hot jet and the ~2°C ambient water creates a gradient, providing habitat for numerous vent animals including molluscs, arthropods and annelids (e.g. Tunnicliffe et al. 2003).

**Cold seeps** constitute areas in the ocean floor, where hydrogen sulfide, methane and other hydrocarbon-rich fluid seepages occur (Tunnicliffe et al. 2003). Cold seeps are distinct from hot vents in that the temperature of the emerging fluid is close to the temperature of the ambient seawater. A recent study in the Monterey Bay collected data on the location of cold seep communities over a 13-year period (Paull et al. 2005a). Upon mapping their data onto the Monterey Bay topography, Paull et al. found that cold seeps are predominantly found below 550 m and often *not* associated with tectonically driven flows through regional faults. According to Paull et al., chemosynthetic biological communities (CBC) are most common on steep slopes, in Monterey Bay, where recent seafloor erosion, exposing methane near the surface, is likely to have occurred (Paull et al. 2005aa; Paull et al. 2005bb).

**Whale carcasses** yield pulses of labile organic matter to the deep-sea floor. A single carcass can sustain a species community, similar to hot vents and cold seeps (Baco and Smith 2003). Bathyal carcasses go through at least three stages, starting with a '*mobile scavenger*' stage, followed by an '*enrichment opportunist'* stage and finally a '*sulphophilic*' stage. The first and second stage last months to years, while the third stage can last for decades, during which the whale skeleton emits sulphides, from the anaerobic breakdown of bone lipids.

The common characteristics of these deep-sea island habitats relate to the chemical nature of the habitats and the adaptations of their inhabitants. Low oxygen and the presence of hydrogen sulfide and sometimes methane are characteristic. Sulphides originate from sulphate reduction, either by microbes or via inorganic processes beneath the seafloor. Methane is derived from reduction of organic matter, either by thermogenic or via biogenic processes (Tunnicliffe et al. 2003). Hydrogen sulphide and other reducing substances present in hydrothermal (Nelson and Fisher 1995), seep (Levin 2005) fluids and emerging from whale bones (Smith and Baco 2003), provide the energy for organic matter synthesis.

The adaptations of deep-sea island inhabitants range from tolerance to low oxygen and high sulphide concentrations, to thermophily (Tunnicliffe et al. 2003). Biological productivity is sustained by the chemosynthesis of organic matter by microorganisms, using energy from chemical oxidations to produce organic matter from $CO_2$ and mineral nutrients (Tunnicliffe 1991; Van Dover 2000). Reducing environments usually host invertebrate inhabitants that have a symbiotic relationship with chemosynthetic autotrophic bacteria. These symbionts convert carbon dioxide into organic matter, providing ‘food’ for themselves and their hosts. Fisher (1990) discusses host-symbiont interactions in detail, including vestimentiferan tube worms, vesicomyid clams and bathymodiolid mussels. The vestimentiferans display a 100% dependence on their symbionts as the mouth and digestive system are completely reduced. The symbionts are housed in a specialized structure known as the trophosome and provided with the necessary metabolites by their host. Vesicomyid clams have a highly reduced digestive tract and symbionts hosted in the gill tissue. $CO_2$ and $O_2$ diffuse directly through the gill, while the clam’s foot, buried in the sediments, takes up hydrogen sulfide. Deep-sea mussels house their symbionts in the gills as well, but have a functional digestive system, supplementing their nutrition through filter-feeding. Thus, the discovery of these island habitats has emphasized the role of invertebrate-bacteria symbioses in exploiting organic and chemical energy sources in the deep sea.

## 1.2 Features of Deep-Sea Island Habitats

Over the last thirty or so years, several hundred new and endemic species have been discovered at hydrothermal vents and seeps (e.g. Sibuet and Olu 1998; Tunnicliffe et al. 1998; Hashimoto et al. 2001). Few species are held in common among the reducing habitats (Smith and Baco 1998), yet many genera and families are shared. To date, the number of common species among vents, seeps and whale falls is less than five (Tunnicliffe et al. 2003). It is these common species that invoked two versions of the 'Stepping Stone' concept, one addressing 'dispersal' (Hecker 1985; Smith et al. 1989) and the other 'evolution' (Distel et al. 2000). The latter states that reducing environments such as whale carcasses and wood falls might have served as stepping-stones for the colonization of cold seeps and hot vents. It received support through a recent phylogenetic analysis of wood and bone associated mussels (Distel et al. 2000). The results show that all samples form a monophyletic lineage that includes all examined vent and seep mussel species but excludes members of the traditional, shallow-water, mytilid subfamilies. Although the internal branching order is not fully resolved, the basal divergence of *Benthomodiolus lignicola*, a species observed exclusively on sunken wood and bone, receives significant bootstrap support. This suggests that wood and bone associations may have preceded vent and seep specialization within this lineage (Distel et al. 2000). Jones et al. (2006), tested Distel et al.'s hypothesis and found data in support of the latter. There also exist data however, on some western Pacific mussel species that occur in both, vents and seeps (Miyazaki et al. 2004) and data on vent to seep habitat reversals, in some Atlantic mussel lineages (Won et al. 2002).

Species richness of the soft-sediment fauna in abyssal sediments is estimated between half (May 1992) and multimillion (Grassle and Maciolek 1992) species. Species richness numbers of island habitats are in the hundreds of species (reviewed in Baco and Smith 2003), implying a much higher diversity on abyssal plains in general. There are exceptions. Baco and Smith (2003) compared macro-faunal diversity on whale bones to existing data for deep-sea soft sediments and found that whale bones from the San Nicolas trough are more speciose than sediments from the Santa Catalina Basin.

The unique chemical nature of island habitats allows for high productivity concurrent with high biomasses. Vent faunal biomasses can be 500 to 1000 times that of the surrounding deep sea (Juniper 2003) and cold seeps can display biomasses that exceed deep-sea estimates by four orders of magnitude (reviewed in Stecher et al. 2003).

### 1.3 Species Distributions in the Northeast Pacific

The distribution of intertidal and subtidal (< 200 m) marine mollusks has been used to delimit marine species provinces for over a century. In the Northeast Pacific, Fisher first defined the Oregonian species province in 1887, with subsequent modifications by Bartsch (1912), Newell (1948) and Hall (1964). Valentine reviewed the information and re-defined the Oregonian province from Point Conception (CA, USA) to Dixon Entrance (AL, USA) (Valentine 1966). A more recent study, using 2838 eastern Pacific molluscan species, substantiated the Oregonian province (Roy et al. 1994).

In the deep sea, hydrothermally active areas are divided into six global biogeographic regions (Van Dover et al. 2002). A different faunal assemblage dominates each of the provinces (reviewed in Tunnicliffe et al. 2003; Shank 2004). The Northeast Pacific stands out from among the six provinces due to its faunal assemblage. The alvinocarid shrimp, for example, occur in every province but the Northeast Pacific province (Shank et al. 1999). In contrast, unlike many other provinces that are colonized by multiple species of a higher taxonomic group (e.g. family, subfamily), the Northeast Pacific appears to display single dominant species. *Ridgea piscesae* is the single species of vestimentiferan tubeworms, present on the Gorda, Juan de Fuca and Explorer Ridges (Southward et al. 1995). Until very recently, *Lepetodrilus fucensis* (McLean 1993), was believed to be the single species of this genus inhabiting the same three ridges. Only recently, Johnson et al. (2006) tested the impact of transform faults on the dispersal of this genus and split the species into two, *L. fucensis* and *L. gordensis*.

Hyland et al. (1991) examined the macro-infaunal communities of the Santa-Maria basin on the California outer continental-shelf and slope at depths from 90 to 565 m. They report highly diverse and abundant macrofauna. Gray (2002) reviewed species richness patterns of marine soft sediments, however both authors do not address biogeographic issues of the common deep sea in the Northeast Pacific.

*Xylophaga washingtona*, a member of the deep-sea wood-boring clam subfamily Xylophagainae, is the only published deep-sea wood-borer, to date, from the Northeast Pacific (Bartsch 1921; Turner 1956; Knudsen 1961; Turner 2002).

### 1.4 Wood in the Deep Sea

Most floating debris in the eastern Pacific is plant material, primarily trees, branches or other tree parts including palm, banana and mangrove trees, as well as bamboo and other types of canes; another significant fraction comprises anthropomorphic wooden debris such as pallets, planks, plywood, boats and parts thereof, rafts and cable drums (Maser and Sedell 1994).

In the north Pacific, floating matter that escapes the inshore oscillations of the tidal currents, enters the open ocean, where it eventually contacts the westward transport of the north Pacific gyre, a great circular vortex (Sverdrup et al. 1946; Karl 1999). Once in the gyre, the wood can remain there for long periods of time and travel great distances, reaching as far as Hawaii. The majority of driftwood on Hawaiian beaches for example, is wood from the Pacific Northwest. Other driftwood comes from the Philippines, Japan or Malaysia (Maser and Sedell 1994).

Several expeditions have reported the presence of wood and other plant material in the deep sea (*Challenger* Expedition, Phillippines and New Guinea; *Blake* Expedition, West Indian waters; *Siboga* Expedition, Indonesian waters; *Galathea* Expedition, 56 different stations; references in (Knudsen 1961)) and it is considered abundant by some authors (Wolff 1979).

Wood has been around since the upper Palaezoic (Carboniferous 354 to 290 mya), which allowed for ample time for, predominately invertebrate, communities to develop micro niches on deep-sea wood. Such wood associated organisms are molluscs (Turner 1973; Waren and Bouchet 1993), annelids (Wolff 1979; Desbruyeres and Laubier 1988), arthropods (Maddocks and Steineck 1987) and echinoderms (Baker et al. 1986; Voight 2005), to name a few.

The importance of deep-sea wood on the ecology of the deep sea has long been recognized by several investigators (e.g. Turner 1973; Wolff 1979; Stockton and DeLaca 1982; Maddocks and Steineck 1987). Wood provides a form of transient and local energy enrichment that significantly increases the biomass of the deep sea benthos for up to several years (Turner 1973). By itself, wood is not immediately usable as a food source. Along with fungi and bacteria, the Xylophagainae (deep-sea wood-boring clam subfamily from the family Pholadidae), provide an essential link in a specialized food chain by converting wood into animal tissue and fecal pellets (Romey et al. 1991; Romey et al. 1994).

In the deep sea, wood is difficult to detect due to its small extent, short residence time, scattered spatial distribution and seasonal occurrence (Wolff 1979). Stockton and DeLaca qualify wood as a 'low quality' food fall because it first needs to be broken down by bacteria and woody plant specialists, before being accessible by non-specialist deep-sea animals (Turner 1978). The Xylophagainae significantly speed up the process of decay through the mechanical breakdown of the wood (e.g. Turner 1973; Hoagland and Turner 1981; Turner 1981).

## 1.5 Experimental Wood blocks

Accessibility and the potential for manipulations are problems for research in the deep sea. Deep-sea islands, as elaborated above, are highly complex and variable, depending on the geological and chemical setting. Deep-sea wood in contrast, is less complex and yet it attracts a community of organisms, much of which is exclusive to wood (Turner 1973; Coan et al. 2000).

Investigators placed experimental wood blocks on the sea floor to survey the effect of wood-falls on community composition (e.g.Hoagland and Turner 1981). The robotic arms of deep-sea submersibles can easily manipulate experimental wood blocks and precisely place them on the sea floor. The ease of deployment and retrieval facilitates the study of long-term processes, such as larval settlement, seasonality, boring and growth processes, and succession phenomena. Species richness and biomass data can be easily collected. Patterns found at other deep-sea island habitats that are intrinsically more difficult to examine, can be explained by the information gained from the wood block systems.

I presented information about the deep sea above, trying to make a case for the use of fauna colonizing deep-sea wood as a proxy system for island habitats in the deep sea.

The simplicity of the system allows us to overcome problems encountered in the deep sea, such as accessibility and the potential for manipulation.

This study is giving special attention to the Northeast Pacific species province.

## 1.6 Objectives

In this study, I aim to assess the species richness of deep-sea wood-boring clams in the Northeast Pacific by means of strategically placed experimental wood blocks, supplemented by opportunistic wood samples, at various depths throughout the Cascadia Basin, the Juan de Fuca Ridge and the Gorda Ridge, by:

1) Assessing the morphological diversity found within the collection area and form morphological taxa based on pre-determined characters and character states.
2) Testing the newly established morphological species taxon hypotheses by employing two genetic markers, the Cytochrome *c* Oxidase 1 gene (CO1) and the 18S rDNA gene.
3) Elucidating the phylogenetic relationships of the potential new species taxa within the NE-Pacific.

# Chapter 2: Morphological Analysis

## 2.1 Introduction

Deep-sea wood attracts multiple phyla including Mollusca, Annelida, Arthropoda and Echinodermata ((Knudsen 1961), personal observation). The subfamily Xylophagainae (Bivalvia, Mollusca) is the only important bioeroder for deep-sea wood, rendering this otherwise 'locked' resource available to an entire deep-sea wood community (Turner 1972).

### 2.1.1 Systematics

The deep-sea wood-boring clam subfamily Xylophagainae comprises three genera: *Xylophaga, Xyloredo* and *Xylopholas.* Together with the shallow-water wood-boring subfamily Martesiinae, and the subfamilies Jouannetiinae and Pholadinae, they form the family Pholadidae. The Pholadidae and the Teredinidae, the common "shipworms", comprise the superfamily Pholadacea in the order Myoida in the subclass Heterodonta (Turner 1972) (Figure 1).

- Subclass Heterodonta
  - Order Myoida
    - Suborder Pholadina Adams and Adams, 1858
      - Superfamily Pholadacea / Pholadoidea Lamarck, 1809
        - Family Pholadidae Lamarck, 1809
          - **Subfamily Xylophagainae Purchon, 1941**
            - **Genus *Xylophaga* Turton, 1822**
            - **Genus *Xyloredo* Turner, 1972**
            - **Genus *Xylopholas* Turner, 1972**
          - Subfamily Martesiinae Grant and Gale, 1931
          - Subfamily Jouannetiinae
          - Subfamily Pholadinae Lamarck, 1809
            - Genus *Barnea*
        - Family Teredinidae Rafinesque, 1815
          - Subfamily Teredininae Rafinesque, 1815

Figure 1: Systematic overview of the subclass Heterodonta, highlighting the deep-sea wood-boring clam subfamily Xylophagainae.

### 2.1.2 Fossil Record

The global fossil record suggests that the suborder Pholadina arose in the Carboniferous (354 to 290 MYA), coinciding with the rise of plants (Gymnosperms) on earth. The pholad subfamilies Xylophagainae and Martesiinae occurred in the Cretaceous (144 to 65 MYA), during the time of the rise of the Angiosperms (flowering plants), and have all survived to the present. Most pholad genera are not recognizable in the fossil record until the Tertiary (65 to 1.8 MYA), although *Martesia*, *Barnea* and *Xylophaga* are found in the lower Cretaceous (144 to 65 MYA). Many taxa of the encompassing order Myoida are mud-boring or rock-boring clams (e.g. geoducks, corbulas, rock-boring angelwings and piddocks). The family Teredinidae is only known from the Paleocene (65 to 54.8 MYA) onwards. Wood boring might have evolved in the deep-sea, from mud boring, in the family Pholadidae, eventually giving rise to the rapid radiation of the Teredinidae in shallow waters. The fossil record is insufficient to prove this theory (Turner 1972) (Figure 2).

The relatively old age of the subfamily Xylophagainae, especially the genus *Xylophaga*, indicates that time is not a limiting factor in the diversification and global distribution of the subfamily.

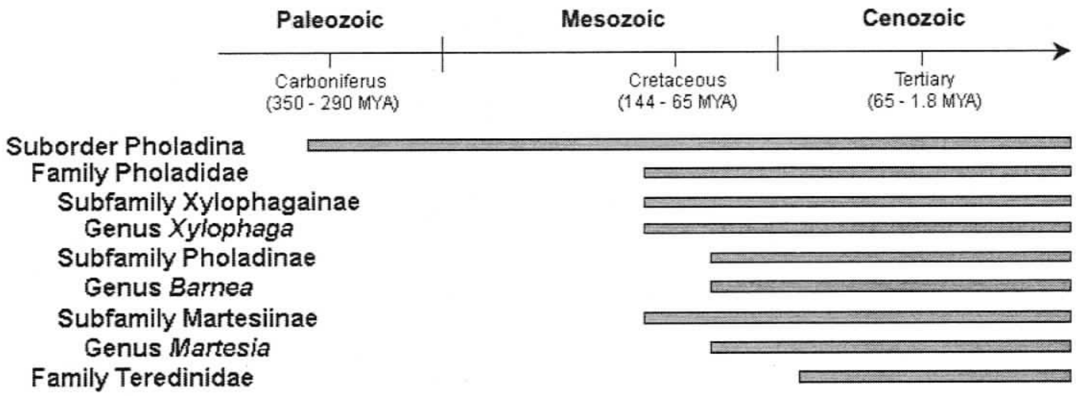


Figure 2: Appearance of wood-boring taxa from Carboniferous in the Paleozoic to the Tertiary in the Cenozoic (Harvey 1996).

### 2.1.3 Global Distribution

The genus *Xylophaga* is globally distributed (Figure 3, Table 1) occurring at depths from 18 to ~7290 m (Knudsen 1961; Turner 2002). The genus *Xylopholas* has been described from one only location off the coast of Florida (Turner 1955). *Xyloredo* is known from two locations, off the Bahamas Islands (Knudsen 1961) and off the coast of California. The Xylophagainae appear to display provinciality. No published wood-boring species occurs in more than one ocean basin. Often they are confined to specific, restricted regions within their ocean basins. The Northeast Pacific, for example, harbours a single described species, *Xylophaga washingtona*. A possible explanation for this could found in their life history strategies (LHS). From the published records of xylophaginid LHS, I deduce that the tendency to retain juveniles attached to the parents is more frequent than the release of planktotrophic larvae (Turner 2002). Planktotrophy is commonly associated with high dispersal abilities. Retaining the young could result in the apparent pattern of provinciality. The global distribution pattern could, however, also be a sampling artifact, considering the low number of research cruises that specifically targeted deep-sea wood.

Figure 3: Distribution map of the described species of the genera *Xylophaga, Xylopholas and Xyloredo.* The genus *Xylopholas* is <u>underlined</u> and the genus *Xyloredo* is in *italics.*

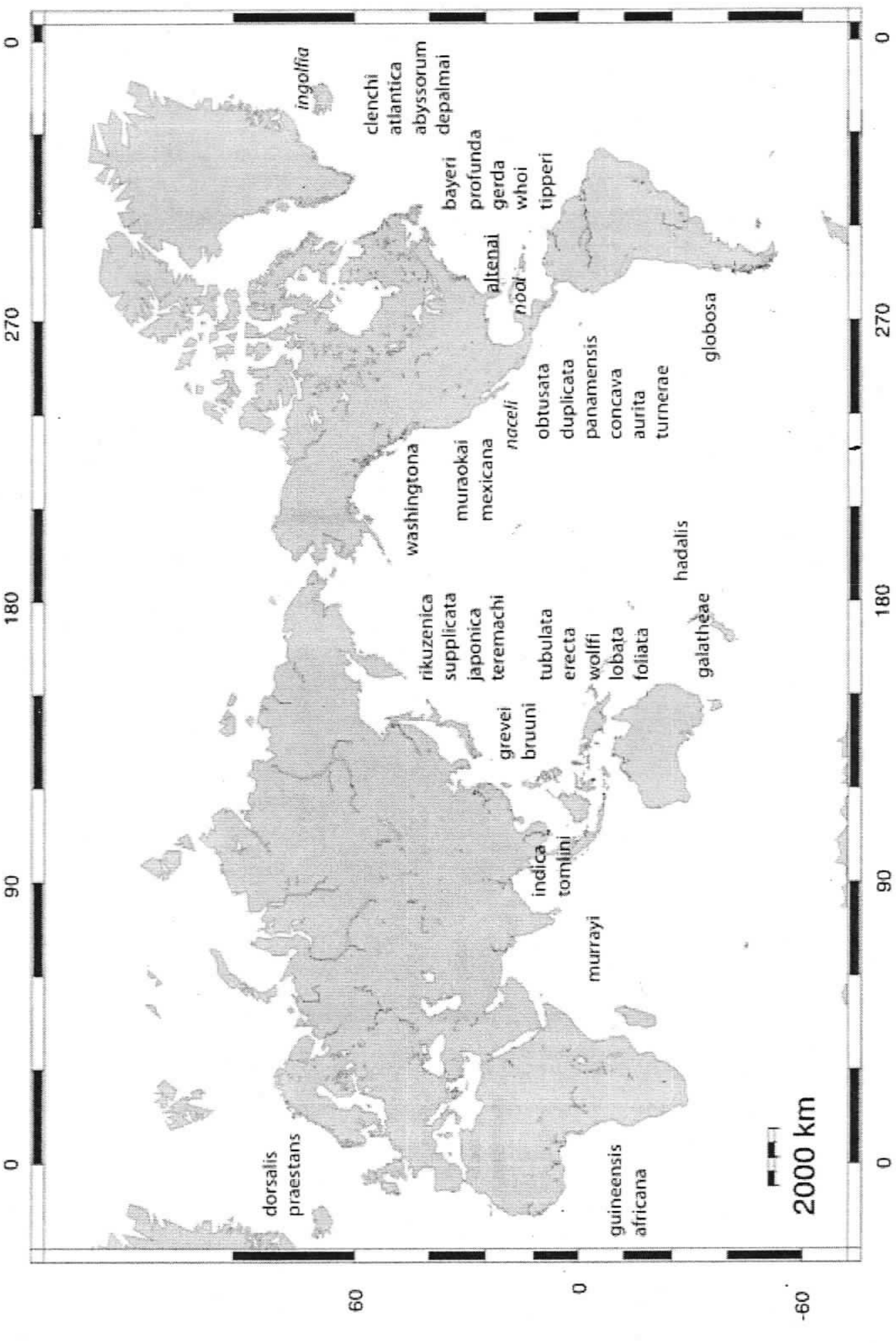

0
270
180
90
0
60
0
-60
ingolfia
clenchi
atlantica
abyssorum
depalmai
bayeri
profunda
gerda
whoi
tipperi
altenai
nodi
globosa
obtusata
duplicata
panamensis
concava
aurita
turnerae
naceli
washingtona
muraokai
mexicana
hadalis
rikuzenica
supplicata
japonica
teremachi
tubulata
erecta
wolffi
lobata
foliata
galatheae
grevei
bruuni
indica
tomlini
murrayi
dorsalis
praestans
guineensis
africana
2000 km

Table 1: Forty-one described species in the subfamily Xylophagainae (Bivalvia: Mollusca), including full scientific name, collection location, ocean basin, depth, latitude and longitude. (Knudsen 1961; Turner 1972)

| Genus | Species | Location | Ocean | Depth | LAT | LON |
|---|---|---|---|---|---|---|
| *Xyloredo* | *ingolfia* | South of Eyrabakki, Iceland | N Atlantic | 1783 | 61 30 N | 22 30 W |
| *Xylophaga* | *dorsalis* | N Norway to Mediterranean | NE Atlantic | 0-2500 | | |
| *Xylophaga* | *praestans* | | NE Atlantic | 30-82 | | |
| *Xylophaga* | *washingtona* | Washington to California | NE Pacific | 18-249 | | |
| *Xylophaga* | *africana* | West Africa | E Atlantic | 2550 | 1 42 N | 7 51 E |
| *Xylophaga* | *guineensis* | West Africa | E Atlantic | 2550 | 1 42 N | 7 51 E |
| *Xylophaga* | *atlantica* | St. Lawrence estuary to Cape Henry | NW Atlantic | 0-3175 | | |
| *Xylopholas* | *altenai* | Fowey Rock, Florida | W Atlantic | 366 | 25 25 N | 79 59 W |
| *Xyloredo* | *nooi* | Bahamas | W Atlantic | 1737 | 25 54 N | 77 49 W |
| *Xylophaga* | *abyssorum* | New Jersey to lesser Antilles | W Atlantic | 253-2456 | | |
| *Xylophaga* | *aurita* | Gulf of Panama | W Atlantic | 915 | 7 15 N | 79 25 W |
| *Xylophaga* | *bayeri* | Fort Lauderdale, Fl | W Atlantic | 152.4 | 26 04 N | 80 04 W |
| *Xylophaga* | *concava* | Gulf of Panama | W Atlantic | 3670-3270 | 5 49 N | 78 52 W |
| *Xylophaga* | *concava* | Gulf of Panama | W Atlantic | 915-975 | 7 22 N | 79 32 W |
| *Xylophaga* | *depalmai* | | W Atlantic | 152.4 | 26 04 N | 80 04 W |
| *Xylophaga* | *duplicata* | Gulf of Panama | W Atlantic | 915 | 7 15 N | 79 25 W |
| *Xylophaga* | *gerda* | Bahamas | W Atlantic | 283.96 | 26 37 N | 78 56 W |
| *Xylophaga* | *obtusata* | Gulf of Panama | W Atlantic | 915 | 7 15 N | 79 25 W |
| *Xylophaga* | *panamensis* | Gulf of Panama | W Atlantic | 915-975 | 7 22 N | 79 32 W |
| *Xylophaga* | *profunda* | Bahamas | W Atlantic | 1722 | 25 54 N | 77 49 W |
| *Xylophaga* | *tipperi* | Fort Lauderdale, Fl | W Atlantic | 152.4 | 26 04 N | 80 04 W |
| *Xylophaga* | *turnerae* | Gulf of Panama | W Atlantic | 915 | 7 15 N | 79 25 W |
| *Xylophaga* | *whoi* | off Cardenas, Cuba | W Atlantic | 914 | 23 21 N | 80 56 W |
| *Xylophaga* | *bruuni* | Mindanao Sea | W Pacific | 1500 | 8 48 N | 124 09 E |
| *Xylophaga* | *erecta* | Sulu Sea | W Pacific | 5050 | 7 54 N | 121 30 E |
| *Xylophaga* | *foliata* | Makassar Strait | W Pacific | 2000 | 3 56 S | 118 26 E |
| *Xylophaga* | *galatheae* | Tasman Sea | W Pacific | 4530 | 31 27 S | 153 33 E |
| *Xylophaga* | *grevei* | Mindanao Sea | W Pacific | 1500 | 8 48 N | 124 09 E |
| *Xylophaga* | *grevei* | Bali Sea | W Pacific | 570-545 | 5 25 S | 117 03 E |
| *Xylophaga* | *grevei* | Banda Trench | W Pacific | 7290-7250 | 5 26 S | 130 58 E |
| *Xylophaga* | *lobata* | Sulu Sea | W Pacific | 5050 | 7 54 N | 121 30 E |
| *Xylophaga* | *tubulata* | Makassar Strait | W Pacific | 2000 | 3 56 S | 118 26 E |
| *Xylophaga* | *wolffi* | Sulu Sea | W Pacific | 5050 | 7 54 N | 121 30 E |
| *Xylophaga* | *japonica* | Shikoku, Japan | NW Pacific | 183 | | |
| *Xylophaga* | *rikuzenica* | Honchu, Japan | NW Pacific | 183-1281 | | |
| *Xylophaga* | *supplicata* | Shikoku, Japan | NW Pacific | 183 | | |
| *Xylophaga* | *teramachi* | Shikoku, Japan | NW Pacific | ? | | |
| *Xyloredo* | *naceli* | Santa Barbara Islands, California | E Pacific | 2072 | 33 46 N | 120 45 W |
| *Xylophaga* | *mexicana* | California to Mexico | E Pacific | 258-805 | | |
| *Xylophaga* | *muraokai* | Santa Barbara Islands, CA | E Pacific | 1720 | 33 44 N | 120 45 W |
| *Xylophaga* | *globosa* | Panama to Chile | SE Pacific | 0-245 | | |
| *Xylophaga* | *hadalis* | Kermadec Trench | S central Pacific | 6660-6770 | 35 51 S | 178 31 W |
| *Xylophaga* | *indica* | | Indian Ocean | 339 | | |
| *Xylophaga* | *tomlini* | Indo-Malayan Sea | Indian Ocean | 1301 | | |

### 2.1.4 Shell Morphology of the Xylophagainae

The shell is globular with a wide anterior opening, partly covered by the antero-dorsal shell extension called the beak (Figure 4-ABC-2). Shell diameters range from 0.5 mm to ~ 16 mm. When viewed from the dorsal side (Figure 4-B), the two shell halves form an interspace called the anterior incision (Figure 4-B-4). The edges of the anterior incision, called the umbonal reflection (Figure 4-BC-3), can be erected or reflected, sometimes adhering to the underlying beak. A characteristic accessory structure, called the mesoplax (Turner 1972), consists of two distinct plates inserted on the umbonal reflection (Figure 4-A-12,D). In some species, the mesoplax can occupy the anterior incision completely, while in others, only part of it. The simplest form of the mesoplax consists of two triangular plates situated horizontally. The edges inserted onto the umbonal reflection are called the basal edges (Figure 4-D-8), the edge where the two plates meet the median edge (Figure 4-D-7) and the remaining the anterior edge (Figure 4-D-6). In a number of species, the mesoplax may be markedly modified. The median part can be raised to a degree that when viewed frontally, the two plates form an angle. In other species, the two plates are longitudinally curved, and yet other species have the lateral parts sharply bent. Finally, some species have a semicircular or 'fan-shaped' mesoplax in a vertical position, forming a dorsal anterior edge.

The shell consists of anterior and posterior sections, separated by the umbonal-ventral sulcus (Figure 4-BC-1), extending from the umbo to the ventral edge of the shell. The articulated umbonal-ventral ridge (Figure 4-E-11) is the corresponding structure on the interior. The anterior section consists of the beak (Figure 4-ABC-2) and the anterior slope. The beak has numerous ridges running parallel to its ventral edge, which continue on the anterior slope as oblique ridges (Knudsen 1961).

The valves of the genera *Xylopholas* and *Xyloredo* are virtually indistinguishable from valves of the genus *Xylophaga*. In all three genera, the visceral mass is completely contained within the valves. Below is a list of characters that distinguish *Xyloredo* and *Xylopholas* from each other and from *Xylophaga*.

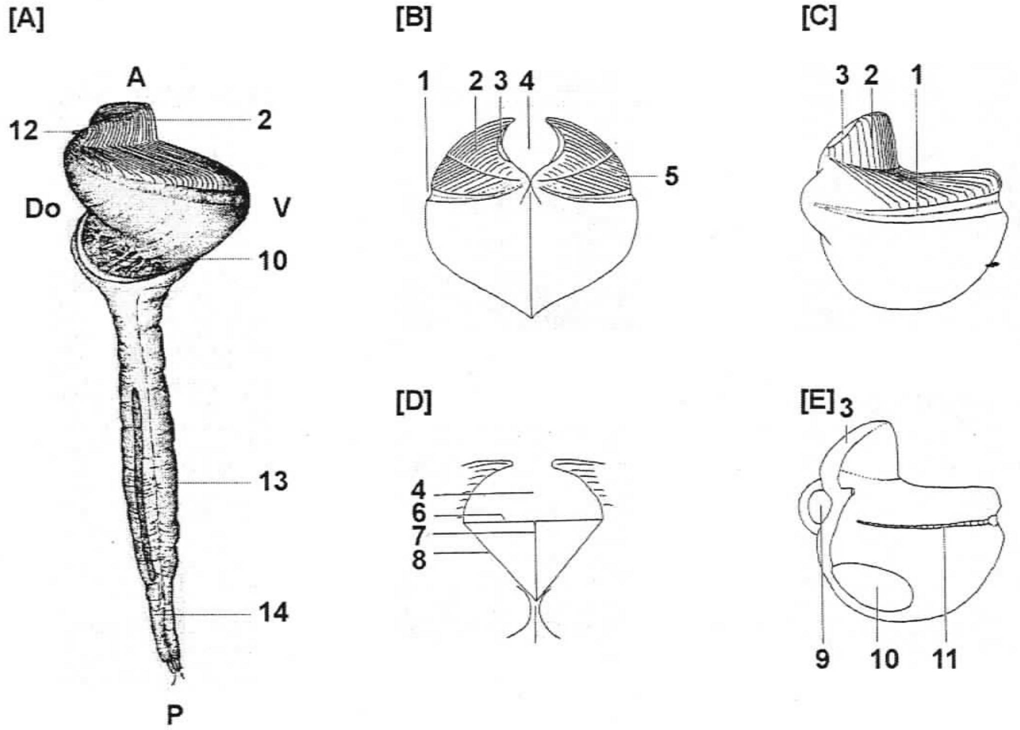


Figure 4: Diagrams of deep-sea woodborer morphology. [A] lateral view [B] dorsal view [C] lateral view – valve exterior [D] dorsal view of mesoplax and anterior incision [E] lateral view – valve interior; 1, umbonal ventral sulcus; 2, beak; 3, umbonal reflection; 4, anterior incision; 5, anterior slope; 6, anterior edge; 7, median edge; 8, basel edge; 9, prodissoconch; 10, posterior muscle scar; 11, umbonal ventral ridge; 12, mesoplax; 13, incurrent siphon; 14, excurrent siphon. Do = dorsal, V = ventral, A = anterior, P = posterior. [A] *Xylophaga gerda* Turner, modified after Turner (2002), [B-E] Modified after (Knudsen 1961).

#### Genus Xylopholas

The protruding portion is covered by a ***periostracal sheath***, which ends in a pair of slightly ***calcified siphonal plates*** (Turner 2002).

#### Genus Xyloredo

The combined incurrent and excurrent canals extend the entire length and attach to the ***calcareous lining*** at the end of the siphons. This ***lining*** is ***distinctly marked with rings*** and covered by a thin, ***outer periostracal layer*** (Turner 2002).

The deep-sea woodborers, known to date, were distinguished and described based on a set or subset of the above-mentioned characters. Often they are known from only one or two locations and, in most cases, from a single piece of wood. These small wood-sample sizes have led to species descriptions based on very small numbers of representatives, that could not address intraspecific morphological variation (Purchon 1941). Of 37 species described by Turner (1997) and Knudsen (1941), 24 are known from fewer than 10 specimens and only seven are known from more than 100 specimens. To understand intraspecific morphological variation, Turner examined the effect of different wood types on the morphological shell characteristics of *Xylophaga washingtona* (Distel and Roberts 1997). All variables except for the wood species were kept the same. The results showed that the accessory plates (mesoplax) remained "remarkably uniform" (p.228) in all species, varying only slightly in length/width proportions. The general shape of the valves remained "rather" (p.228) constant whereas the most variable characters were the size of the valves and the number of denticulated ridges on the anterior slope in relation to the length of the valves. Regardless of wood species, no noticeable variation other than size was found in the siphons (Distel and Roberts 1997). Consequently, a combination of the mesoplax and siphonal characteristics is the most reliable set of characters to distinguish members of the subfamily morphologically.

### 2.1.5 Internal Anatomy

Wood-boring clams have the same basic anatomy as other members of the order Myoida, with a major difference being the elongation of the body to accommodate boring

(Turner 1973). An additional highly specialized outpocketing of the stomach called the caecum functions as a storage area for wood-chips created by the boring of the clams. Presumably, its main function is to release wood fragments into the stomach in a continuous stream. The labial palps are greatly reduced (Waterbury et al. 1983; Distel and Roberts 1997). The ctenidia are formed by a single outer demibranch on either side of the visceral mass. The marginal groove is absent and there is no ciliary sorting mechanism. The lack of a ciliary sorting mechanism in the gills suggests inefficacy with respect to suspension feeding (see below: feeding).

The gills house gram-negative, bacterial endosymbionts ranging from 0.4 – 0.7 μm in width and up to 5 μm in length (Purchon 1941). Their location within the clam host as well as their morphology is similar to the location and morphology of the endosymbionts of the Teredinidae (see below: feeding) (Turner 1973). The foot is developed and actively used in the boring process (see below: boring).

### 2.1.6 Feeding

Turner (1997) speculated that the Xylophagainae could perform a role similar to that of the termites in terrestrial systems, by converting the refractory cellulosic carbon, deposited in wood and other plant material, to a more readily available form. We know that this holds true for the Teredinidae. The role of the teredinid endosymbionts, as cellulose digesters and atmospheric nitrogen fixers, was discovered through *in vitro* cultivation. The symbiosis is considered to be essential for the feeding strategy of the Teredinidae, as wood is rich in carbon and energy, but deficient in nitrogen (Knudsen 1961; Distel and Roberts 1997). Like the teredinids, the Xylophagainae ingest wood particles while boring (Distel and Roberts 1997; Distel et al. 2002). To date, the role of wood in the diet of the Xylophagainae has not been tested experimentally. As mentioned above, Distel and Roberts (2004) examined two species of the genus *Xylophaga* and found endosymbionts that resemble the shipworms' (Teredinidae) endosymbionts, both morphologically and in their anatomical location within the gills, suggesting a similar role to the latter. Both termites and shipworms (Teredinidae) utilize cellulolytic and nitrogen-fixing symbionts to survive on wood as the sole food source (references in Hoagland and Turner 1981). The feeding strategies of the Xylophagainae are still poorly understood.

Although the connection between the caecum and the cellulolytic bacteria in the gills has not been experimentally proven, a feeding symbiosis, supplemented by suspension and / or deposit feeding, is presumed (Purchon 1941; Knudsen 1961; Turner 1973). Hoagland and Turner (1941) suggested a cessation of woodboring in favour of suspension feeding as a result of crowding or lack of the resource wood.

### 2.1.7 Boring Mechanism and Borehole Densities

Boring is a mechanical process, executed by the anterior adductor, the foot, siphon and mantle (reviewed in Turner 1954). New 'recruits' to a wood block form approximately 300 to 500 µm, cup-shaped, indentations in the wood block surface. As the juveniles burrow deeper into the block, they grow in size and length, resulting in a pear-shaped burrow, to which the individuals are confined for life. The opening to the burrow is only marginally enlarged through unknown processes. The maximum diameter of a burrow opening I observed was ~ 750 µm.

The posterior regions of the burrows are often filled with wood particles produced by the boring. These particles often form a tube, referred to as a 'chimney' that adheres to the posterior part of the shell. Burrow sharing is reported, however no damage to shell or soft-tissues has been observed (Knudsen 1961).

Members of the subfamily Xylophagainae occur in densities up to 150 individuals per $cm^2$ (Romey et al. 1994).

### 2.1.8 Life History Strategies

Very little is known about the life history strategies of the Xylophagainae. *Xylophaga dorsalis* (Turton) is a protandric hermaphrodite (Turner and Yakovlev 1983). Purchon (1976) advocates the possibility of self-fertilization in this species and proposes hermaphroditism in other members of the genus. This has not been scientifically tested yet. Brood protection was observed by Turner (1994) in *X. atlantica* Richards, where the young were held in a brood pouch until the late veliger stage. This is followed by a planktonic larval stage. Knudsen (1991) reported on an additional nine species that show brood protection. There are no records of free-spawning in the Xylophagainae.

To date, no study has addressed the age of sexual maturity in deep-sea woodborers. Given the opportunistic nature of deep-sea wood-boring clams, the common perception is that sexual maturity is reached fairly quickly (Culliney and Turner 1976). Extreme adult adaptations (e.g. parasitic dwarf-males) are known from the shallow-water woodborers (Teredinidae) (Parker and Tunnicliffe 1994) and proposed in the Xylophagainae (Takuma Haga, personal communication).

Culliney and Turner (1968) examined the larval development of *X. atlantica*. Internally fertilized eggs developed into free-swimming larvae within 30-40 hours at 9°C. The swimming larvae displayed a temperature preference of around 11-13°C. Within four days, straight-hinge veligers averaged 62 μm in length. The subsequent umbo-veligers ranged from 150 to 255 μm in length and displayed a characteristic velum. 50 days post fertilization, pediveligers appeared in the culture dishes, averaging ~ 280 x 268 μm. The first signs of metamorphosis showed after 57 days. Despite the larvae continuing to live for an additional six months, metamorphosis was never completed in culture.

Romey et al. (1976) studied the effects of environmental factors on the growth rates of *X. atlantica* by comparing the growth rates at two different depths (100 and 200 m). Growth rates varied greatly depending on the time of the year, substrate, site and crowding. Growth at the deeper site (0.246 mm / day) was a lot higher than that from the shallower site (0.015 mm / day), which they attributed to the fact that the average temperature at the 200 m site, was higher than the average temperature at the 100 m site.

Romey et al. (1976) examined the recruitment patterns of *X. atlantica* over a two-year period. Average recruitment densities of *X. atlantica* in test panels varied from 0 to 30 animals / $cm^2$. At the 100 m site, peak settlement was observed between September and December, with a smaller peak from June to September. The recruitment rates declined between January and May. In contrast, at the 200 m site, the rates remained at relatively high levels during February to April. Low recruitment was observed from May through December.

Although planktotrophy is reported in the literature (Romey et al. 1991), it is surprising to see the high number of species that have a reproductive strategy that does not support larval dispersal via a planktonic stage. It seems that a space-limited environment such as patchily distributed and ephemeral wood blocks would attract widely dispersing taxa to minimize competition for space and food. A study assessing the life history strategies of inhabitants of Cobb seamount, an isolated, space limited but non-ephemeral habitat, 510 km off the coast of Oregon, showed a predominance of short-lived planktonic and brooding species, indicating that the potential for survival is higher when retaining the offspring despite space limitations, compared to sending them into an uncertain future (Turner 2002). No study has examined the correlation between deep-sea wood abundance and the type of life history strategy. A simple expectation is that in an area of high wood abundance (e.g. river delta or estuary), a planktonic larval phase would be preferred, as sending the young off to a nearby piece of wood limits crowding on the parental wood block, with the inverse being the case in an area of low wood abundance. Here the young would be retained (brooded) since the likelihood of them reaching another piece of wood is low.

### 2.1.9 Summary

The deep-sea wood-boring clam subfamily Xylophagainae, lives on a patchily distributed habitat that is unpredictable in time and space. Over evolutionary time, the Xylophagainae have developed exclusivity in deep-sea wood and other plant material. Wood blocks become increasingly crowded over time and consequently riddled with boreholes, leading to a lack of suitable space for the initiation of boring by new recruits. Adult clams, normally located within the secure confines of a wood block, are exposed to predation as the wood block starts to disintegrate.

The Xylophagainae occur globally in depths ranging from a few tens of meters to multiple thousands of meters. They display provinciality in that specific species only occur in one ocean basin and are confined to certain areas within that basin. This is possibly due to the life history strategies of many Xylophagainae where the young can be found attached to the adult, suggesting a lecithotrophic larval stage, brood protection and poor dispersal capabilities. According to the literature, the Northeast Pacific harbours only one species, *Xylophaga washingtona*, a representative of the Xylophagainae with a free-swimming larval stage (Turner 1973; Turner 2002). The single other species reported to have a free-swimming larval stage is its most close relative, *X. atlantica* (Tipper 1968). This information leads to a simple expectation that the populations of *X. washingtona* of various sites within the Northeast Pacific maintain gene flow amongst each other, impeding a process of adaptive radiation (speciation) within the Northeast Pacific.

The current knowledge on pholad diversity and global distribution together with the specific knowledge of the situation in the Northeast Pacific lead me to formulate the following hypotheses:

### 2.1.10 Hypotheses

$H_0$: All wood samples collected from within the Northeast Pacific harbour *X. washingtona,* in similar densities, regardless of location and depth.

$H_A$: Wood samples from the Northeast Pacific contain multiple, morphologically distinct species.

At this point it is important to note that I do not have the intention to describe new species from the Northeast Pacific. Dr. Janet Voight from the Field Museum in Chicago is working on the species descriptions. The goal of this chapter is to create reasonable morphological bins, which will consequently allow me to perform a molecular analysis (Chapter 3). The characters I am using to bin the specimens morphologically, are a subset, and by no means encompassing, of the characters used by Dr. Voight for the species descriptions.

## 2.2 Materials and Methods

### 2.2.1 Experimental Wood Blocks

In general, the experimental wood blocks were transported to the ocean bottom in a lidded biobox by manned and un-manned submersibles (Table 2). Several workers prepared the experimental wood blocks used in this study. Dr. Janet Voight (The Field Museum Chicago, IL) used 4 x 4 x 18 in (10 x 10 x 45 cm) untreated fir wood blocks and Craig Smith (U of Hawaii at Manoa) used 200 kg fir blocks. For samples deployed by the Tunnicliffe lab, two 2 x 4 x 12 in (5 x 10 x 30 cm) untreated fir blocks were used together. For my deployments, I used untreated 4 x 4 x 8 in (10 x 10 x 20 cm) fir blocks. Fir is abundant in the temperate forests of northwest America and consequently represents a naturally occurring habitat for the Xylophagainae. The experimental wood blocks were placed into mesh bags to prevent the loss of bio-eroded wood material upon recovery. For easy handling of the wood block by the submersibles' robotic arms, I attached a monkey's fist knot (Figure 5-A). To prevent the drifting of the wood blocks I attached up to 1 lb of lead to the underside of the wood blocks. The blocks were individually marked and the deployment sites were marked with floating, white bucket lids to facilitate the retrieval of the blocks (Figure 5-B). The wood deployed by Bob MacDonald (IOS, BC) was part of the superstructure of borehole 1026B of the Ocean Drilling Program.

### 2.2.2 Wood Locations

Wood blocks were deployed along the Gorda and Juan de Fuca Ridges as well as in the Cascadia Basin using the manned deep-sea submersible ALVIN (WHOI, USA) and the remotely operated vehicles ROPOS (CSSF, Canada), Jason II (WHOI, USA) and Tiburon (MBARI, USA) (Table 2, Figure 6). The deployment locations were predetermined by the Chief Scientists of the particular research cruises. The specific wood block locations were carefully selected to represent common, muddy, deep sea without the influence of hydrothermal vent activity. Deployment depths varied from 1540 to 3222 m. The wood retrieved by Shana Goffredi (MBARI) at Mendocino consisted of a single twig. The wood retrieved at 'Hecate Strait' and 'Ucluelet' were both opportunistic bycatches from bottom trawls.

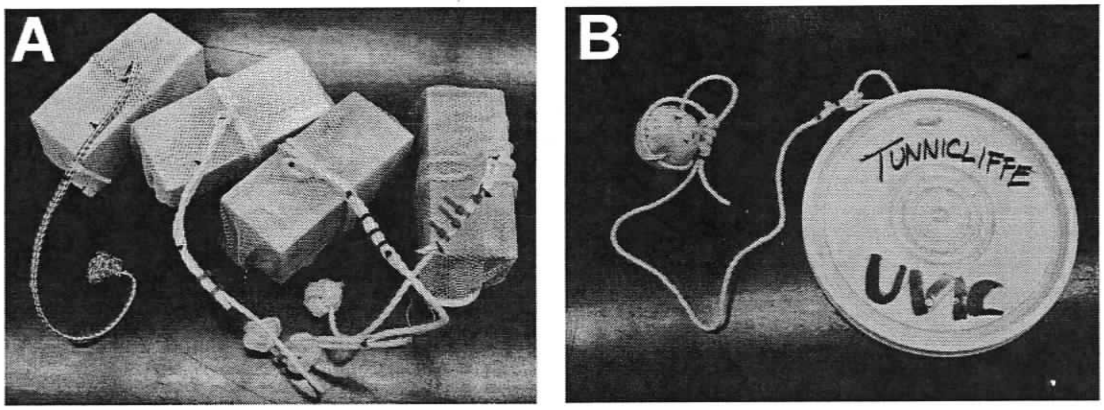
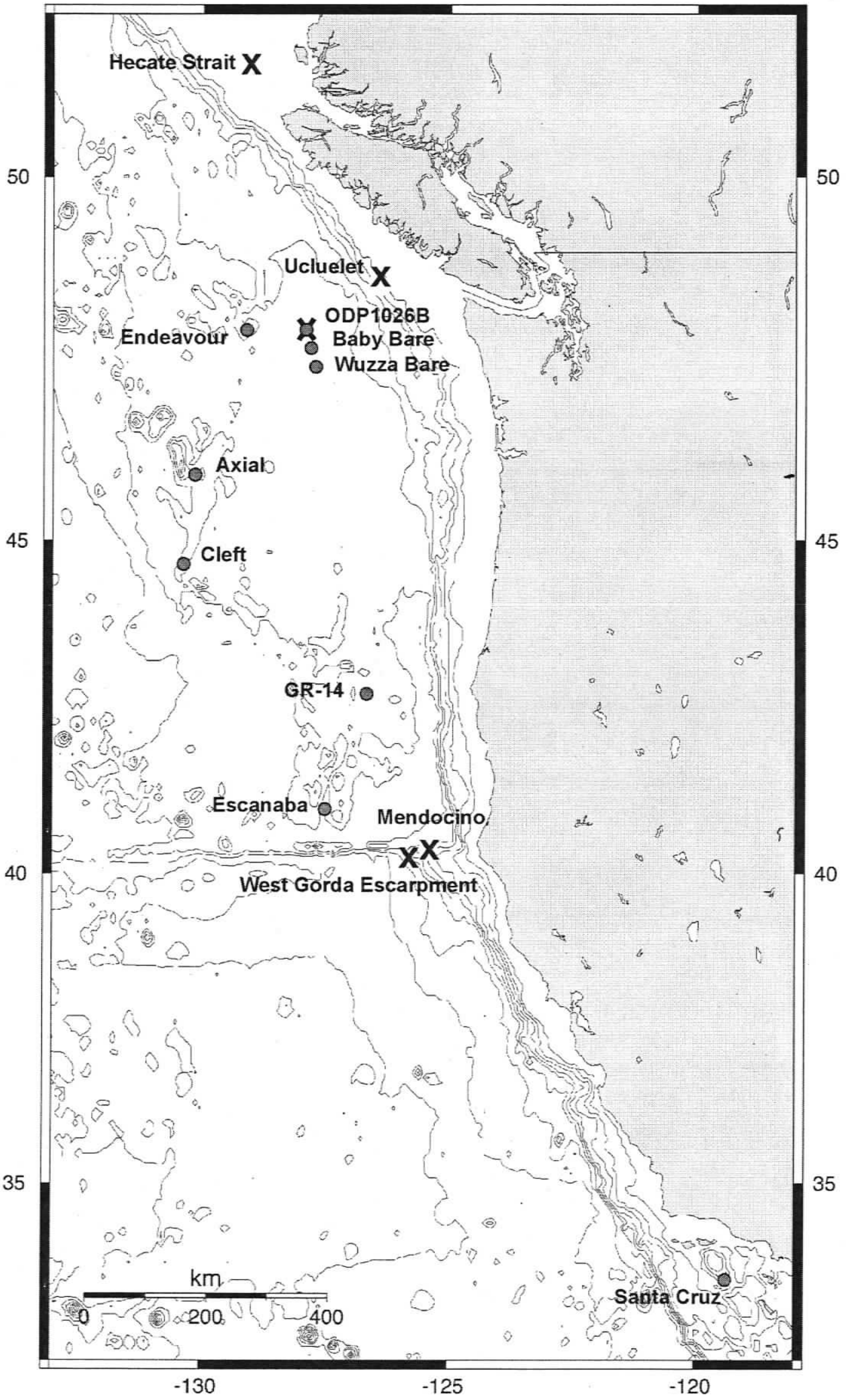


Figure 5: [A] Four wood blocks ready for deployment. The meshbag prevents the loss of wood fragments upon recover of the deteriorating wood block. One lb of lead was inserted in the meshbag to prevent the drifting of the wood blocks. [B] A lead ball attached to a plastic bucket lid served as an underwater marker for easy detection upon retrieval.

Table 2: Wood deployment locations, collection ID, depth, latitude and longitude, date deployed and retrieved, # of blocks recovered, time spent at bottom and dive #. [A] Experimental wood blocks [B] Opportunistic wood finds. An (*) indicates non-deployed wood. (**) indicate blocks deployed by Dr. Voight (The Chicago Field Museum, USA). (***) indicate samples from Bob MacDonald (IOS, BC). (****) indicate sample from Shana Goffredi (MBARI, USA). (*****) indicate samples from Dr. Craig Smith (University of Hawaii at Manoa, USA).

| Location | Collection ID | Depth [m] | Lat | Long | date deployed [m/y] | date recovered [m/y] | recovered (n) | Time at Bottom (months) | Dive |
|---|---|---|---|---|---|---|---|---|---|
| **A. Experimental Wood blocks** | | | | | | | | | |
| Hecate Strait | WB-C | 205 | 53.135 | 130.468 | Jun-02 | Sep-02 | 1 | 3 | Mooring |
| ODP1026B | WB-I | 2661 | 47.6788 | 127.8555 | Jul-02 | Jul-03 | small pieces | 12 | JASON 065** |
| Endeavour | WB-F | 2250 | 47.9427 | 129.096 | Jul-02 | Jul-03 | small pieces | 12 | JASON 068** |
| Endeavour | WB-G | 2194 | 47.9427 | 129.096 | Aug-02 | Jul-03 | 2 | 11 | ROPOS 710 |
| Endeavour | WB-P | 2213 | 47.9427 | 129.096 | Sep-02 | Sep-04 | 3 | 24 | ALVIN 4045** |
| Wuzza Bare | WB-Q | 2658 | 47.4847 | 127.6915 | Sep-02 | Sep-04 | 7 | 24 | ALVIN 4046** |
| Baby Bare | WB-H | 2641 | 47.7105 | 127.7882 | Jul-02 | Jul-03 | small pieces | 12 | JASON 062** |
| Axial | WB-E | 1540 | 45.9333 | 130.1333 | Sep-02 | Sep-03 | 7 | 12 | ROPOS 740** |
| Axial | WB-D | 1540 | 45.9333 | 130.1333 | Jul-01 | Jul-02 | 1 | 12 | ROPOS 662 |
| Axial | WB-J | 1540 | 45.9333 | 130.1333 | Jul-01 | Jul-02 | 8 | 12 | ROPOS 662 |
| Cleft | WB-A | 2100 | 44.6633 | 130.355 | Jul-99 | Jul-00 | 4 | 12 | ROPOS 542 |
| Cleft | WB-B | 2100 | 44.6633 | 130.355 | Jul-99 | Jul-01 | 1 | 24 | ROPOS 619 |
| GR-14 | WB-O | 2721 | 42.7559 | 126.7096 | Jul-02 | Sep-04 | 2 | 26 | ALVIN 4044** |
| Escanaba | WB-N | 3222 | 40.999 | 127.4934 | Jul-02 | Aug-04 | 2 | 25 | ALVIN 4043** |
| Santa Cruz | WB-CS | 1672 | 33.3769 | 119.3653 | Oct-99 | Mar-05 | subsamples | 54 | TIBURON 827***** |
| Station P | WB-K | 3800 | 50 | 145 | Aug-02 | Jun-03 | 3 | 11 | Mooring |
| **B. Opportunistic Wood Collections** | | | | | | | | | |
| Hecate Strait | WB-J1 | 100 | 51.45 | 129.04 | ? | 2005 | log | ? | Bottom trawl* |
| Ucluelet | WB-T1 | 600 | 48.556 | 126.436 | ? | 2004 | log | ? | Bottom trawl* |
| ODP1026B | WB-R | 2658 | 47.6788 | 127.8555 | 2004 | 2005 | plywood | 12 | ALVIN 4151*** |
| Mendocino | WB-L | 1588 | 40.3705 | 125.3806 | ? | 2001 | twig | ? | TIBURON 352-J5**** |
| West Gorda Escarpment | WB-M | 2440 | 40.401 | 125.773 | ? | Aug-01 | twig | ? | TIBURON 348* |

Figure 6: Collection locations. Contour lines = 500 m intervals. Circles mark wood block locations. 'X's mark opportunistic wood findings.


Hecate Strait
Ucluelet
ODP1026B
Endeavour
Baby Bare
Wuzza Bare
Axial
Cleft
GR-14
Escanaba
Mendocino
West Gorda Escarpment
Santa Cruz
km
0
200
400
50
45
40
35
-130
-125
-120

### 2.2.3 Retrieval

The wood blocks that were recovered by a submersible were placed into the lidded biobox and transported to the surface. Once on the research vessel, the wood blocks were kept submerged in cold seawater and temporarily stored in onboard cold rooms at ~5 °C before further processing. The wood pieces from the bottom trawls were hauled to the surface in fishing nets. Once on deck, the wood was carried to the onboard freezer room and frozen in their entirety. The ODP wood (ALVIN 4151) was kept in an onboard fridge for several days, before being transferred to a freezer on shore.

### 2.2.4 Extraction and Preservation

A variety of woodworking tools was used to extract the pholads from the wood blocks. The most successful method was to split the blocks along the grain with a hammer and chisel. The specimens were immediately transferred into the preservative (Table 3). Further specimens and/or wood blocks or parts thereof were frozen. The remaining wood pieces were either, frozen, air-dried or preserved. Specimens were selected for preservation by randomly picking easily extractable individuals from the split wood blocks. I could not extract individuals smaller than ~0.5 mm due to their small size. Many individuals were damaged during the process of splitting the wood or when too forcefully extracted from the wood using forceps. This rendered certain identifying characters useless. Table 3 summarizes the number of individuals picked from the wood blocks of the various locations.

Table 3: Wood block code, location and preservative used for sub-sampled specimens. An '*' following the wood block code indicates wood blocks I processed at sea.

| Wood Block Code | Site Name | # Blocks Subsampled | # Individuals | Size of Wood Block | 1st Preservative | 2nd Preservative |
|---|---|---|---|---|---|---|
| WB-J1 | Hecate Strait | 2 | 60 | ~20 x 10cm | 95 % EtOH, frozen | |
| WB-T1 | Off Ucluelet | 2 | 30 | ~25 x 10cm | 95 % EtOH, frozen | |
| WB-I | OPD1026B | | 198 | little junks | 95 % EtOH | |
| WB-R | ODP1026B | 3 | 41 | 30 x 30 cm plywood | 95 % EtOH, frozen | |
| WB-F | Endeavour | | 184 | little junks | 70% EtOH | |
| WB-P* | Endeavour | 3 | 39 | 4 x 4 x 45 cm | 95 % EtOH, frozen | |
| WB-Q* | Wuzza Bare | 4 | 184 | 4 x 4 x 45 cm | 95 % EtOH, frozen | |
| WB-H | Baby Bare | | 6 | little junks | 70% EtOH | |
| WB-E | Axial | 4 | 156 | 4 x 4 x 20 cm | 80% EtOH | |
| WB-A | Cleft | 1 | 75 | 2 x 4 x 20 cm | 70% EtOH, frozen | |
| WB-B | Cleft | 2 | 128 | 2 x 4 x 20 cm | 7% Formalin | 95% EtOH |
| WB-O* | GR-14 | 2 | 58 | 4 x 4 x 45 cm | 95 % EtOH, frozen | |
| WB-N* | Escanaba | 2 | 73 | 4 x 4 x 45 cm | 95 % EtOH, frozen | |
| WB-L | Mendocino | | 1 | Tree branch | 70 % EtOH | |
| WB-CS | Santa Cruz | - | 20 | - | 95% EtOH | |

### 2.2.5 Examination and Documentation

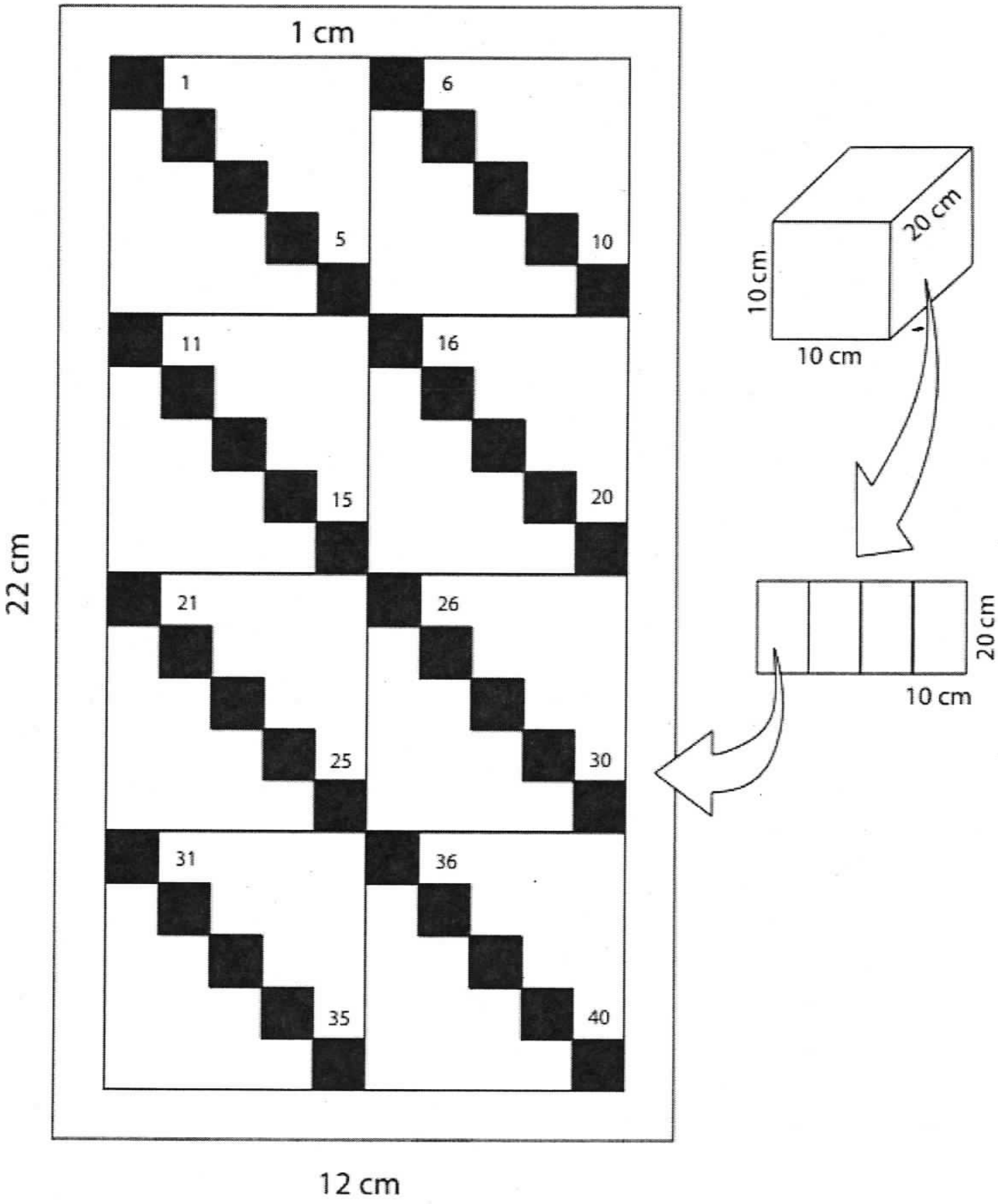
I used a stereo dissecting-microscope to examine the specimens. Digital images were taken through the dissecting-microscope, using a Nikon Coolpix 995 with an ocular lens adapter. I imitated a confocal microscopy approach by focusing on sequentially deeper focal plains for each picture. I then imported the resulting images as layers into Adobe Photoshop CS 2 and used the eraser tool to erase the out-of-focus areas in each of the layers, resulting in mostly in-focus images of the specimens.

I digitized pencil drawings of the specimens using Adobe Illustrator CS 2. All computer work was conducted on a Mac Powerbook G4 with OS X 10.3.9.

### 2.2.6 Borehole Densities

We extrapolated clam densities on 11 blocks from four locations within the study area (Escanaba, A4043, 1 blocks; GR-14, A4044, 1 blocks; Endeavour, A4045, 2 blocks; Wuzza Bare, A4046, 7 blocks). All four longitudinal sides of the experimental wood block were sub-sampled using an adapted version of an established protocol (Tunnicliffe et al. 2003). A 12 x 22 cm rectangular stencil was subdivided into 8 quadrats of 5 x 5 cm with a one cm margin on the outer periphery (Figure 7). A series of five 1 x 1 cm quadrates were cut from the stencil on a diagonal through each of the eight quadrats. 20 of the 40 available 1-$\text{cm}^2$ quadrats were randomly chosen for borehole counts by using a random number generator.

Figure 7: Stencil used for sub-sampling surface area of experimental wood blocks. 20 quadrates were randomly selected for borehole counts.


1 cm
1
5
6
10
11
15
16
20
21
25
26
30
31
35
36
40
22 cm
12 cm
20 cm
10 cm
10 cm
20 cm
10 cm

## 2.3 Results

### 2.3.1 Wood Bioerosion

Upon recovery, the obvious difference among the wood blocks within and among locations, was the degree of bioerosion by wood-boring clams. Even blocks deployed for less than a year displayed high woodborer densities. I did not detect any differences between the experimental wood blocks and the opportunistic wood recoveries. The outer surfaces of the 11-12 months wood blocks were mainly intact, unlike the 24 months samples, which had either fallen apart or were doing so upon recovery. Figure 8-AB shows wood blocks deployed at Marker 33 on Axial Seamount (WB-E). Clam siphons are protruding from the wood block surface upon which a majid crab feeds (*Macroregonia macrochira*). A wood block from Endeavour (WB-P), displayed a degree of bioerosion, where large clams (~ 8 mm) were exposed to the ambient seawater, or had fallen out of their boreholes, due to the loss of wood structure (Figure 8-C). Figure 8-D shows a section of a plywood sample (WB-R). Note how the clams stopped boring, when reaching the glue layer, after the first sheet of plywood.

Table 4 summarizes the site locations, years deployed and retrieved, deployment depths, times spent at the bottom as well as the number of wood blocks and their approximate sizes. It also contains the wood-boring clam 'yield' per collection site. Despite an approximately 12-month deployment, collections WB-K (Station Papa) and WB-C (Hecate Strait) did not show any signs of boring activity. The wood blocks at Station Papa were attached to a mooring, approximately 2 m above the sea floor. The wood blocks from Hecate Strait were attached to a mooring as well (height above sea floor unknown).

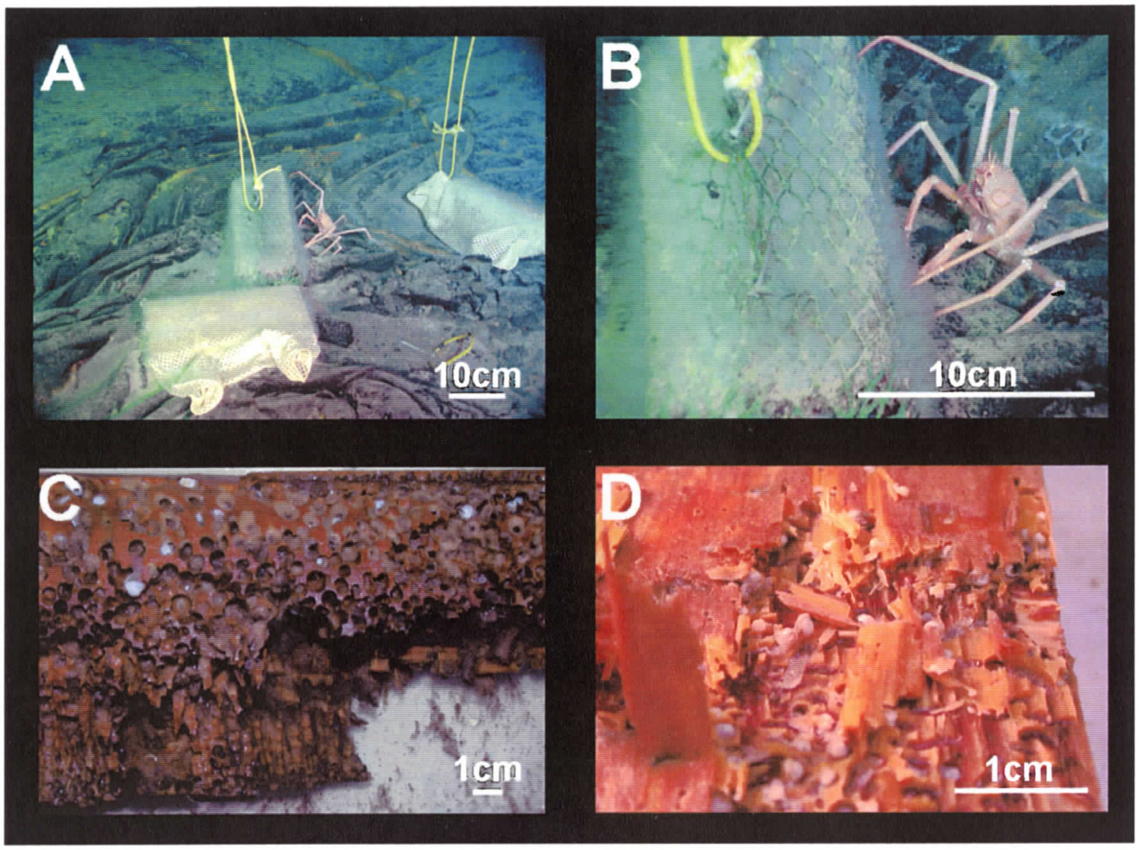


Figure 8: Bioerosion on recovered wood blocks. [A, B] Wood blocks deployed on Axial Seamount (WB-E). [C] WB-P [D] WB-R

Table 4: Collection codes, location, year deployed and retrieved, time spent at bottom, number of blocks and approximate block sizes. * indicates blocks I processed at sea.

| Code | Location | Time spent at Bottom (months) | # Blocks recovered | Size | Observations |
|---|---|---|---|---|---|
| WB-K | Station Papa | 11 | 3 | 10 x 10 x 45 cm | No clams, wood > 2 m above sediment |
| WB-J1 | Hecate Strait | ? | 2 | ~20 x 10 cm | Bottom trawl, lots of clams, wood heavily deteriorated |
| WB-C | Hecate Strait | 12 |  | little junks | No clams, wood comes from mooring |
| WB-T1 | Ucluelet | ? | 2 | ~25 x 10 cm | Bottom trawl, lots of clams, wood heavily deteriorated |
| WB-I | OPD1026B | 12 |  | little junks | Only small fragments of wood preserved, but lots of tiny clams |
| WB-R | ODP1026B | 12 | 3 | 30 x 30 cm | plywood , lots of clams, poorly preserved, was not intended to be experimental but functional |
| WB-F | Endeavour | 12 |  | little junks | Lots of clams |
| WB-P* | Endeavour | 24 | 3 | 10 x 10 x 45 cm | Lots of clams, wood heavily deteriorated |
| WB-G | Endeavour | 11 | 2 | 10 x 10 x 45 cm | Main field, N of S&M, very few boreholes |
| WB-Q* | Wuzza Bare | 24 | 4 | 10 x 10 x 45 cm | Lots of clams, wood heavily deteriorated |
| WB-H | Baby Bare | 12 |  | little junks | Lots of clams |
| WB-E | Axial | 12 | 4 | 10 x 10 x 20 cm | Lots of clams, wood heavily deteriorated |
| WB-D | Axial |  | 1 | 10 x 10 x 45 cm | 'crack vent', dried out upon recovery, few tiny holes |
| WB-J | Axial |  | 8 | 5 x 10 x 45 cm | 'ashes near crack', few holes |
| WB-A | Cleft | 12 | 1 | 5 x 10 x 20 cm | Lots of clams, wood heavily deteriorated |
| WB-B | Cleft | 24 | 2 | 5 x 10 x 20 cm | Very few clams, heavily deteriorated wood |
| WB-O* | GR-14 | 24 | 2 | 10 x 10 x 45 cm | Large clams, wood mostly intact |
| WB-N* | Escanaba | 24 | 2 | 10 x 10 x 45 cm | Very large clams, wood mostly intact |
| WB-L | Mendocino | ? | - | 1 specimen | Only one specimen |
| WB-M | West Gorda Escarpment | ? | - | Twig | No clams |
| WB-CS | Santa Cruz | 54 | - | 20 specimens | Donation from Dr. Craig Smith |

### 2.3.2 Morphological Characters

I discovered a variety of clam morphotypes in both the experimental wood blocks and opportunistic wood findings, from the various locations. I created a character matrix based on the morphological analysis of 1271 specimens. In accordance with previous pholad researchers, I chose the most stable characters to distinguish the morphological taxa. In this case, stable refers to minimal phenotypic plasticity caused by different environmental factors as outlined in the Introduction and in Turner's 1955 study (Voight 2006). These characters are: the incurrent siphon cirri (present or absent), the excurrent siphon cirri (present or absent), the relative length of the incurrent siphon cirri to the excurrent siphon cirri (longer or shorter), the position of the excurrent siphon with respect to the incurrent siphon (equal length, sub-terminal, shorter), the shape of the mesoplax (eyebrow-shaped, shallow triangle, steep triangle) as well as the build of the mesoplax (lightly or strongly calcified) (Table 5, Figure 9).

Table 5: Characters and character states used to distinguish different morphotypes. [1] (Voight 2005); [2] Voight, personal communication

| Character Name | Description | State | Authority | Comment |
|---|---|---|---|---|
| In | Incurrent siphon cirri | Absent = 0<br>Present = 1 | 1 | non-ambiguous |
| Ex | Excurrent siphon cirri | Absent = 0<br>Present = 1 | 1 | non-ambiguous |
| Ex Siphon | Position of excurrent siphon with respect to incurrent siphon | Equal length = 0<br>Subterminal = 1<br>Shorter = 2 | 1, 2 | generally not ambiguous |
| Mesoplax | Mesoplax (accessory plate) build | Lightly calcified = 0<br>Strongly calcified = 1 | this study | subjective categorization |
| M Shape | Shape of the mesoplax | Eyebrow-shaped = 0<br>Shallow triangle = 1<br>Steep triangle = 2 | 1, 2 | various grades of eyebrow-shape that were not distinguished |

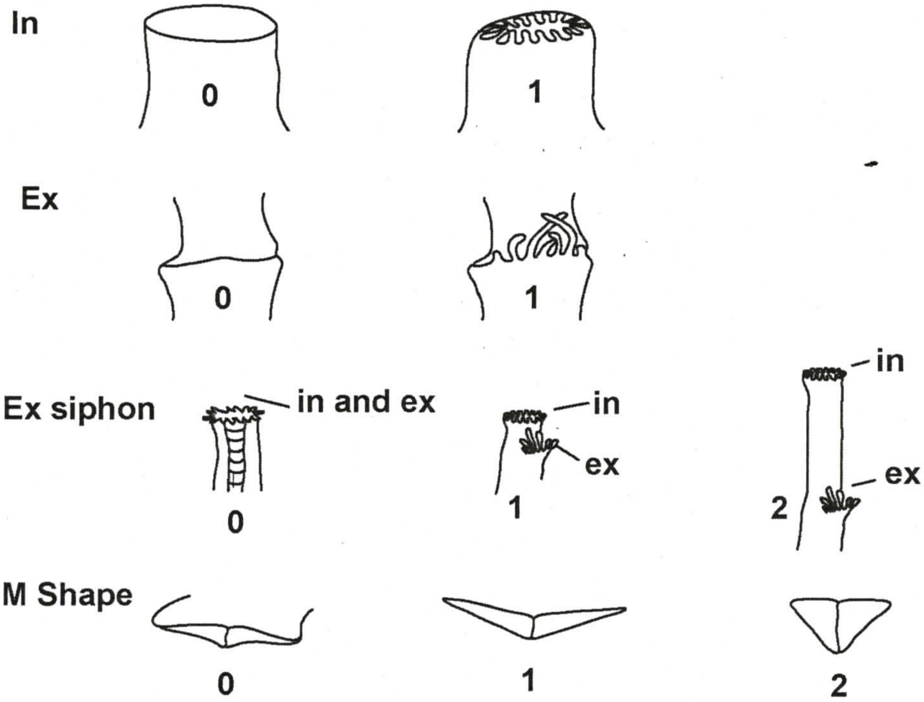


Figure 9: Visual representation of character states outlined in Table 5 and Table 6.

### 2.3.3 Morphological Taxa

Based on the above-mentioned characters, and in collaboration with Dr. Voight, I established eight new morphological taxa (B, F, G, M, R, T, U, X). Taxon Q (Table 7) comprises a group of individuals that closely resemble each other but are not distinct enough from any of the newly established morphological taxa to warrant the establishment of an additional taxon. I was not able to associate these specimens without a doubt, with any of the newly established morphological taxa, due to missing or ambiguous characters.

Specimens collected from all locations, (Figure 6) were morphologically classified into the newly established taxa, yielding the following specimen collection (Table 7, Figure 10). Taxon names followed by a '?' indicate that the specimen is most likely the given letter taxon, however allocation is not certain due to one or two missing or unclear characters. Figure 11 illustrates to scale all newly discovered morphological taxa.

Table 6: Taxa are separated morphologically based on the characters and coded character states (Figure 9, Table 5). "Other" acts as characters; however, they could not be coded.

| Character | In | Ex | Ex Siphon | Mesoplax | M Shape | Other |
|---|---|---|---|---|---|---|
| Taxon Name | | | | | | |
| B | 0 | 0 | 2 | 1 | 2 | Excurrent lip |
| U | 1 | 1 | 0 | 0 | 0 | Flower like ring of cirri at siphon |
| G | 1 | 1 | 2 | 0 | 0 | Short siphon |
| F | 1 | 1 | 2 | 0 | 0 | Very long excurrent cirri |
| R | 0 | 0 | 1 | 1 | 1 | Excurrent lip |
| T | ? | ? | ? | 1 | 2 | Dwarf-males |
| M | 1 | 1 | 1 | 1 | 0 | Inflated siphons |
| X | 0 | 0 | 0 | 0 | 0 | Siphonal plates |

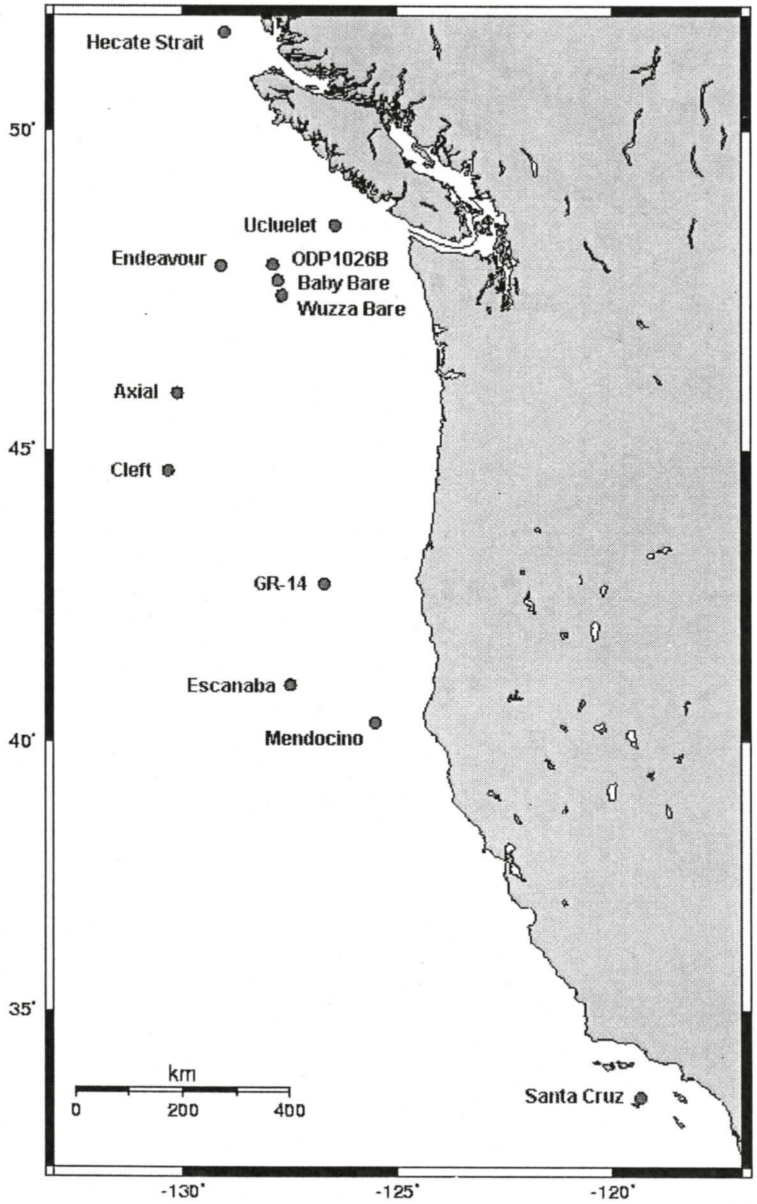
### 2.3.4 Morphological Taxon Distribution

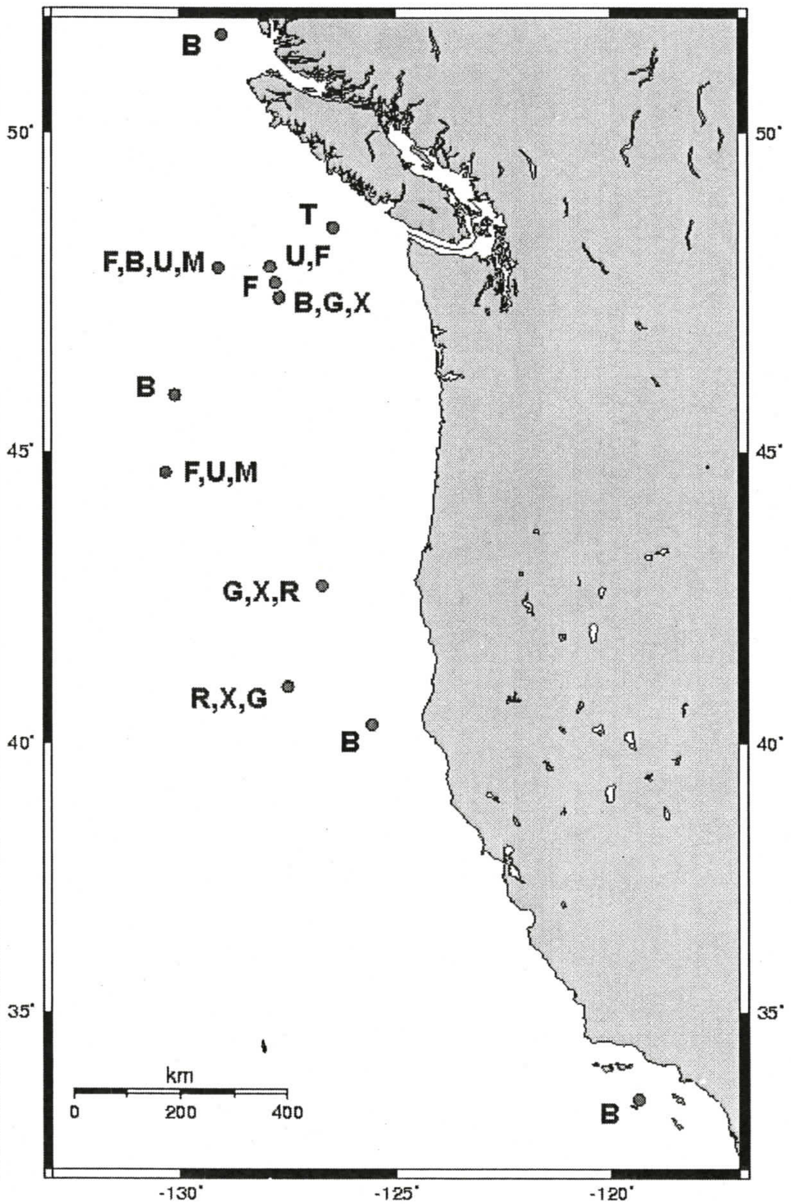
Figure 10 shows the distribution of the morphological taxa within the study area. Initially, it seemed as if every wood block yielded an individual morphotype. I did however find additional morphotypes within wood blocks after closer examination of numerous specimens. Generally, one morphotype appears to dominate a given wood block, with other morphotypes (up to four in total) present in smaller numbers (Table 7). Exceptions to this pattern are the Endeavour collection (WB-F) where taxon B and F are present in almost equally high numbers, and the Endeavour collection (WB-P), where two thirds of the specimens are B and one third F (Table 7).

Table 7: Summary of relative numbers of morphological taxa of Xylophagainae sampled from a single location. A '?' following a taxon letter indicates individuals that cannot be associated to any taxon with certainty due to poor preservation. See comments about Taxon Q in text.

| Location | Dive # | Collection Code | Months deployed | Taxon | # | Total |
|---|---|---|---|---|---|---|
| Hecate Strait | Trawl | WB-J1 | ? | B | 60 | 60 |
| Ucluelet | Trawl | WB-T1 | ? | T | 30 | 30 |
| ODP 1026B | J065 | WB-I | 12 | U | 197 | 198 |
| | | | | F | 1 | |
| Endeavour | J068 | WB-F | 12 | F | 99 | 184 |
| | | | | B | 70 | |
| | | | | ? | 15 | |
| Endeavour | R710 | WB-G | 11 | F | 53 | 59 |
| | | | | U | 6 | |
| Endeavour | A4045 | WB-P | 24 | B | 25 | 39 |
| | | | | F? | 9 | |
| | | | | M | 5 | |
| Wuzza Bare | A4046 | WB-Q | 24 | G | 20 | 184 |
| | | | | G? | 3 | |
| | | | | B | 19 | |
| | | | | X | 1 | |
| | | | | Q | 141 | |
| Baby Bare | J062 | WB-H | 12 | F | 6 | 6 |
| Axial | R740 | WB-E | 12 | B | 156 | 156 |
| Cleft | R542 | WB-A | 12 | F | 75 | 75 |
| Cleft | R619 | WB-B | 24 | U | 127 | 128 |
| | | | | M | 1 | |
| GR-14 | A4044 | WB-G | 11 | X | 6 | 58 |
| | | | | G | 28 | |
| | | | | Q | 5 | |
| | | | | ? | 18 | |
| | | | | R | 1 | |
| Escanaba | A4043 | WB-N | 25 | R | 54 | 73 |
| | | | | R? | 9 | |
| | | | | G? | 2 | |
| | | | | X | 7 | |
| | | | | X? | 1 | |
| Mendocino | T352 | WB-L | ? | B? | 1 | 1 |
| Santa Cruz | T827 | WB-CS | 54 | B | 20 | 20 |

Figure 10: Left: Map indicating collection locations and names. Right: Map indicating morphological taxa found at locations. See Figure 11 for an illustration of the newly established morphological taxa.


Hecate Strait
Ucluelet
Endeavour
ODP1026B
Baby Bare
Wuzza Bare
Axial
Cleft
GR-14
Escanaba
Mendocino
Santa Cruz
km
0
200
400
50˚
45˚
40˚
35˚
-130˚
-125˚
-120˚


B
T
F,B,U,M
U,F
F
B,G,X
B
F,U,M
G,X,R
R,X,G
B
B
km
0
200
400
50˚
45˚
40˚
35˚
-130˚
-125˚
-120˚

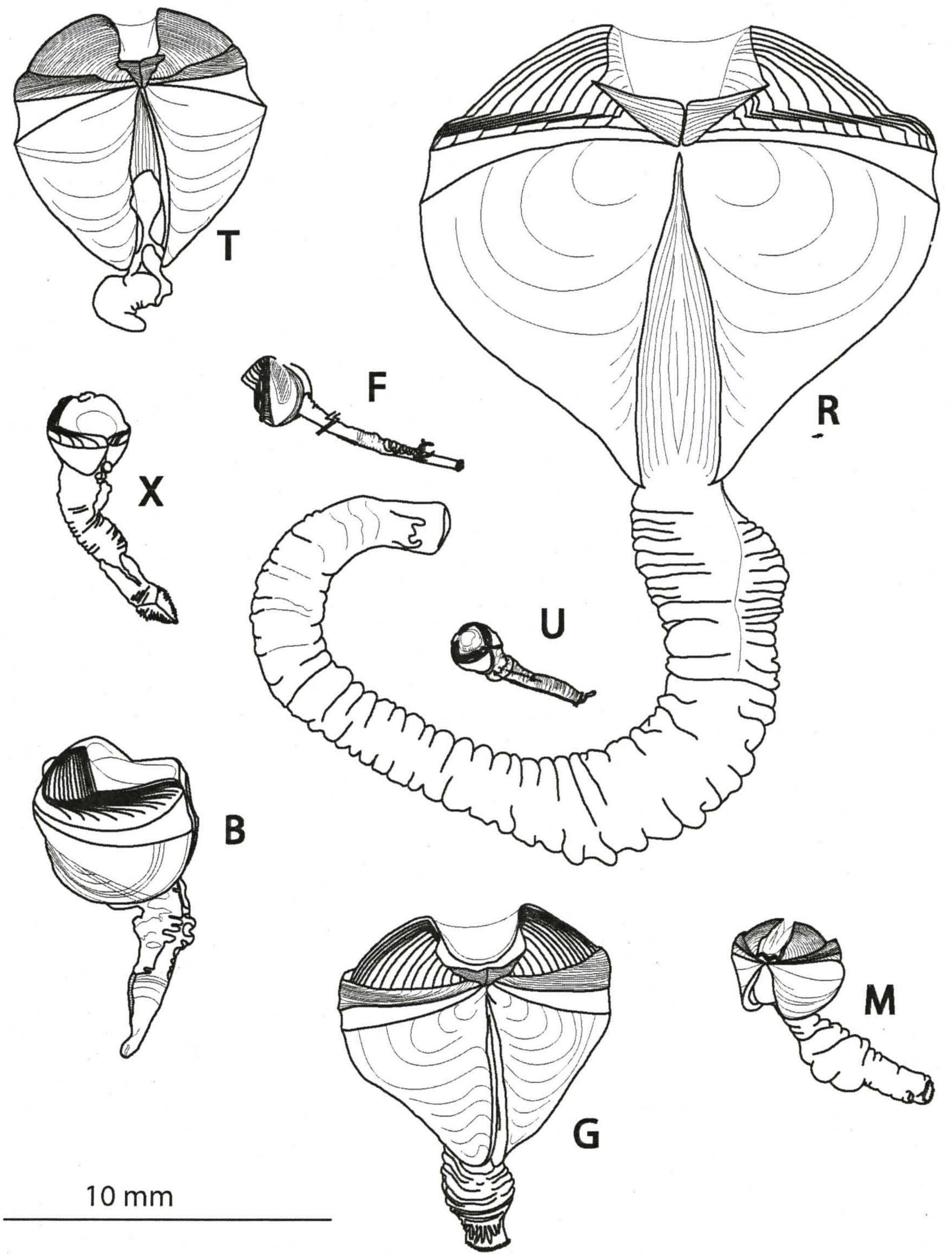


Figure 11: Illustrations of newly discovered morphological taxa, drawn to scale. Letters indicate morphological taxon names.

### 2.3.5 Taxon Working Descriptions

#### Taxon F (Plate 1)

This taxon was found at Endeavour, ODP1026B, Baby Bare and Cleft (Table 7, Figure 10). It is characterized by an ear-shaped, lightly calcified mesoplax, short cirri on the incurrent siphons, and long cirri on the excurrent siphons. The length of the siphon is multiple times the body diameter. I observed fecal pellets in the burrows of Taxon F, although in small quantities. The maximum diameter encountered was ~ 2.4 mm.

#### Taxon B (Plate 2)

This taxon was found in Hecate Strait, at Endeavour, Wuzza Bare, Axial and Santa Cruz (Table 7, Figure 10). It is characterized by a triangular, heavily calcified mesoplax as well as a complete absence of cirri on the incurrent siphon. The excurrent siphon shows a small lip rather than cirri. The length of the siphon is multiple times the body diameter. Fecal pellets were very prominent in this taxon, forming sleeping-bag-like linings of the entire burrow. The maximum body diameter encountered was ~ 6 mm.

#### Taxon R (Plate 3)

This taxon was found at Escanaba and GR-14 (Table 7, Figure 10). It is characterized by a wide, triangular, heavily calcified mesoplax as well as a complete absence of cirri on the incurrent siphon. The excurrent siphon is subterminal. With a maximum diameter of 16 mm, this taxon is the largest found in the 24 months deployment period of this study. The length of the siphons is 1.5 – 2 x the body diameter. Fecal pellets were not observed.

#### Taxon U (Plate 4)

This taxon was found at Endeavour, ODP1026B and Cleft (Table 7, Figure 10). It is characterized by an eyebrow-shaped, lightly calcified mesoplax, as well as a flower-like ring of cirri on the siphons. At the base of the siphon, clear tissue inflations formed round ‘bubbles’, which I found in this taxon only. The incurrent siphon is a small structure protruding from the excurrent siphon. This taxon was found in very low numbers at all three sites. The length of the siphon is 1.5 – 2 x the body diameter. I did not observe fecal pellets. The maximum body diameter encountered was ~ 2.5 mm.

### Taxon T (Plate 5)

This taxon was found at Ucluelet only (Table 7, Figure 10). It is characterized by triangular, heavily calcified mesoplax as well as the presence of juveniles/dwarf-males on the adult specimens. No preserved specimens had intact siphons. The features that distinguished Taxon T from Taxon B were a different overall body shape (more oval shaped posterior and anterior slopes) and a much higher number of denticulated ridges on the anterior slope and beak. Fecal pellets were observed. The maximum body diameter encountered was ~ 15 mm.

### Taxon M (Plate 6)

This taxon was found at Endeavour and Cleft (Table 7, Figure 10). It is characterized by an eyebrow-shaped strongly calcified mesoplax with prominent growth rings. The incurrent siphon has a wide opening with very short cirri. The excurrent siphon is subterminal with short cirri as well. The length of the siphons is 2 – 3 x the body diameter. Taxon M occurred in very low numbers (< 10) at each site. I did not observe any fecal pellets. The maximum body diameter encountered was ~ 8 mm.

### Taxon G (Plate 7)

This taxon was found at GR-14 and Wuzza Bare only (Table 7, Figure 10). It is characterized by a wide, bow-like, lightly calcified, mesoplax. Furthermore, a short incurrent siphon, with medium length cirri and a subterminal excurrent siphon with long cirri. The length of the siphon is 0.5 – 1 x the body diameter. Fecal pellets were not observed. The maximum body diameter encountered was ~ 11 mm.

### Taxon X (Plate 8)

This taxon was found at Escanaba, GR-14 and Wuzza Bare (Table 7, Figure 10). It is characterized by a very fragile, translucent mesoplax as well as siphonal plates with cat-like hooks, characteristic for the genus *Xylopholas.* The length of the siphons is 2 – 3 x the body diameter. These specimens occurred in very low numbers (< 10) at both sites. I did not observe fecal pellets. The maximum body diameter encountered was ~ 2.5 mm.

### Taxon Q

I cannot provide a description for Taxon Q. The specimens, even though very similar within the Taxon, share characters with multiple other morphological taxa, described above. It remains an interim taxon that requires further investigation.

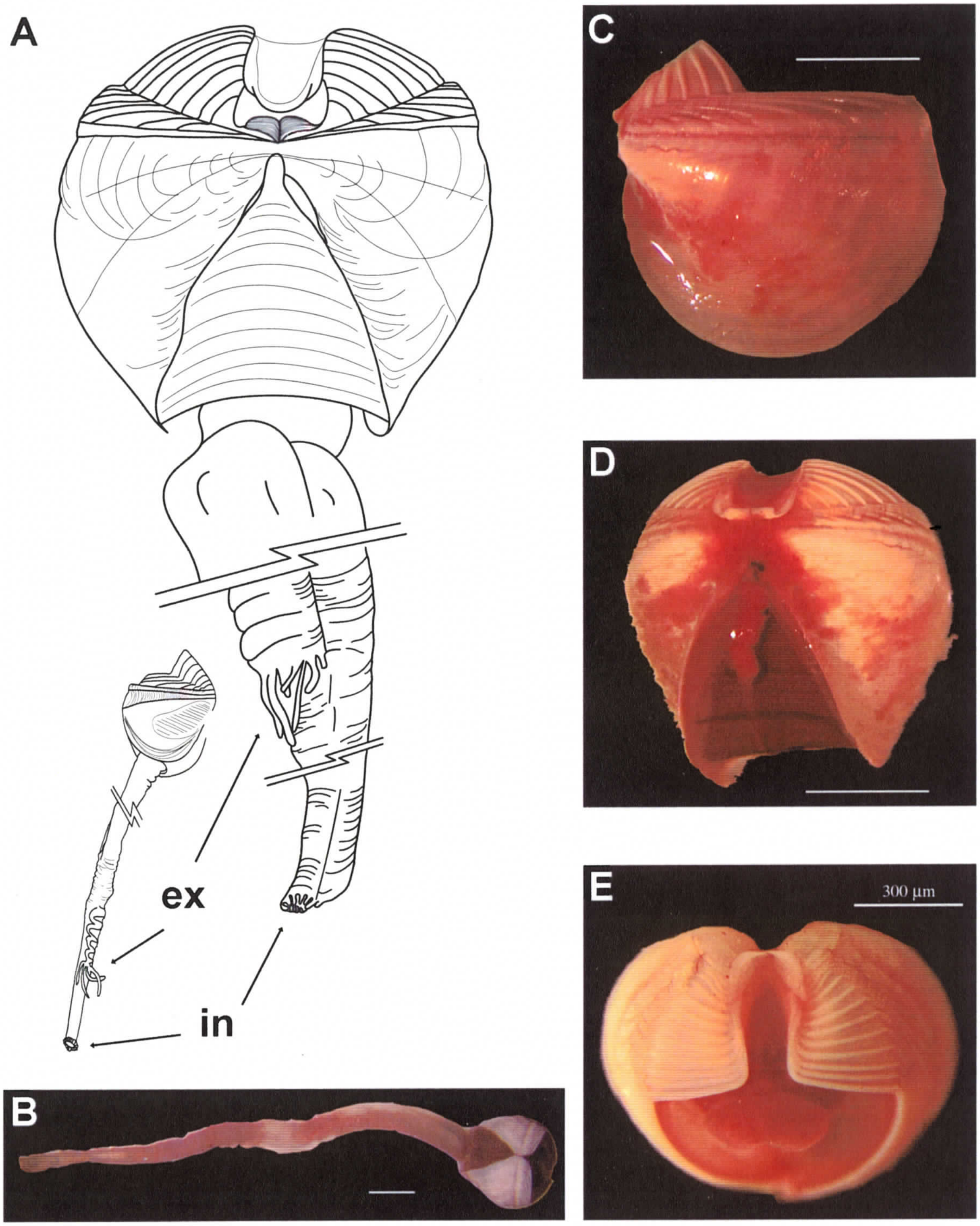


Plate 1: Taxon F [A] Illustration. Lightning bolts indicate truncation of siphon. ex = excurrent siphon, in = incurrent siphon [B] overall body shape [C] side view [D] dorsal view [E] anterior view. Scale bar = 1 mm unless otherwise specified.

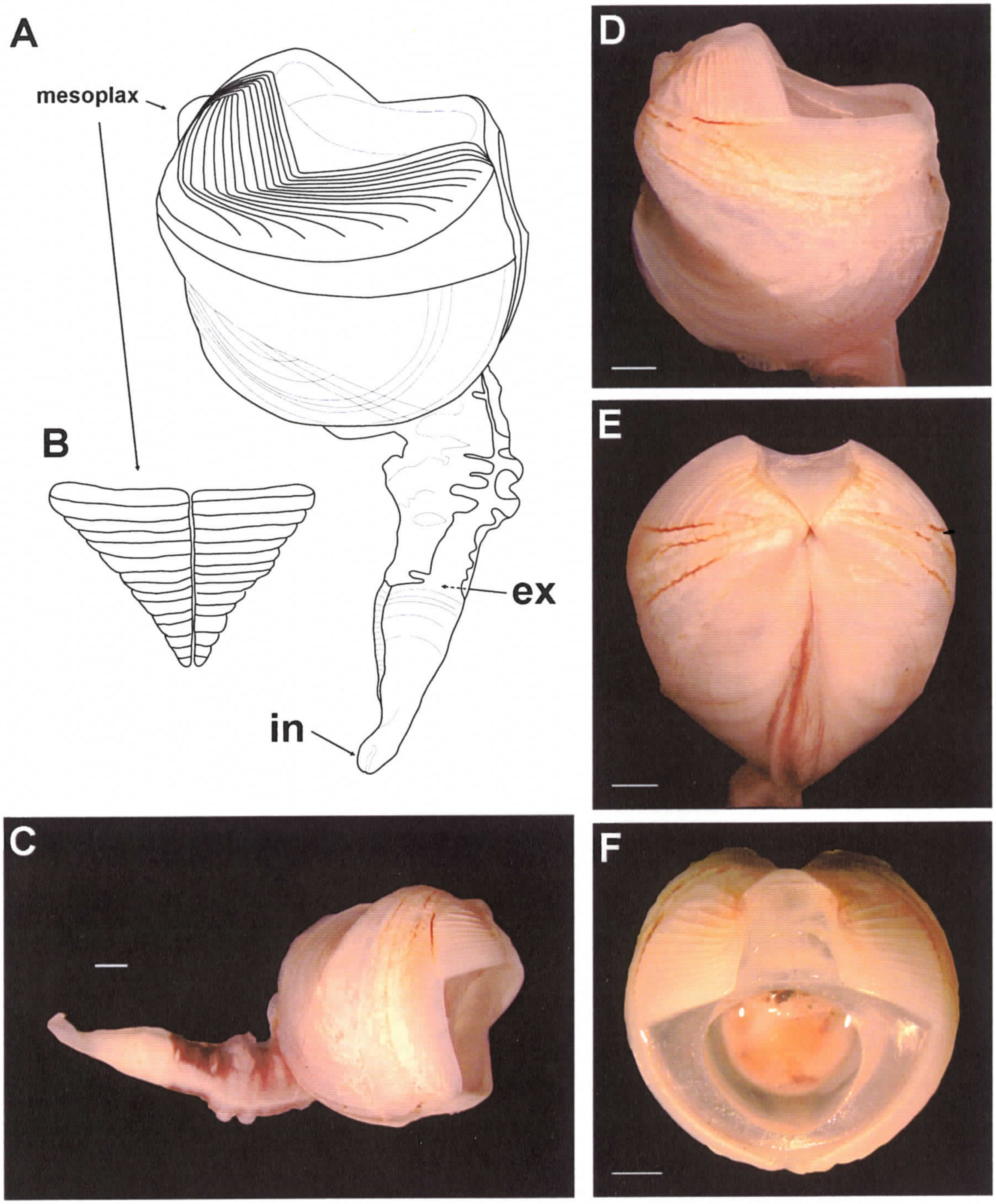


Plate 2: Taxon B [A] Illustration. ex = excurrent siphon (not visible in illustration, located on reverse side), in = incurrent siphon [B] mesoplax detail illustration [C] overall body shape [D] side view [E] dorsal view [F] anterior view. Scale bar = 1 mm.

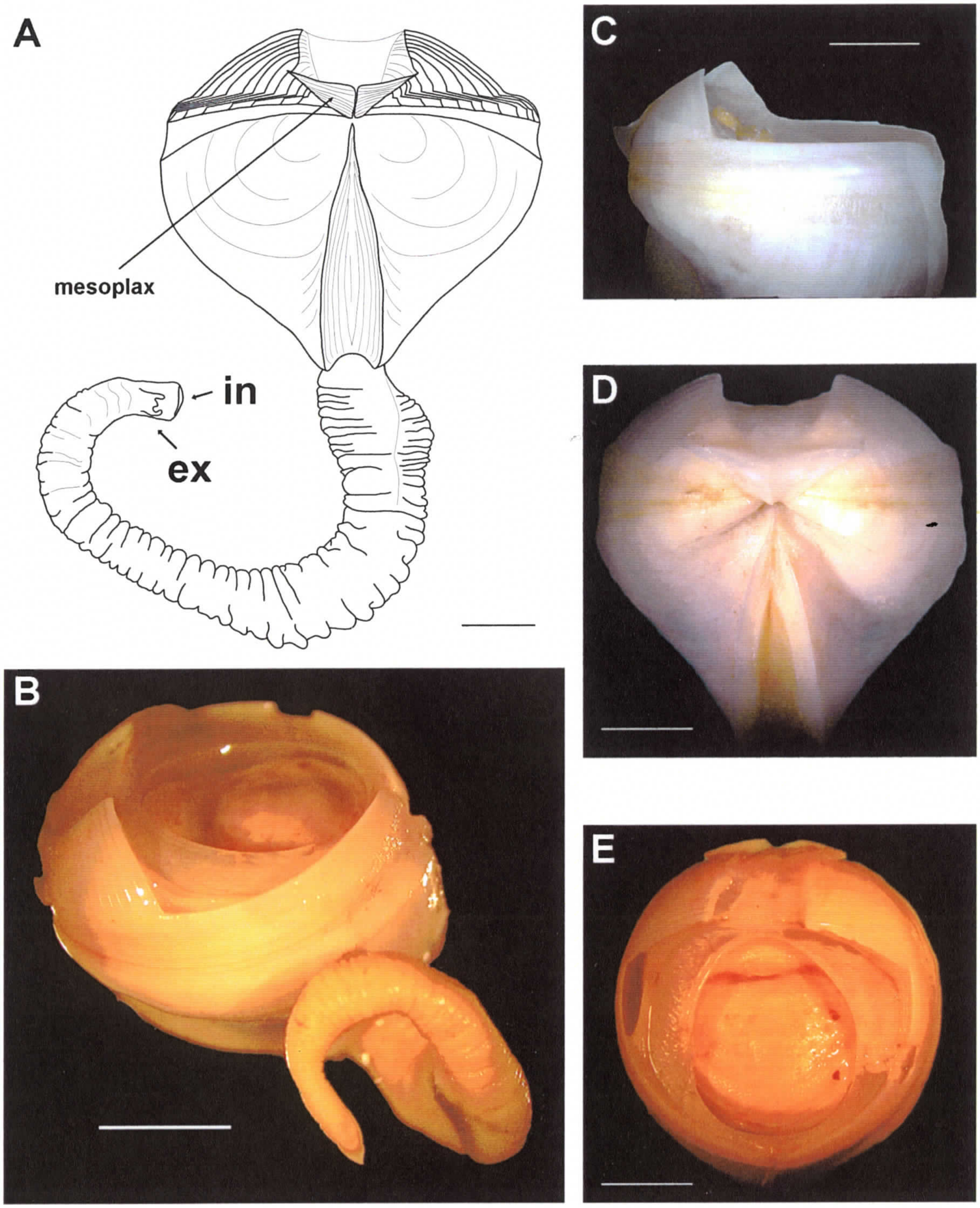


Plate 3: Taxon R [A] Illustration. ex = excurrent siphon, in = incurrent siphon [B] overall body shape [C] side view [D] dorsal view [E] anterior view. Scale bar = 5 mm.

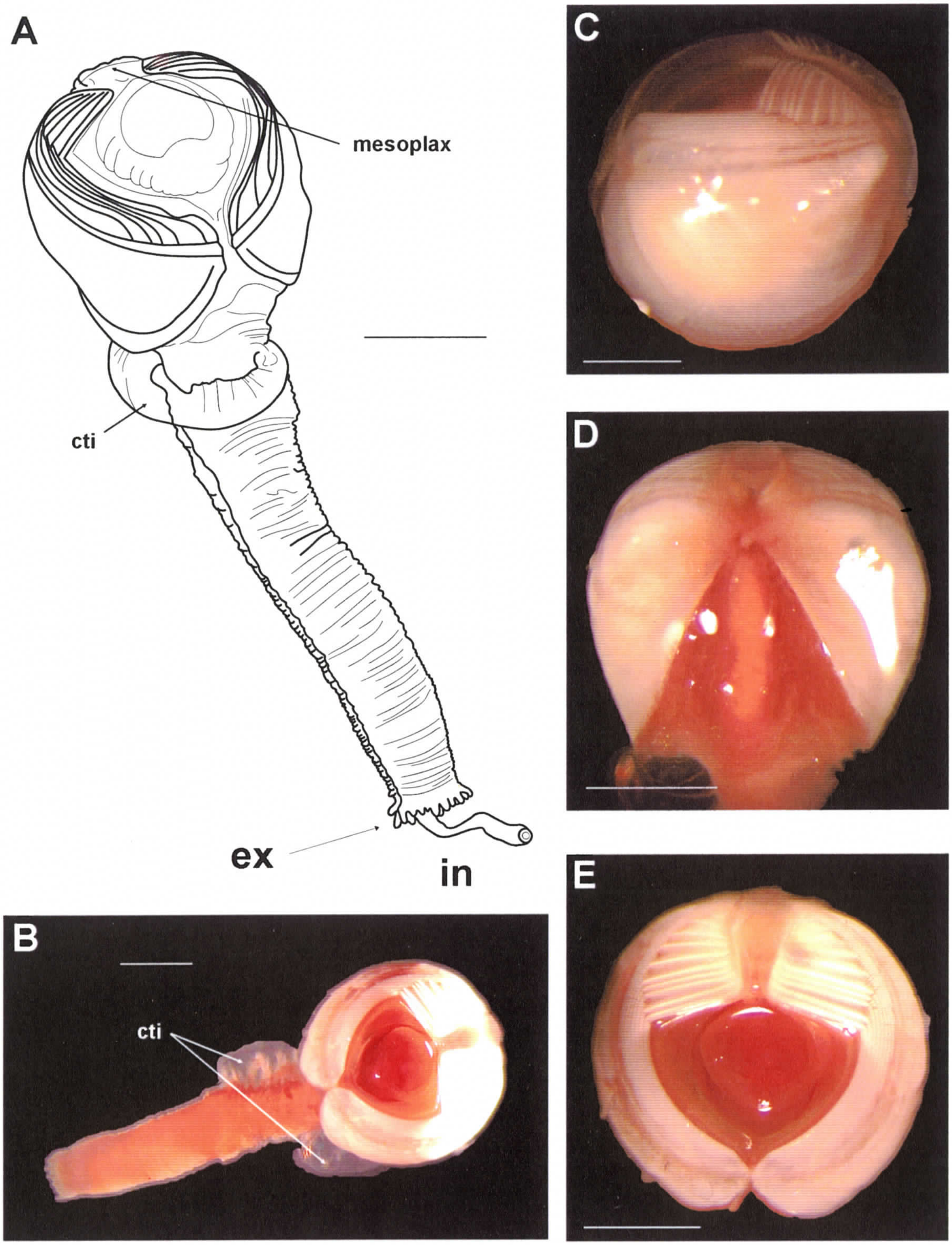


Plate 4: Taxon U [A] Illustration. ex = excurrent siphon, in = incurrent siphon [B] overall body shape [C] side view [D] dorsal view [E] anterior view. cti = clear tissue inflation at base of siphon. Scale bar = 1 mm.

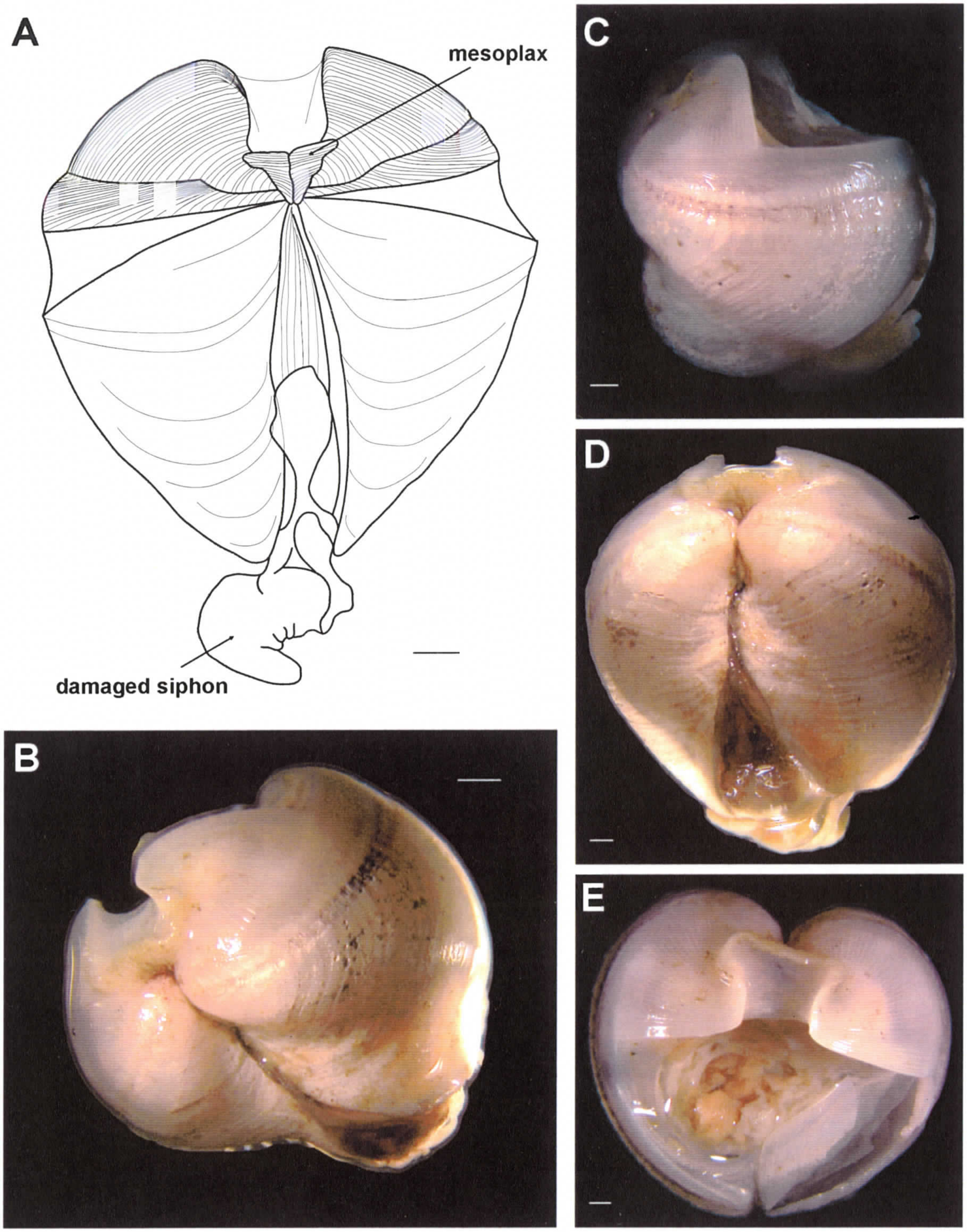


Plate 5: Taxon T [A] Illustration. Note: siphon is missing [B] overall body shape [C] side view [D] dorsal view [E] anterior view. Scale bar = 1 mm.

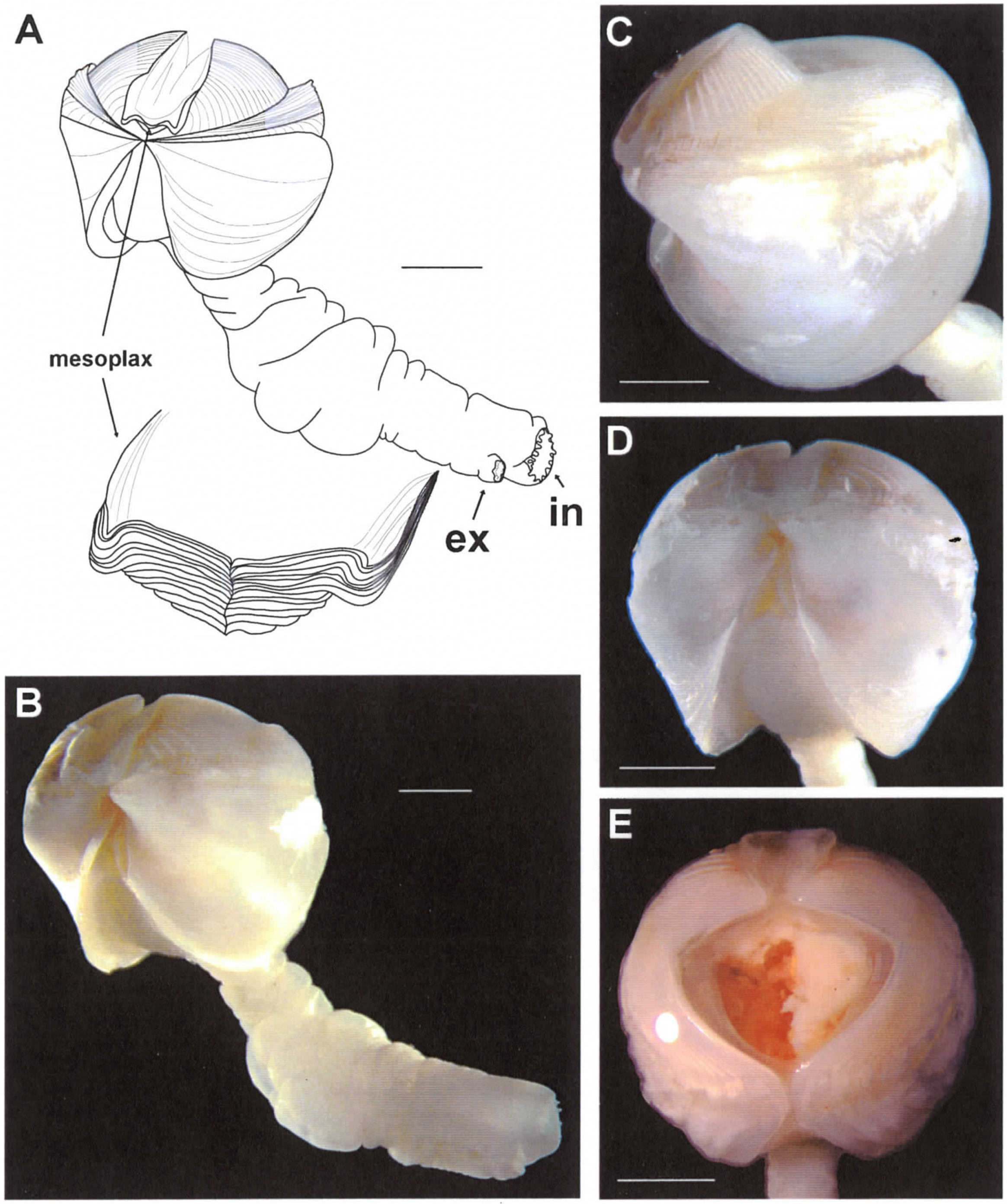


Plate 6: Taxon M [A] Illustration. ex = excurrent siphon, in = incurrent siphon. [B] overall body shape. [C] side view. [D] dorsal view. [E] anterior view. Scale bar = 1 mm.

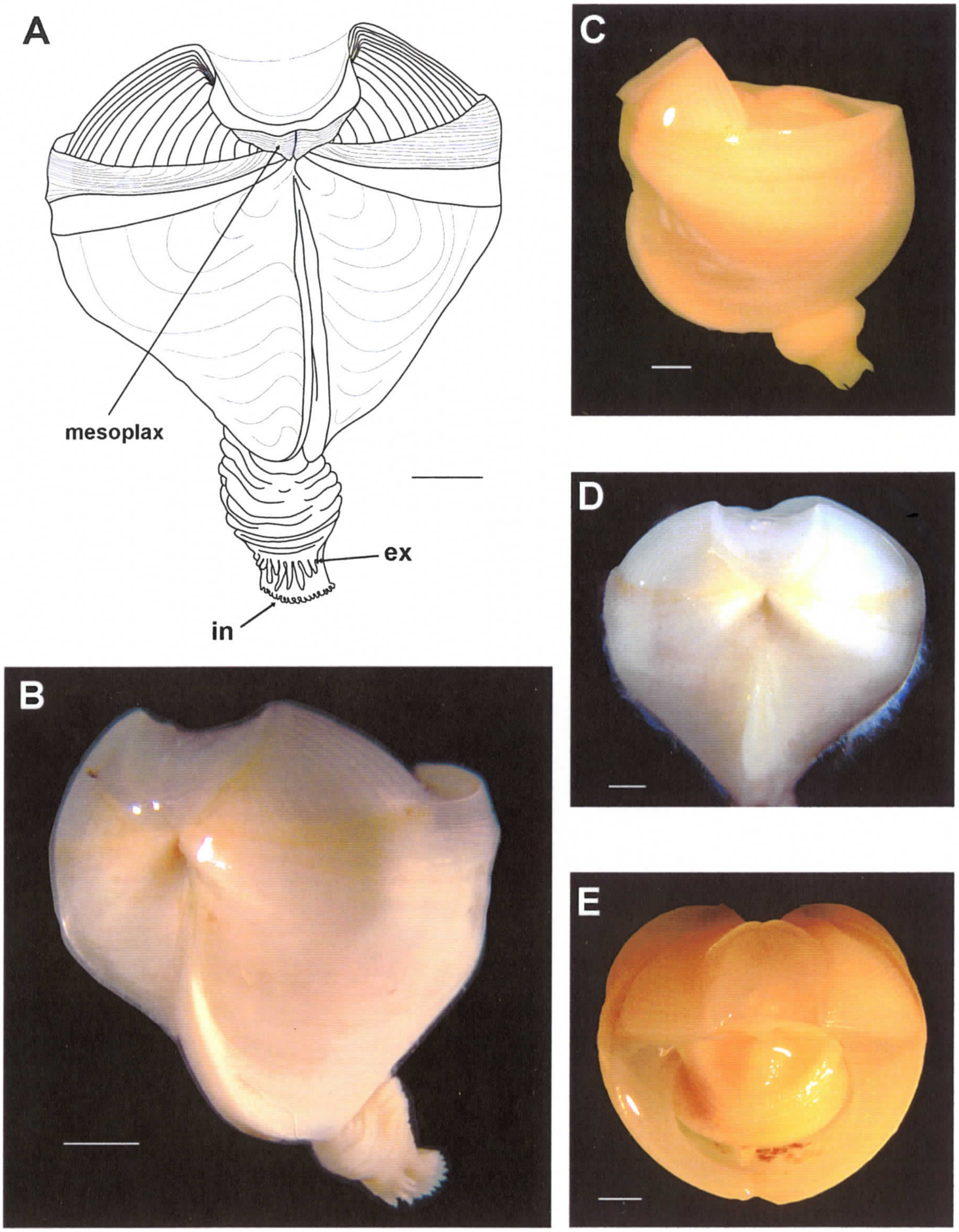


Plate 7: Taxon G [A] Illustration. ex = excurrent siphon, in = incurrent siphon [B] overall body shape [C] side view [D] dorsal view [E] anterior view. Scale bar = 1 mm.

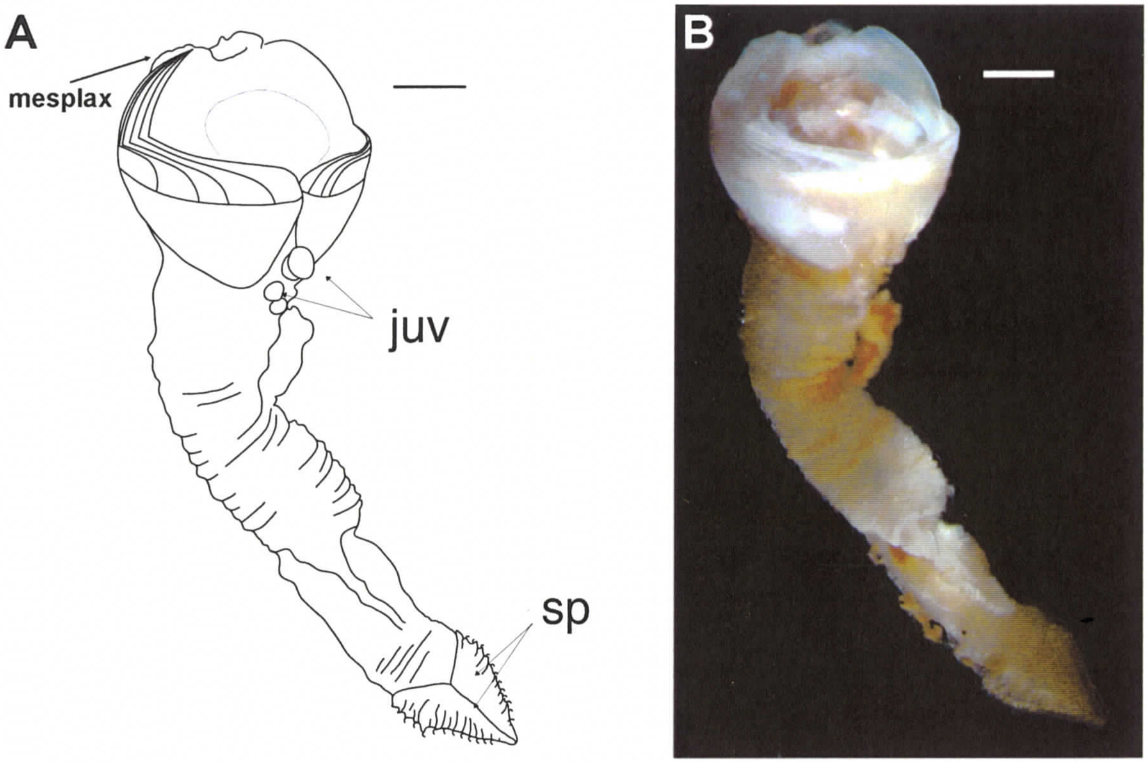


Plate 8: Taxon X [A] Illustration. juv = juveniles attached to outside of shell. sp = siphonal plates [B] overall body shape. Scale bar = 1 mm.

### 2.3.6 Boring Pattern

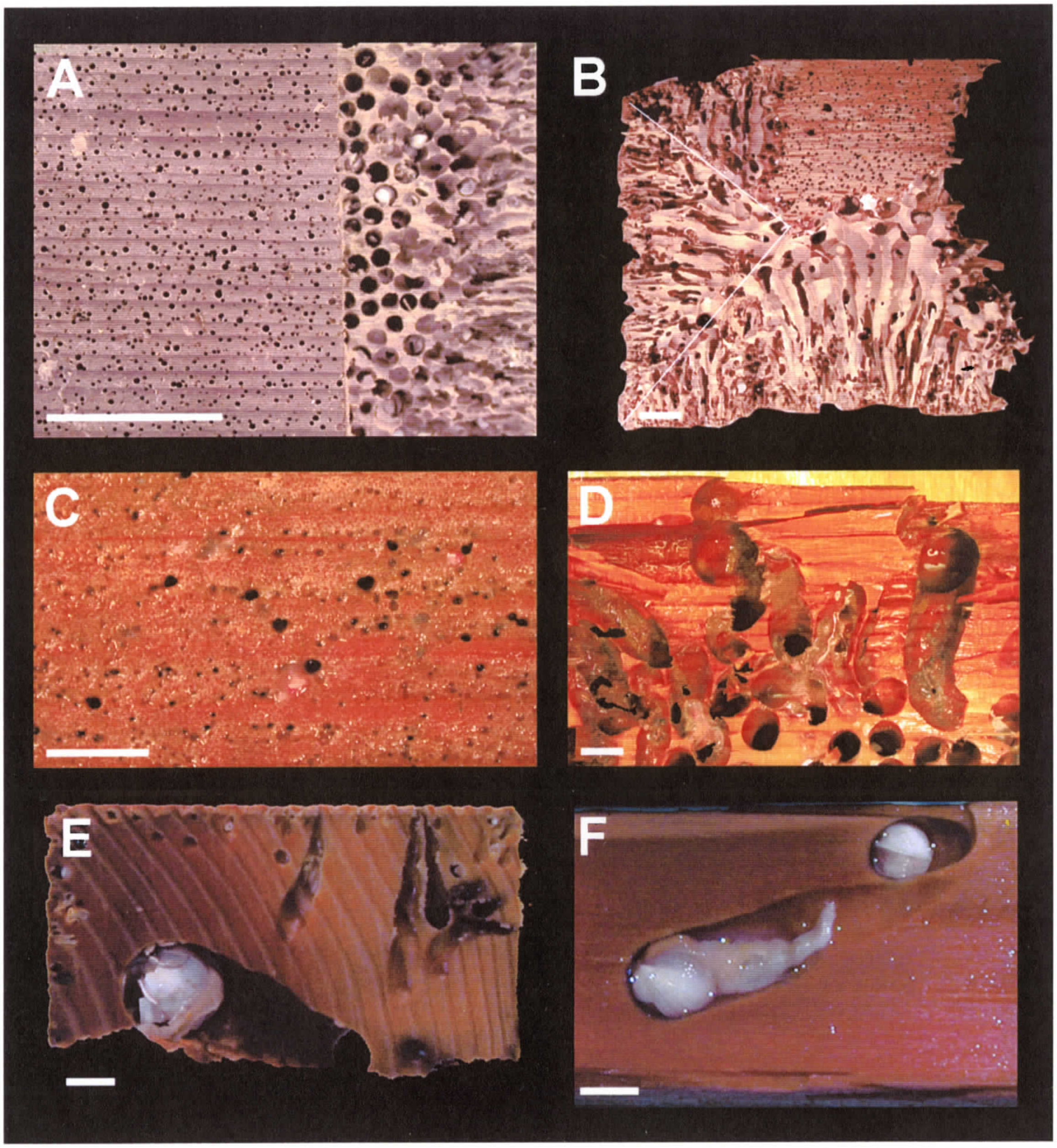
A general, un-tested trend I observed was, that higher borehole densities on the wood surface were loosely correlated with the degree of organization of the boring pattern (Figure 12). High-density wood blocks like the samples from Cleft (WB-A, B) and Axial (WB-E) were highly organized with a 90° boring angle to the outer surface. The burrows were so densely packed, that they regularly broke through into the neighbouring burrow. Taxon F and B are prominent examples displaying this pattern.

Wood blocks with medium densities displayed a less organized boring pattern, where individuals changed their path from a straight line to an S-bend or C-turn, often following the softwood part of the growth ring (Figure 12-D, Figure 13-C). Break-throughs among the burrows are less frequently observed.

In the low-density wood blocks, individuals were found boring into the wood in arbitrary directions, sometimes paralleling the outside surface (Figure 12-F). Taxon R is a prominent example displaying this pattern. I could not discern any obvious boring patterns, yet on some wood blocks with very low densities, aggregates of woodborers could be observed (Figure 13-ABD).

I also observed a correlation between burrow depth and body size with respect to crowding. Individuals from Cleft were heavily crowded (Figure 12-AB). The largest animals with a body diameter of ~ 2 – 3 mm resided ~ 20 mm within the wood. The smallest individuals (~ 0.3 mm) were situated directly below the surface of the wood block, with the intermediates in between. GR-14 on the contrary displayed very low borehole densities (0.8-1.2 bh / $cm^2$). Here I found up to 8 mm wide individuals situated directly below the surface of the wood block (Figure 12-F).

Figure 12: High [A, B], medium [C, D] and low [E, F] woodborer density experimental wood blocks. [A] Cleft (WB-A), left half of the image shows the outer surface, right half of the image shows a layer ~ 6 mm below the outer surface. Note the sharp line between the borers from the right and the borers from the top. [B] Cleft (WB-A), top view of a heavily disintegrated wood block. Note the left-over outer surface in the top right side of the image. Also, note the 90° angle of attack of the wood block, creating a straight, 45° intersection line (white lines) among the borers from the different sides. [C] Endeavour (WB-P), medium density wood block outer surface [D] Endeavour (WB-P), medium density wood block boring pattern. Note S-bend type burrows on the right of the image. [E] Escanaba (WB-N), low density wood block. Note the size difference among the inhabitants of this wood block as well as the arbitrary burrow directions. [F] Escanaba (WB-N), low density wood block. Note the parallel to the outer surface boring direction and the large individual just below the wood surface in the upper right corner. Scale bar = 1 cm. Dominant taxa: [A] and [B] = F; [C] and [D] = B; [E] and [F] = R


A
B
C
D
E
F

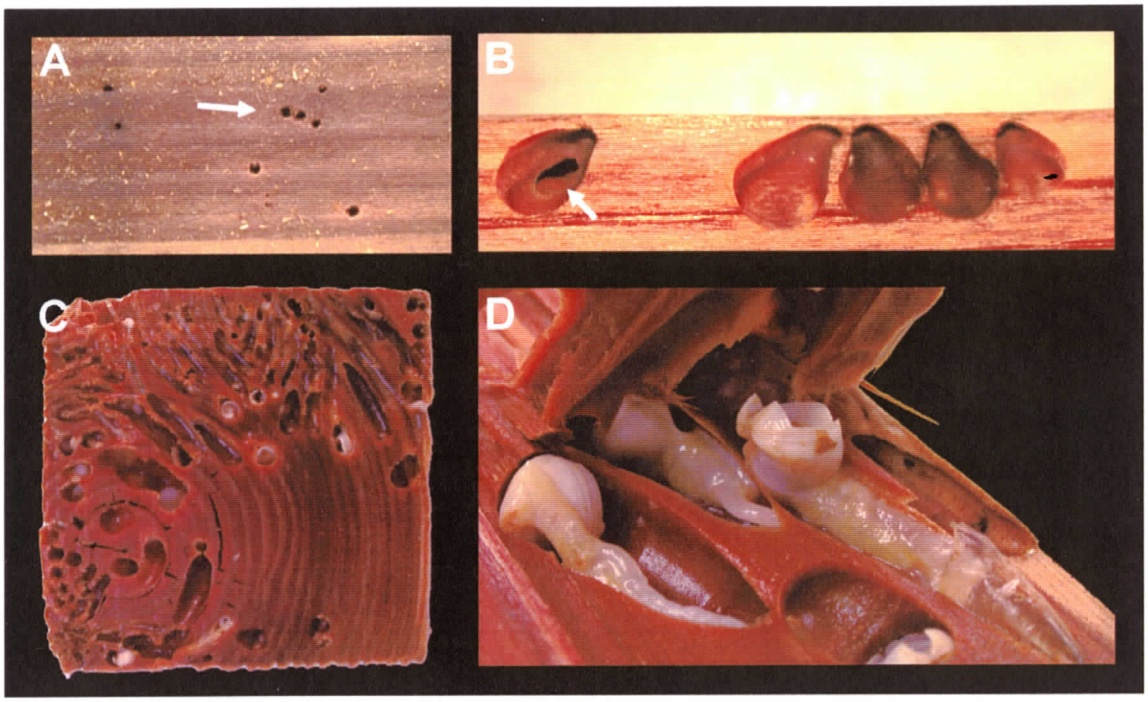


Figure 13: Boring peculiarities [A] Outer surface with aggregation of boreholes (white arrow). [B] Split open wood block. Note the burrow aggregate on the right as well as the breakthrough into the neighbouring burrow on the left (white arrow). [C] Slice through a 4 x 4 in wood block. Note how the inhabitants tend to follow the soft part of the growth ring. [D] Wood block split open showing the woodborers paralleling the outer surface. The burrows in this case are not breaking into one another

### 2.3.7 Borehole Densities

I did not collect borehole density data systematically for all experimental wood block collections. In Taxon F, the maximum density I counted was ~ 65 specimens / $\text{cm}^2$ (WB-A). In Taxon B, the maximum was ~ 50 specimens / $\text{cm}^2$ (WB-P). In Taxon G, the maximum was ~ 29 specimens / $\text{cm}^2$ (WB-O) and in Taxon R (WB-N), the maximum density was 37 / $\text{cm}^2$. Maximum densities for Taxa U, M, T and X are not known.

Borehole density data were systematically collected from a total of 11 wood blocks from four locations. The data are summarized in Table 8. From North to South, Wuzza Bare was the highest with 64 boreholes (bh) / $\text{cm}^2$, Endeavour showed an average of 32 bh / $\text{cm}^2$, GR-14 was the lowest with 1 bh / $\text{cm}^2$ and Escanaba tied with Endeavour at 37 bh / $\text{cm}^2$. The woodborer patterns on the various wood blocks were not equal within and between sites as can be seen from the density value ranges between sites, and standard deviations within sites (Table 8).

Figure 14 shows a 3D plot of averaged boring density data from Wuzza Bare (n wood blocks = 7). The plot shows the woodborers' preference for the top and the upper half of the left and right side. The bottom is not colonized.

Table 8: Summary of borehole density data.

| Site | # blocks | Dominant taxon | Range bh/cm2 | average bh/cm2 | std |
|---|---|---|---|---|---|
| Wuzza Bare | 7 | Q | 14-130 | 64 | 41.9 |
| Endeavour | 2 | B | 10-45 | 32 | 19.4 |
| GR-14 | 1 | G | 0.8-1.2 | 1 | 0.3 |
| Escanaba | 1 | R | 24-49 | 37 | 17.2 |

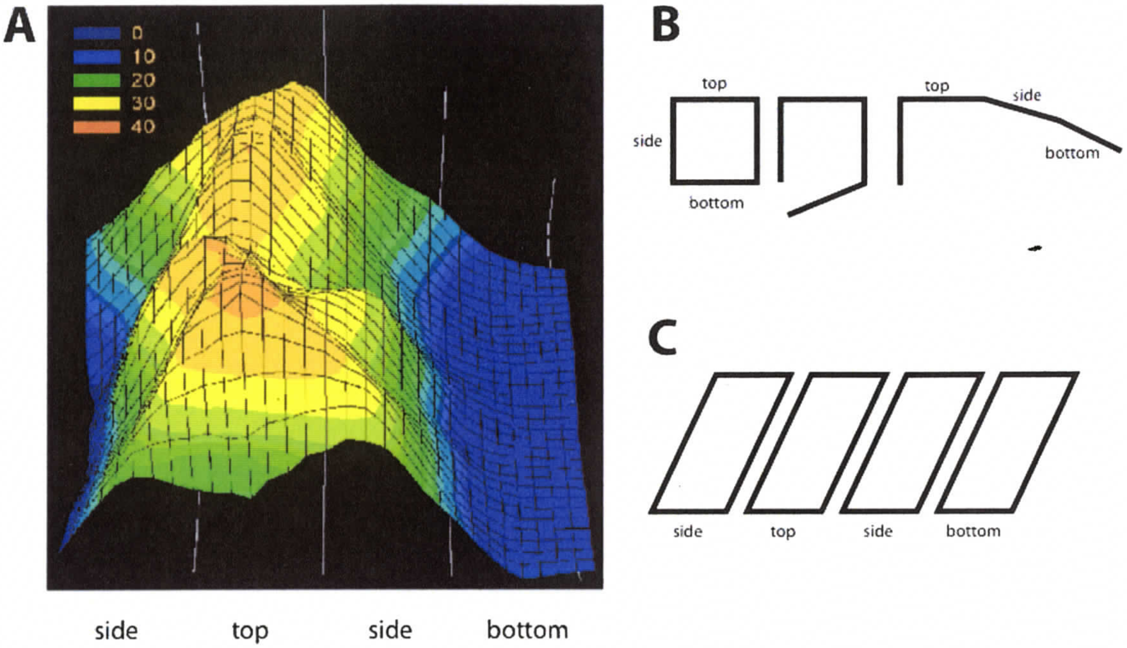


Figure 14: [A] 3D, averaged boring density data plot (seven wood blocks from Wuzza Bare, WB-Q). Legend = number of woodborers per colour. Dark blue = 0; light blue = 10; green = 20; yellow = 30; orange = 40 [B, C] Illustrations explaining the layout of [A]. Maximum boring densities are found on the top and upper half of the sides. Minimum densities (0) are found on the bottom.

## 2.4 Discussion

### 2.4.1 Wood Bioerosion

Upon examination of the wood blocks, observed differences among collection sites were: boring densities, boring patterns, maximum diameter of the clams as well as the depth in the wood block that the clams were located at. The blocks that were not colonized at all (WB-C, WB-K) were not in contact with the sea floor. Wood boring clams settle in highest numbers within the first 20 cm above the sea floor (Turner 1973). Recruitment numbers decline 50 cm above bottom (Santhakumaran 1984). It is likely that the dispersing woodborers could not reach the wood from these two locations, as the blocks were attached > 5 m and > 3 m respectively, above the seafloor. However, their absence does not mean the species are not present. Further sampling is needed for clarification.

Collections WB-D, G and J all showed very few and very small boreholes. This could be attributed to the fact that these wood blocks were the only collections located in or on the circumference of hydrothermally active venting areas: WB-D on Axial Volcano (Crack Vent, ~3 m), WB-G in the Endeavour Mainfield (north of S&M, ~20 m) and WB-J on the Axial Volcano (Ashes Ventfield near Crack, ~ 5 m). This leads me to formulate the hypothesis that woodborers have a low tolerance for a chemically challenging environment such as the one experienced in the proximity of active vents, where reducing compounds are ubiquitous in the superheated waters, ejected from the earth's crust (Haderlie 1983). An alternative explanation could be predation. Hot vents are known to attract mobile predators such as majid crabs (Decapoda, Majiidae) and polynoid worms (Polychaeta, Polynoidae). Although circumstantial evidence exist (Romey et al. 1991; 1994), these hypotheses remain to be tested experimentally.

The remaining collections (Table 4) all yielded high numbers of wood borers. Some of these remaining collections were located on the continental shelf (WB-J1, T1), the continental slope (WB-L, M), the abyssal plain (WB-I, R, Q and H) and in deep-sea troughs (WB-CS, O, N). WB-F, P, E, A and B were all located outside of venting areas (50-100 m). The high numbers further support the hypothesis that vent proximity does not allow for large wood-boring clam populations.

All one-year deployments (except for the two mentioned above) yielded high clam densities. Fast occupation rates and high densities are phenomena previously reported by Turner (1921), Santhakumaran (1980), Haderlie (1956) and Romey et al. (1972; 2002). I cannot say with certainty whether the number of clams in the second year increases or decreases. In some instances (e.g. WB-P) there is an increase in population size, however in others (e.g. WB-B), there is a sharp decrease. The decrease in population size seems to be correlated with wood deterioration. WB-B is such an example. Previously occupied by Taxon F, it is now occupied by a very low number of Taxon U and M. I observed the same succession phenomenon at Endeavour. In 2003 (WB-F), Taxon F dominated over Taxon B. No representatives of Taxon M were present. In 2004 however (WB-P), Taxon B was dominating and Taxon M was present in low numbers. The specimens classified as 'F?' were ambiguous. Hence, it is possible, that Taxon F is a fast recruiter that arrives first on a wood block, but becomes out-competed by Taxon B, M or U due to unknown reasons to date.

Intra-site bioerosion variability among wood blocks is minimal, for any given location. The observed differences are most likely due to touching wood block surfaces and wood block surfaces oriented towards the sea floor, as these surfaces could not be used for settlement.

### 2.4.2 Morphological Characters

The most informative characters to distinguish the taxa morphologically are the incurrent (In) and excurrent siphon (Ex) cirri presence or absence. It is likely that these two characters are not independent as there is no case where one of the siphons has cirri, when the other does not. The relative length of the excurrent siphon in relation to the incurrent siphon (Ex Siphon) was informative in most cases but had considerable variation in Taxon F. Here the excurrent siphon ranged from subterminal (just below the incurrent siphon) to only one third the length of the incurrent siphon. The mesoplax build (Mesoplax) and mesoplax shape (M shape) were also useful, although the interpretation of the stage of calcification (light or strong) is subjective. The same holds true for the shape of the mesoplax. The difference between a shallow (1) and a steep (2) triangle was obvious but there were multiple degrees of eyebrow-shaped (0) mesoplaxes in the collection.

Dr. Voight is currently working on the species descriptions. If I were to do the official species descriptions, I would, in addition to the characteristics described above, analyze internal shell characters such as the umbonal ventral ridge (Figure 4-E-11), the anterior and posterior muscle scars (Figure 4-A,E-10) as well as the beak (Figure 4-BC-2) and umbonal reflection (Figure 4-C-3). Workers used all or a subset of these characters to describe species of the Xylophagainae (Knudsen 1961; Turner 1972; Maser and Sedell 1994; Turner 2002).

### 2.4.3 Morphological Taxa

Due to one or two missing and/or ambiguous characters, a few specimens could not be confidently assigned to one of the newly established taxa. The reasons for the missing characters were poor preservation, lysed tissues due to freezing and thawing, mechanical destruction during the extraction process, or the miniscule size of the specimens. I allocated those specimens to the morphological taxon most closely resembling the present characters. These specimens were highlighted by a '?' following the taxon letter.

One of the two additional morphological taxa in my collection, Taxon T, stems from an opportunistic bottom trawl off Ucluelet, BC. Dr. Voight does not have any specimens of this taxon in her collection. Very few individuals represent the second additional morphological Taxon M. Dr. Voight had noticed them in her collection, but disregarded these as 'heavily stressed' individuals of Taxon F. Based on the morphological characters I used (.

Table 6), I have to disagree with Dr. Voight's classification and keep the morphological Taxon M separate for now.

Dr. Voight and I agree on the morphological Taxa B, F, G, R, U and X. Taxon X is most easily distinguished as it falls into a different genus, *Xylopholas*. The defining character of the genus, the siphonal plates, are clearly visible in the digital photographs and highlighted in the illustration Plate 8. I found Taxon X in three locations (Wuzza Bare, GR-14 and Escanaba) in very low total numbers (N=14). A characteristic of the siphonal plates of the genus *Xylopholas,* are cat-like hooks. I believe these hook-structures could have led to a sub-sampling artifact, where the siphonal plates were separated from the individual and remained in the burrow during the extraction from the wood. I could not discern the remaining characters due to the small size of the specimens or, if visible, they were too similar to other morphological taxa.

Furthermore, I have been unable to assign any of the newly discovered morphological taxa to described species. This might be because many species descriptions are minimalistic and do not provide enough morphological detail to discern clearly, newly discovered taxa.

I found Taxon B at five locations (Hecate Strait, Wuzza Bare, Endeavour, Axial, Santa Cruz) and in high numbers. Of all morphological taxa, this taxon resembles the species description of *X. washingtona* most. According to Dr. Voight, however, there are minute differences separating the two. There are no ambiguities (as elaborated upon previously) regarding this taxon – it forms a distinct grouping of specimens.

Taxon U was found at ODP1026, Endeavour and Cleft. The flowerlike arrangement of the incurrent and excurrent siphons of these small animals (< 2 mm) is very distinct and unambiguous.

Taxon R was restricted to the Gorda Ridge, occurring at GR-14 and Escanaba only. Their siphonal characteristics and the unique mesoplax build made distinction of this taxon straightforward. Damaged specimens that were missing siphonal characters or the mesoplax were ambiguous, leading to a few specimens classified as R?.

Taxon F occurs in four locations (OPD1026, Endeavour, Baby Bare, Cleft). Their siphonal characters are very distinct from other taxa. Some ambiguous specimens were classified as F? at Cleft and Endeavour.

Taxon G occurred at Wuzza Bare and GR-14. Their large size together with very short siphons rendered them easily distinguishable. No ambiguities exist in this taxon.

The *coded* characters I used to distinguish the morphological taxa were not specific enough to distinguish Taxa F and G. The additional character called 'other' was crucial for the distinction of these two taxa, since morphologically, the siphons of Taxon F and G are similar in that they both have short incurrent siphon cirri and long excurrent siphon cirri (Table 6. Figure 9). The main difference is the body size of Taxon G compared to its siphon length versus the body size of Taxon F compared to its siphon length. Taxon G has a short siphon (~ 0.7 x body diameter). Taxon F has a long siphon (< 8 x body diameter). It is possible that the short siphon of Taxon G is simply retracted. Wrinkles on the siphon support this hypothesis (Plate 7). While analyzing the morphological characters of these two taxa, I also compared the features with the species description and illustrations of *Xylophaga muraokai* TURNER (Newell 1979). Based on the geographic distribution of *X. muraokai* (California, USA) and the similarities between Turner's illustrations (Deburgh and Singla 1984) and my own observations, I hypothesized that Taxon F represent juveniles of Taxon G and Taxon G could be *X. muraokai*. I discussed this idea with Dr. Voight who assured me that Taxa F and G are morphologically different. Both taxa are furthermore morphologically different from *X. muraokai.*

Taxon Q constitutes a group of individuals from two locations (Wuzza Bare, GR-14) that are very similar to each other. I was not able to confidently assign these specimens to any of my morphological taxa, due to missing and/or ambiguous characters.

Throughout the study, I did not distinguish the life stages of the animals under examination. One reason for making distinct morphological taxon attributions could result from looking at different life stages (juveniles versus adults). Failure to distinguish different life stages of a single taxon could lead to formation of multiple taxa. This is especially possible for morphological taxa F and G, as previously discussed. One hypothesis is that Taxon F is the juvenile stage of Taxon G. The genetic analysis might reveal the relationship between the two taxa. Unfortunately, a benchmark sequence for *X. muraokai*, does not exist. The possible assignment of Taxa F and G to *X. muraokai* must be addressed in the future.

*X. muraokai* and *X. mexicana* are the closest species, geographically, to my study area. Dr. Voight and I have both come to the conclusion that neither of our morphological taxa R, U, M and T represent either *X. muraokai* or *X. mexicana*. I previously elaborated on the case of Taxa F and G.

Turner (1991) proposed six species categories, based on morphological mesoplax and siphon characters. I was not able to divide my morphological taxa into these categories. The definitions of Turner's categories are too vague at times, as to allow for the allocation of specimens with uncertain character states. Ambiguous character states such as missing siphons, missing mesoplaxes, etc. were frequently encountered in this study.

In summary, all eight morphological taxa are distinguishable from each other and, cannot be associated without a doubt to any described species. There are a few specimens that lack all necessary characters to associate them with an existing morphological taxon. These specimens, about 5% of the total examined, were marked with a '?'. Furthermore, the association of Taxon Q needs further clarification. In Chapter 3, I explore their identity further, by analyzing the 18S rDNA and the Cytochrome *c* Oxidase 1 genes.

### 2.4.4 Morphological Taxon Distribution

Distributions of the morphological taxa are summarized in Figure 10. Taxon B is the most widespread in this study, occurring at the North-most collection location (Hecate Strait (WB-J1)) and the South-most collection location (with Santa Cruz (WB-CS)). I did not find any specimens of Taxon B on the Gorda Ridge. The Gorda Ridge sites were also among the deepest locations in this study, potentially excluding Taxon B (and others), while creating a niche for Taxon R, which is exclusively found on the Gorda Ridge.

Taxon F is ubiquitous in the Cascadia Basin and on the Juan de Fuca Ridge, often sharing locations with Taxon B. According to Dr. Voight, Axial yields Taxon B and F. In my collection (WB-E) I could not find any representatives of Taxon F.

The distributions of taxa U, T, M, X and G do not present a discernable pattern. It is important to note that, despite 11 sampling sites, the study area is vastly undersampled. Distribution patterns remain purely speculative at this point.

### 2.4.5 Boring Pattern

I observed a loose correlation between borehole densities and burrow pattern, ranging from organized to unorganized.

Low-density borehole wood blocks occasionally display burrow aggregates, where 3 - 5 individuals initiated boring in close proximity to one another, boring away from each other, while inching deeper into the wood (-CD). My interpretation of this observed pattern ranges from coincidence to the possibility that larvae might like to associate upon the initiation of boring. There might be an advantage to knowing the exact position of your immediate neighbours upon boring initiation, rather than 'stumbling' upon their burrow once inside the wood block.

In high-density woodblocks, crowding may exert a boring pressure on the inhabiting individuals. Individuals possibly perceive their neighbours, which makes them bore deeper to gain access to a secure spot deep within the wood block. Following the morphological classification, I now know that the individuals from Cleft and GR-14 do not fall into the same morphological taxon. The differences in boring habit (deep, straight (WB-A,B; Cleft) versus shallow arbitrary (WB-G; GR-14)) might be the result of an occupation of a different microniche on the wood blocks. This micro-niche remains to be proven, examined and characterized.

Knudsen (1983) reported an "upper bore-hole sharing" phenomenon, where multiple individuals extend their siphons into a communal excurrent chimney. Similar to his findings, I did not detect any soft-body tissue damage that would indicate any combative behaviours among the inhabitants.

From the Teredinidae, we know that their need to grow causes them to bore continuously. The boreholes of a species from the Northeast Pacific (*Bankia setacea*) can reach diameters of 22 mm and 1.8 m in length (reference in Turner 1973). Not all carved wood is ingested but flushed out of the burrow as wood chips. I observed some taxa of the Xylophagainae (morphological Taxa F, B and T) with a wood-chip lining of the burrow, while others did not show this character. The wood chip lining could be a result of the above-mentioned boring competition among individuals. Faster boring creates a surplus of wood chips. An alternative explanation lies in the poorly understood feeding habits of the Xylophagainae. Certain members of the Xylophagainae may have a symbiosis with cellulolytic bacteria, similar to the Teredinidae. An alternate scenario might be that bacteria are 'farmed' exterior to the animal body. The wood-chip lining could represent culturing grounds for bacteria, which, in turn, could be harvested by the woodborer. I propose this hypothesis based on: a) the fact that bacterial harvesting is known from the Mollusca (*Hydrobia ulvae* (Turner 2002); endocytosis of bacterial cells in *Lepetodrilus fucensis* (Bock 2004)) and b), fundamental siphonal character differences among the morphological taxa with respect to siphonal cirri. Cirri are fleshy appendages, located at the incurrent and/or excurrent siphon openings. In my mind such fingerlike appendages could be used for selectively choosing food items. No data exist that could prove or disprove this theory to date.

### 2.4.6 Borehole densities

For density measurement purposes, I am assuming that one borehole equals one woodborer. However, observations in this study, and the literature, suggest that woodborers can share a common entry hole on the wood surface under crowded circumstances. Thus, density estimates are minima.

There exist obvious differences in borehole densities among locations. Wuzza Bare, a location in the Cascadia Basin, representing abyssal plain, produced the highest borehole counts (max. 130 / $cm^2$). Endeavour (max. 45 / $cm^2$), GR-14 (max. 1.2 / $cm^2$) and Escanaba (max. 49 / $cm^2$) are all located on ridge areas. A wood deployment, which targeted hydrothermal venting at an active area at Axial (Crack Vent, WB-D), yielded very few woodborers. The maximum density counts at Wuzza Bare are extremely high (> 1 clam / $mm^2$). High density values had been reported previously by Romey et al. (< 30 / $cm^2$) (2003), Haderlie (< 50 / $cm^2$) (1995) and Turner (< 150 / $cm^2$) (2002).

The density distribution over an experimental wood block was not surprising, with the highest numbers on the top surface, sharply declining, when moving down the side surfaces and the lowest number on the underside of the wood block. To date, we only know the dispersal method of two species of deep-sea woodborers (*X. washingtona* and *X. atlantica*). Whatever the method of dispersal, and consequently recruiting a 'fresh' piece of wood, may be, the woodborers seem to favour the top surfaces that are in close proximity to, but not in direct contact with, the sea floor. Potential reasons for this could be: access to oxygen-rich waters, reduction of sediment particles filling up the burrows due to micro-turbulences or ease of attachment upon arrival on the wood, just to name a few. Figure 8-AB shows wood blocks, heavily populated by wood-boring clams. The white 'fur-lining' of the blocks constitutes hundreds of extended clam siphons. The reason for their extensions has not been examined. Choosing the topside, however, would allow access to suspended particles in the water column.

My data derive from four locations only and I cannot say whether density differences stem from the location-specific variables or can be attributed to the presence of different taxa. This phenomenon remains to be studied.

### 2.4.7 Summary

The encountered morphological taxon richness came as a surprise given that, to date, only one species (*Xylophaga washingtona* Bartsch) had been known and described from the Northeast Pacific. Since phenotypic plasticity is reported in the Xylophagainae (Sokal and Crovello 1970), the first step is to confirm whether the encountered morphological diversity described above, in fact represents species richness, and not phenotypic plasticity within species.

Subjecting a subset of the specimens from all morphological taxa to the same treatment and classifying the outcome gives an independent perspective. If the resulting classes match the previously established morphological taxa, an independent variable confirms the first classification. The uniform treatment I used was to extract and amplify two genetic markers, the Cytochrome *c* Oxidase 1 gene (CO1) and the 18S rDNA gene. Chapter 3 elaborates on the outcome.

## Note in Proof

Subsequent to the completion and defense of this work, a re-examination of the CO1 data in Chapter 3 revealed that it is unlikely to be of acceptable quality for citation. The extracted DNA may not be that of the bivalves in question.

# Chapter 3: Genetic Analysis

## 3.1 Introduction

I have shown the existence of eight new morphological taxa (Chapter 2). The goals of the following work are to a) use two independent genetic markers to assess the intra-morphological-taxa genetic variation and elaborate on their 'species status' (see below: Species Concepts) and b) to investigate the phylogenetic relationships among the potentially new species.

I am collaborating with the Consortium for Barcoding of Life (CBOL), which uses the CO1 sequence as a unique identifier for the world's species. I am furthermore presenting 18S sequence data for selected specimens from the Northeast Pacific.

I am using the 18S sequence data alone and in conjunction with CO1 sequence data, to investigate the phylogenetic relationships among the deep-sea woodborers.

### 3.1.1 Species Concepts

The existence of 'species' has long been recognized. The definition of the word 'species' however, remains problematic. The source of confusion resides in the distinction of the **species concept**, **species category** and the **species taxon**. The **species concept** is part of evolutionary theory and consequently independent of systematic theory (Van Halen 1976). The **species category** is the basic level recognized in the Linnaean hierarchy (species, genus, family, etc.). The **species taxon** is derived from the species category. It represents a taxonomic group at the level of the species category. They are never defined but rather recognized and delimited with a description, allowing workers to identify other individual organisms as members of this species taxon.

Most workers assume the species concept (e.g. biological species concept) to be the species category. This is not the case however, since the species taxon must be applicable to all organisms, including asexual ones. The biological species concept for example, applies to sexual organisms only.

Workers tend to not disclose the particular species concept employed in their studies, rendering the interpretation of the species somewhat arbitrary (Simpson 1961; Templeton 1989; Wiley and Mayden 2000). Consequently, for this study, a clear definition for the species taxon and its underlying evolutionary species concept are necessary.

### Similarity Concepts

The Phenetic Species Concept (PhSC) (Mallet 1995) in its most extreme form, uses phenetic resemblance as the sole species criterion. The PhSC is rarely used today and heavily criticized on numerous grounds. Due to the lack of an objective measure of total similarity, different sexes or ontogenetic stages could easily be assigned into different species. A phenogram, even if correctly representing phylogenetic relationships, will display a nested hierarchy of organisms. The problem of deciding, what level of clustering corresponds to the species rank, remains. In the case of DNA data, one could envision a certain level of divergence (e.g. 10%) corresponding to the species rank, however this is not practical since different genetic markers will result in differing similarity scores for the same organism. The statement: ‘Organism X belongs in species Y’ is consequently un-testable. Under a very generous interpretation of the PhSC, most life on earth could be considered one species, whereas under a very restrictive interpretation of the concept, every organism could be considered a separate species.

### Cohesion Concepts

The following concepts all use cohesion as the major criterion to delimit the species rank. A species in these cases is a group of organisms sharing features that cause them to remain coherent entities through time. The cohesion mechanisms can either be present in each individual (e.g. because of shared developmental homeostasis or a common ecological niche) or because of interactions between individuals (e.g. gene flow). If cohesion is the result of gene flow, the cohesion species concepts will demarcate the same species as the interbreeding species concepts (see below). Examples of species concepts from this category are the Ecological Species Concept (EcSC) (Mayr 1940), the Cohesion Species Concept (CSP) (Mishler and Brandon 1987), the Evolutionary Species Concept (ESC) (Lee 2003; Bock 2004) and the Genotypic Cluster Species Concept (Lee 2003). Similar to the shortcomings of the similarity concepts above, the cohesion concepts share the problem of a lack of objective criteria for the demarcation of species. “‘Organism X belongs in species Y’ is unfalsifiable, since the boundaries of species Y can be moved arbitrarily by subjective changes in the amount of cohesion appropriate for the species rank" (Mayr 1940, p.181f). As a consequence of the above quote, under the cohesion concepts, there is no objective criterion that universally demarks the species category. Consequently, the species recognized in the literature do not have properties qualitatively distinguishing them from other entities, whether infraspecific (e.g. sub-species) or supraspecific (e.g. genus, family, .. , phylum).

### Monophyly Concepts

Under the Monophyletic Species Concept (MSC) (Mayr 1942) all species must be monophyletic entities. Since multiple levels can be present in a monophyletic clade, other criteria must be employed to demark the clades that should be assigned the species status. Such criteria are: the amount of support for the clade, the number of synapomorphies (shared derived character), the geographical distribution, phenotypic, genetic and ecological distinctness ('cohesion'), geological age and their biological importance. Yet, the problem of where to draw the line remains. The criteria for assigning the species status remain vague, and more problematically, also characterize higher taxa (Dobzhansky 1937). Recent work, advocating the recognition of only monophyletic basal taxa, even emphasizes that these do not differ from other clades and thus should not be called species (Rossello-Mora 2003).

### Interbreeding Concepts

Interbreeding species concepts use interbreeding to demarcate the species rank. Under these concepts, species at a given point in time are groups of organisms that interbreed with each other but are reproductively isolated from other species (e.g. traditional Biological Species Concept). Like all other concepts (similarity, cohesion, monophyletic inclusiveness), interbreeding is not a discrete, but continuous variable. Two taxa can be totally panmictic, totally isolated and everything in between (Bock 2004). Multiple variations of Mayr and Dobzhansky's BSC (Bock 1986; Avise 2000; Bock 2004) were published over the years and discussed (e.g. Hebert et al. 2003). Bock (1991) recently stated a flaw in the original postulation of the BSC, namely that reproductive isolation serves as the demarcating character among species. Fact is, that species can be reproductively isolated, which renders them genetically isolated, the inverse however is not true. Genetic isolation does not necessarily mean reproductive isolation. The horse and the donkey are genetically isolated, but reproduce when given the chance (Hillis and Dixon 1991). Bock modifies the BSC as following: "A species is a group of actually or potentially interbreeding populations which are genetically isolated in nature from other such groups." (Hillis and Dixon 1991).

### 3.1.2 CO1

The Cytochrome *c* Oxidase 1 gene (CO1) is a mitochondrial candidate gene. It codes for the first subunit of the terminal enzyme of the respiratory chain that reacts with oxygen to create energy. Mitochondrial genes are maternally inherited. From generation to generation, random mutations occur, some of which become fixed in the population. DNA-specific repair mechanisms are not 100% effective and do not 'correct' all mistakes in the changed sequence. It is these differences in mitochondrial gene sequences that allow us to distinguish same from different.

This system is comparable to the male driven last-name inheritance process in certain human societies (Hillis and Dixon 1991). Here individual letters of the last name change and get passed on to the next generation. Point mutations in mitochondrial genes, especially in the third position of the codon, are rarely detrimental and in many cases do not affect the enzyme's performance. Its function as such, even if affected, is not as integral as e.g. the transcription-responsible 18S gene.

In contrast to this commercial barcode, known from e.g. grocery stores, distinctive DNA barcodes are generated through the accumulation of random mutations between reproductively isolated groups. With a 2% per million year rate (Brown et al. 1972) of sequence evolution, a 600 bp segment of DNA will provide 12 characteristic nucleotide differences between two species, given these have been separated for one million years. As with UPC codes, the DNA barcode approach requires a database, linking the unique barcode to a particular species name, previously described through traditional taxonomic approaches.

Mitochondrial DNA is a good candidate for this work as it occurs in multiple copies per mitochondrium, of which there are hundreds per eukaryotic cell. The high number of copies makes the DNA extraction process more likely to be successful.

### 3.1.3 Ribosomal DNA

The comparison of ribosomal DNA (rDNA) sequences has been used to infer phylogenetic history on numerous levels, from the basal lineages of life to relationships among closely related species or populations (Hillis and Davis 1988). rDNA constitutes a set of genes encoding ribosomal RNAs (rRNA) that are responsible for the direction of protein synthesis from messenger RNA (mRNA). Ribosomes are composed of two subunits, with each comprising distinct rRNAs and ribosomal proteins. The rDNA array of a eukaryote nuclear genome consists of one to numerous copies of a transcription unit, separated by non-transcribed spacers (NTS). The transcription unit consists of an externally transcribed spacer (ETS) followed by the small rRNA subunit (18S), the 5.8S and the large subunit (28S). Two internal spacers (ITS-1 and ITS-2) separate the 18S and 5.8S and 5.8S and 28S respectively (Figure 15). The different regions evolve at different rates. As a consequence, for any systematic question, a specific region can be targeted that yields the resolution needed. Islands of highly conserved basepair sequences are essential for the creation of universal primers, applicable across taxa. (Baverstock and Moritz 1990). Generally, the best DNA regions for phylogenetic studies, are those that are more than 70% but less than 100% similar among taxa (Giribet and Distel 2003).

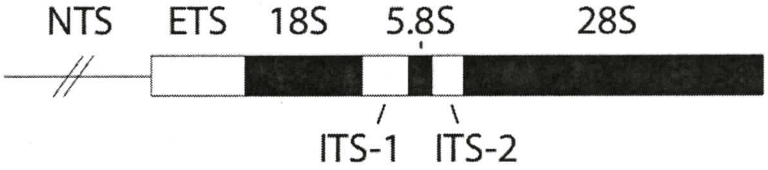


Figure 15: rDNA array of a eukaryote. NTS = non-transcribed spacer separating the different copies of the array; ETS = externally transcribed spacer; 18S = small ribosomal subunit; ITS-1 and 2 = internal spacers; 28S = large ribosomal subunit. Modified after (Parker et al. 1998)

### 3.1.4 18S

The 18S rRNA gene is an approximately 1800 bp gene. Together with 30 ribosomal proteins, they constitute the eukaryotic small ribosomal subunit. Protein synthesis is a fundamental life property. Consequently, rRNAs are universally present, in all living systems. Much of the 18S gene sequence is conserved across taxa, which renders it useful in determining phylogenetic relationships. This gene is among the slowest evolving sequences found in living organisms; and studies of high-level phylogenetic relationships are possible. The mechanism for this 'slowness' was unciear, until the accumulation of 18S sequences in large databases allowed for across-taxa comparisons. While comparing ribosomal RNA of *Xenopus laevis* and *X. mulleri*, Brown et al. (2006) noticed that the multiple copies of the gene were not evolving independently. Each copy of an rRNA gene is usually very similar within individuals and species, with differences only accumulating in certain parts of the gene. This low variation within individuals and species indicates that the multiple copies are homogenized. This process is called 'concerted evolution'. Concerted evolution affects phylogenetic analyses. For example, intraspecific variation is greatly reduced compared to what would be expected based on interspecific variation. This makes it possible to use small sample sizes (Chomczynski and Sacchi 1987; Folmer 1994), which is convenient, in the field of deep-sea biology, where samples are scarce (Chapter 1).

Over the past forty years, a substantial database of 18S gene sequences from a wide variety of organisms has accumulated and is available for download from the world wide web. Currently, there are 110216 published 18S sequences on the NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide). In the same database, there are five 18S sequences from the family Pholadidae, only one of which is from a member of the subfamily Xylophagainae (*Xylophaga atlantica* [AY070123]). Its sequence was published in 2003 as part of a larger molluscan study (Giribet and Distel 2003). It serves as the benchmark for the following work.

Within taxa, the 18S gene sequence is mostly highly conserved. This makes it a suitable candidate for higher level phylogenetic work from the genus level up and less suitable for the distinction of species (Giribet et al. 1996).

### 3.1.5 Hypotheses

**$H_0$**: The morphological taxa all possess identical CO1 and 18S sequences.

**$H_{A1}$**: The morphological taxa all possess unique CO1 *and* unique 18S sequences that clearly distinguish them from one another.

**$H_{A2}$**: The morphological taxa all possess unique CO1 sequences but do not possess unique 18S sequences.

$H_0$ implies that neither the 18S nor the CO1 gene sequences represents useful genetic markers for the distinction of the species of woodborers of the Northeast Pacific or, that the morphological diversity encountered represents phenotypic plasticity within one woodborer species. In this case a re-evaluation of all morphological features as well as additional genetic markers would be needed.

The implications of $H_{A1}$ are that the eight morphologically distinguishable taxa represent eight undescribed species from the Northeast Pacific. This would increase the number of known species in the Northeast Pacific by 900% from one to nine.

$H_{A2}$ implies that the unique CO1 sequences support the species status of the morphological taxa. The 18S data would support subfamily and genus level relationships, but the 18S gene does not provide a high enough evolutionary resolution to distinguish the morphological taxa on a species level.

## 3.2 Materials and Methods

### 3.2.1 Samples

**CO1 sequencing:** I sent 260 tissue samples from deep-sea woodborers from 11 locations (Figure 16) to the Barcoding of Life consortium (CBOL) at the University of Guelph, ON, Canada. The tissue samples originate from clearly identified morphological taxa, as well as samples that could not be associated with any of the newly established morphological taxa, earlier marked by '?' (Chapter 2). See Table 9 for details.

Every specimen, from which a tissue sample was sent to CBOL, was photographed and received a unique identifier, linking the tissue sample with the original specimen. The original specimens are kept as voucher specimens, at the University of Victoria.

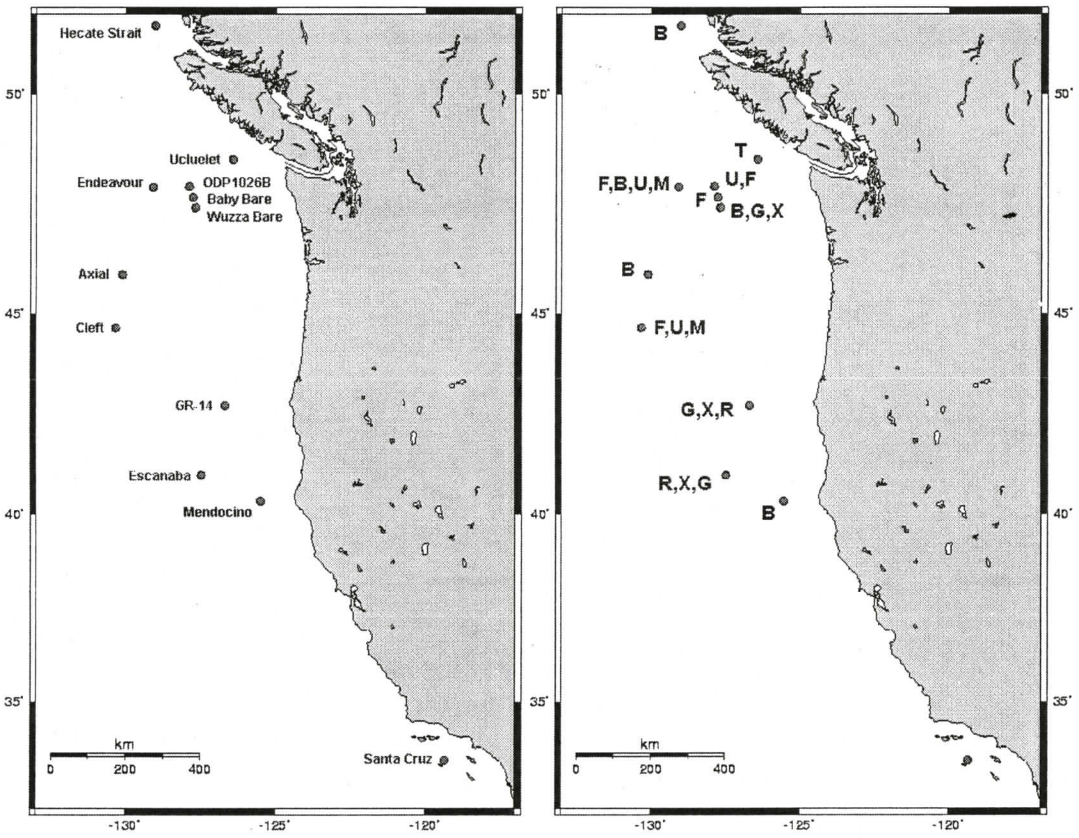


Figure 16: Left map indicates names of collection locations. Right map indicates morphological taxa used for CO1 sequencing.

Table 9: Summary of morphological taxa per location, used for CO1 sequencing. A '?' following the taxon letter, indicates a morphologically ambiguous taxon. 'Xyloph~~r~~againae' represents a group of six specimens that I could not associate with any of the established, morphological taxa

| Taxon | # | Location | lat | long | [m] |
|---|---|---|---|---|---|
| B | 8 | Axial, ROPOS 740 | 45.9333 | 130.1333 | -1540 |
| | 12 | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| | 6 | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| B? | 8 | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| | 2 | Axial, ROPOS 740 | 45.9333 | 130.1333 | -1540 |
| | 15 | Hecate Strait, dredge | 51.4516 | 129.04 | -100 |
| | 7 | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| | 1 | Mendocino | 40.373 | 125.373 | -1588 |
| *Bankia setacea* | 6 | Hecate Strait, dredge | 51.4516 | 129.04 | -100 |
| F | 17 | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| | 4 | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| F? | 6 | Baby Bare, JASON 062 | 47.7105 | 127.7882 | -2641 |
| | 6 | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| | 8 | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| | 1 | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| | 10 | OPD1026B, Alvin 4151 | 47.7626 | 127.7592 | -2658 |
| G | 7 | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| G? | 6 | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| | 8 | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| M | 1 | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| M? | 6 | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| Q | 1 | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| Q? | 6 | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| | 31 | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| R | 10 | Escanaba, Alvin 4043 | 40.9990 | 127. 4934 | -3222 |
| R? | 4 | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| T | 6 | Ucluelet Dredge | 48.5560 | 126.436 | -600 |
| T? | 4 | Ucluelet Dredge | 48.5560 | 126.436 | -600 |
| U | 10 | Cleft, ROPOS 619 | 44.6633 | 130.355 | -2100 |
| | 3 | ODP1026B, JASON 065 | 47.6788 | 127.8555 | -2661 |
| U? | 5 | Cleft, ROPOS 619 | 44.6633 | 130.355 | -2100 |
| | 6 | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| | 9 | ODP1026B, JASON 065 | 47.6788 | 127.8555 | -2661 |
| X | 7 | Escanaba, Alvin 4043 | 40.9990 | 127. 4934 | -3222 |
| | 1 | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| X? | 6 | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| Xylophagainae | 6 | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |

**18S sequencing:** Of the 1271 specimens isolated from the woodblocks, I extracted genomic DNA from a total of 114 individuals from ten taxa (the eight morphological taxa B, F, G, R, T, U, X, the temporary taxon Q and *X. washingtona* [Royal BC Museum]). I predominantly used siphon tissue to extract DNA. Only when the siphon was missing, did I extract the DNA from foot tissue. I used small quantities of siphon or mantel tissue. The specimens were placed back into the fixative and kept as voucher specimens. I conducted 545 amplification trials. I chose 39 successfully amplified individuals from eight locations for sequencing (Figure 17).

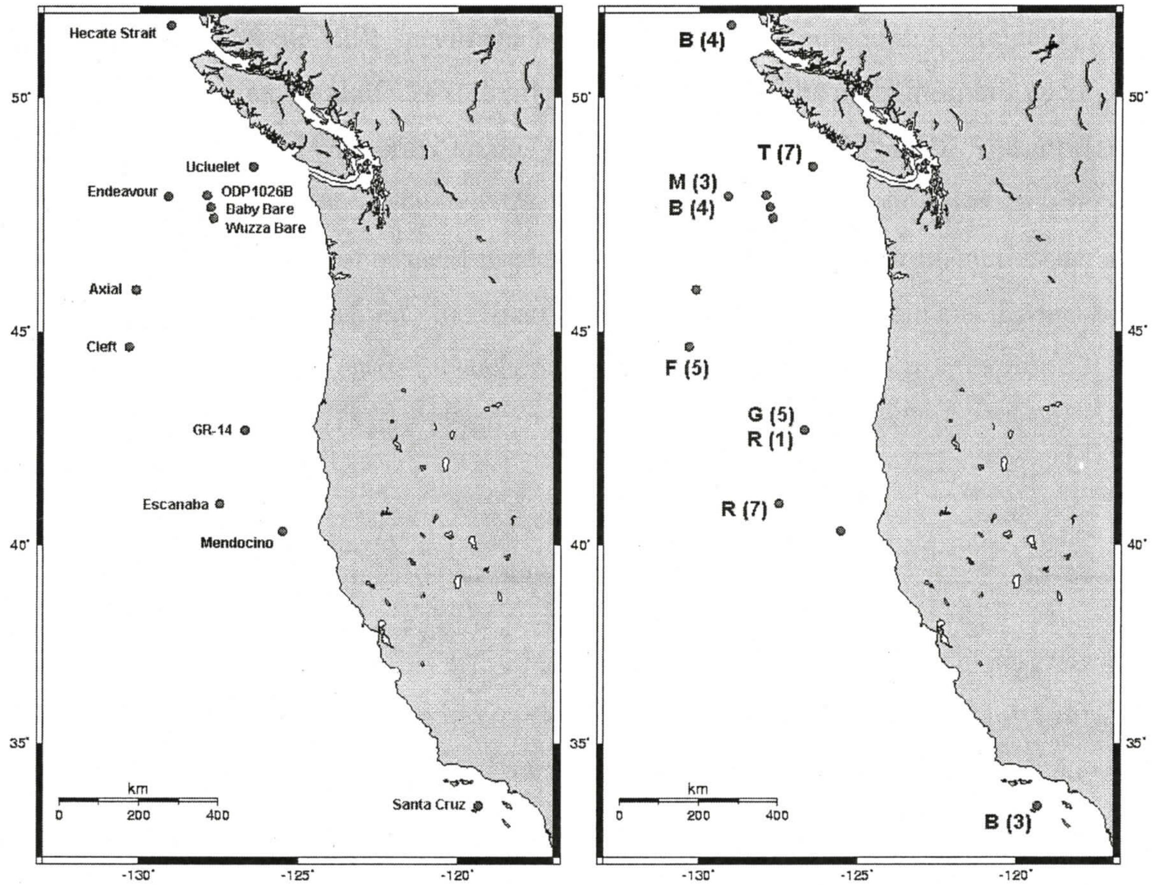


Figure 17: Left map indicating the name of the collection locations. Right map indicating the taxa used for 18S rDNA sequencing.

### 3.2.2 DNA Extraction

#### 3.2.2.1 CO1

The Canadian Centre for DNA Barcoding at the University of Guelph, Ontario, performed these DNA extractions. A 96 well, automated system, was used that simultaneously extracts 94 samples (Whiting et al. 1997). The step-by-step protocols can be found in the Appendix.

The data is uploaded to BOLD and accessible via the internet (www.barcodinglife.com). Following the policy of CBOL, the data is password protected until the primary researcher published the original work. Post publication, the data is available to anybody with an internet connection.

#### 3.2.2.2 18S

I experimented with a variety of extraction protocols to maximize DNA yield. I used the standard phenol-chloroform extraction protocol (Hajibabaei et al. 2006) for my first extractions, followed by the CTAB extraction protocol (M. Hills, personnal communication). I obtained the best results with the Qiagen DNeasy Tissue Kit protocol (Catalogue # 69504). The step-by-step protocols can be found in the Appendix B.

### 3.2.3 DNA Amplification

#### 3.2.3.1 CO1

The CO1 gene was amplified in a mixture of 6.25 μl 10% trehalose, 2 μl ddH20 1.25 μl 10X buffer, 0.625 μl 50 mM $MgCl_2$, 0.125 μl 10 μM forward primer, 0.125 μM reverse primer, 0.0625 μl 10 mM dNTPs, 0.06 μl Platinum TAQ Polymerase (Invitrogen) (5 U / μl) and 2 μl DNA. Total volume = 12.5 μl.

Bufferless e-gels (Invitrogen) were used for amplification. The PCR protocol consists of an initial denaturing step at 94°C for 2 min followed by 30 amplification cycles (96°C for 30s, 55°C for 15s, 60°C for 4 min).

Table 10: Primers used in amplification and sequencing of the CO1 gene. *(Burland 2000) **Steinke, D, personal communication, unpublished.

| Primer | 5′-3′ |
|---|---|
| LC1490* | GGTCAACAAATCATAAAGATATTGG |
| HCO2198* | TAAACTTCAGGGTGACCAAAAAATCA |
| crustdF1** | GGTCWACAAAYCATAAAGAYATTGG |
| crustDR1** | TAAACYTCAGGRTGACCRAARAAYCA |

### 3.2.3.2 18S

The 18S rDNA loci were PCR-amplified in two (1400 bp and 850 bp or 950 bp and 1450 bp) overlapping fragments using primer pairs 1f-bi and 5f-9r or 1f-bi and 3f-9r respectively (Figure 18; Table 11).

To maximize PCR product yield, I used two slightly varying PCR protocols for amplification. The first is a published protocol by Giribet and Distel (1994). It consists of an initial denaturing step at 94°C for 60s, 35 amplification cycles (94°C for 15s, 49°C for 15s, 72°C for 15s) and a final step at 72°C for 6 min. The second protocol is an alteration of the above with an initial denaturing step at 94°C for 60 s, 35 amplification cycles (94°C for 15 s, 45°C for 20 s, 72°C for 45s) and a final step at 72°C for 6 min. Both protocols were executed in a GeneAmp, PCR system 2700 (Applied Biosystems).

The total reaction volume of 50 µl consisted of 25µl READYMIX$^{TM}$ TAQ (Sigma, P4475), 6 µl $MgCl_2$, 16 µl $ddH_2O$, 1µl of each primer (forward and reverse) and 1 µl of DNA template (ranging between 10 and 100 ng / µl).

Table 11: Primers used in amplification and sequencing of the 18S rDNA gene. *(Maddison and Maddison 2003) **(Posada and Crandall 1998) ***Stoeckle, MA

| Primer | 5′-3′ |
|---|---|
| 1f * | TACCTGGTTGATCCTGCCAGTAG |
| 3r * | AGGCTCCCTCTCCGGAATCGAAC |
| 3f * | GTTCGATTCCGGAGAGGGA |
| 5r * | CTTGGCAAATGCTTTCGC |
| 5f * | GCGAAAGCATTTGCCAAGAA |
| bi ** | GAGTCTCGTTCGTTATCGGA |
| bif *** | TCCGATAACGAACGAGACTC |
| 9r * | GATCCTTCCGCAGGTTCACCTAC |

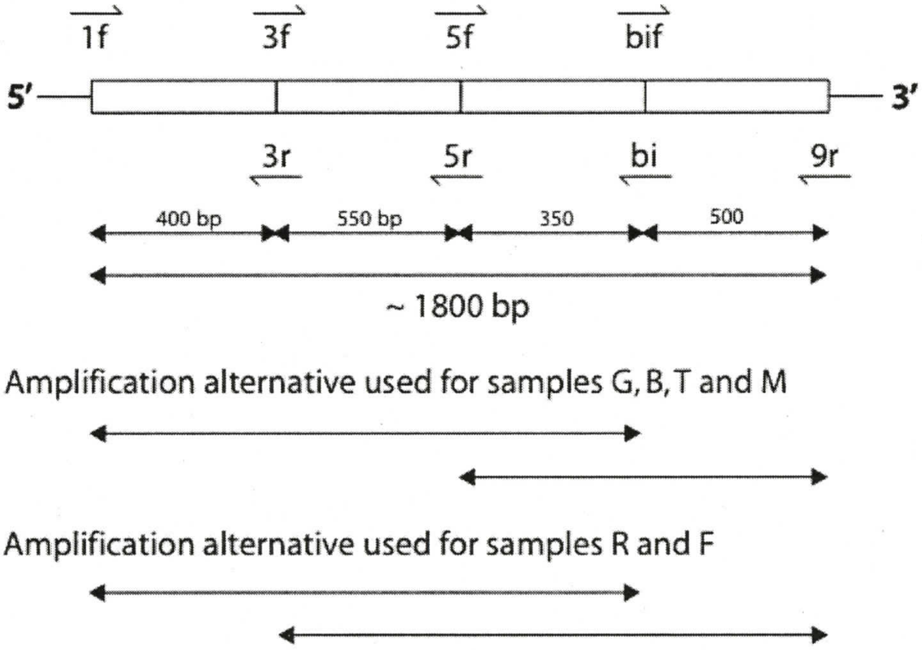


Figure 18: 18S small ribosomal subunit. The entire gene can be amplified in sections through the use of the primers indicated by arrows in polymerase chain reactions. Both amplification alternatives were tried for Taxa U, X and Q but failed to amplify.

### 3.2.4 PCR Product Purification

DNA fragments from the PCR reaction need to be purified from primers, nucleotides, polymerases and salts before sequencing, to obtain satisfactory sequencing results.

I purified the 18S PCR product using the Qiagen DNA Purification Kit (Catalogue # 28104). The full protocol can be found in the Appendix B.

Sequence purification is not necessary in the automated CO1 sequencing process (Steinke, D, personal communication).

### 3.2.5 Sequencing

Automated, fluorescent, 18S rDNA sequencing was performed on a Beckman Coulter CEQ 8000 DNA Sequencer, by the University of Victoria Centre for Biomedical Research DNA Sequencing Facility.

Sequencing of the CO1 gene was performed on an Applied Biosystems 3730 capillary sequencer, by the Canadian Centre for DNA Barcoding, University of Guelph, ON (Swofford 2002).

### 3.2.6 Sequence Editing

I have not personally inspected the trace files of the CO1 sequencing results. Employees of the Centre for Barcoding of Life (University of Guelph, ON) checked the sequences for errors and stop codons. Only sequences that fulfill the standards of CBOL were included in the dataset (Steinke, D, personal communication).

I used DNA Star: Seqman (Ronquist and Huelsenbeck 2003) to inspect the 18S rDNA trace files visually and make changes to the sequence, where the automatically produced sequence was ambiguous or incorrect. I automatically aligned the sequences using clustalw (Huelsenbeck and Ronquist 2001) and visually edited the alignment in MacClade (Saitou and Masatoshi 1987). Sequence alignment assumes the positional homology of bases. Ambiguous areas of alignment may hence alter the results. They were deleted from the alignment. I used Modeltest (Fitch 1977) to determine the best base-substitution model for the data. I used PAUP* (Graur and Li 2000) for the maximum parsimony (MP), maximum likelihood (ML) and neighbour-joining approaches (NJ) and

MrBayes (Cavalli-Sforza and Edwards 1967; Graur and Li 2000) for Bayesian analysis (B).

### 3.2.7 Outgroup

I used *Petricola pholadiformis* (Lamarck 1818), a mud-boring clam from the subclass Heterodonta, as the outgroup for the genetic analysis. This choice is based on the presumed evolutionary history of the Xylophagainae, as descendents of rock and mud-boring clams (see Chapter 2).

### 3.2.8 Sequence Analysis

I analyzed the 18S and CO1 sequences independently as well as in conjunction with each other, employing four different tree inference methodologies, described below.

For the combined analysis, I concatenated the 18S sequence with the matching CO1 fragments and re-ran the four phylogenetic analyses.

#### 3.2.8.1 Neighbour-Joining

The neighbour-joining tree-building algorithm (Huelsenbeck et al. 2001) attempts to find the shortest (minimum evolution) tree. This is accomplished by sequentially finding neighbours that minimize the total length of the tree. The algorithm starts with a star-like tree. Two OTUs are grouped and their distance is calculated. The two OTUs with the shortest distance are combined and considered a new composite OTU, to which the remaining single OTUs can be compared. This procedure is continued until all N-3 internal branches are found.

#### 3.2.8.2 Maximum Parsimony

Maximum Parsimony is a tree-building algorithm adapted for nucleotide data (Rannala and Yang 1996), based on the principle of "smallest number of evolutionary changes" (e.g. nucleotide substitutions). Discrete character states are assumed and the shortest pathway leading to those character states is chosen as the best tree. Using this approach often produces multiple trees require the same amount of character state changes (equally parsimonious).

A site is defined as invariant if all operating taxonomic units (OTU) possess the same character state at this site. Variable sites are categorized as informative and uninformative. A nucleotide site is informative only if it favours a subset of trees over the other possible trees. Generally a site is only informative when there are at least two different kinds of nucleotides at the site, each of which is present in at least two of the sequences.

The first step in a MP algorithm is to identify the informative sites. The next step is to calculate the number of substitutions for each possible tree and in the final step, to sum the number of changes over all the informative sites for each possible tree. The tree associated with the smallest number of changes is the preferred tree.

It is important to note that the informative sites that support the internal branches are deemed to be synapomorphies (similarity shared due to ancestry), whereas all the other informative sites are deemed to be homoplasies (similarity NOT due to inheritance). (Ronquist and Huelsenbeck 2003)

#### 3.2.8.3 Maximum Likelihood

The maximum likelihood tree building algorithm (Huelsenbeck and Ronquist 2001) is based on the probability of observing the data (e.g. the nucleotide sequences) under a given tree and a specified model of character state changes (e.g. the substitution pattern). The goal is to find the tree with the highest likelihood amongst all the possible trees. The question asked is: What is the probability that an assumed tree could have generated the data under an assumed model of nucleotide substitution? (Graur and Li 2000)

#### 3.2.8.4 Bayesian Inference

Bayesian inference is based on a quantity called posterior probability of a tree (Graur and Li 2000). It can be interpreted as the probability that the tree is correct. The tree with the highest probability is chosen as the best estimate of phylogeny (Hebert et al. 2003).The direct calculation of posterior probabilities is next to impossible. Numerical methods for its approximation exist. The Markov Chain Monte Carlo (MCMC) is the most useful algorithm for the Bayesian approach. First, a random number seed proposes a tree. A new tree is subsequently proposed by stochastically perturbing the current tree. The new tree is then either accepted or rejected with a certain probability. If the new tree is accepted, it will be subjected to further perturbation. I used MrBayes (Mayden 2002;

Rossello-Mora 2003) for the Bayesian analysis of the woodborer data. This software computes two independently seeded trees and uses the standard deviation of the split frequencies to determine the validity of the resulting trees. If the standard deviation of the two independently calculated scenarios falls below 0.01, the resulting posterior probability values are deemed significant and the MCMC can be halted.

#### 3.2.8.5 The Bootstrap Value

The bootstrap (bs) is a computer algorithm designed to re-sample data from the original sample set to infer node reliability or strength in MP-trees. *n* pseudo-samples are generated (this study: 1000) by re-sampling informative sites from the original sample. A tree is constructed for every site sampled. The resulting trees are scored 0 or 1 if they reject or support the original hypothesis set forth in the tree resulting from the original sample. The numbers are added up for all *n* trees. The bs-value is considered a confidence value in the node. Since there are computational problems associated with this methodology (references in Mayden 2002), only values > 95% are considered very reliable. Branches with low values are often collapsed and termed unresolved (Rossello-Mora 2003).

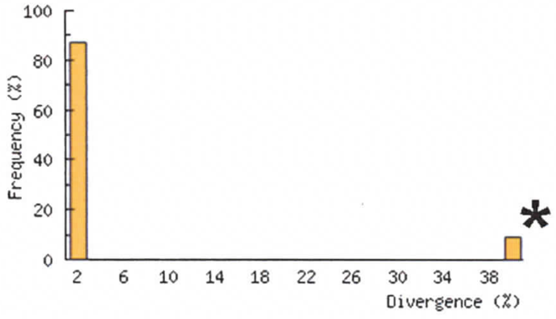
## 3.3 Results

### 3.3.1 Cytochrome *c* Oxidase 1

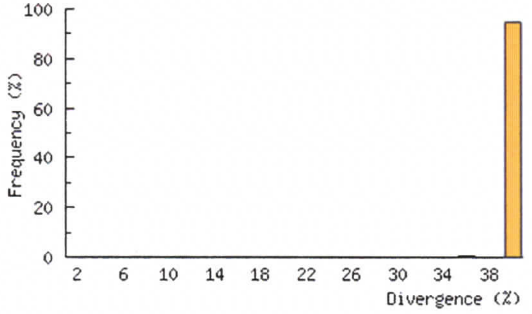
Of the 260 tissue samples sent to CBOL, 116 amplified successfully. The remaining samples did not amplify despite numerous changes to the primers and protocols. Of 11 submitted morphological taxa, seven have been sequenced to date, representing 116 sequences. Figure 19 summarizes the genetic distance in percent within species, genus and family [A, B, C] and shows examples of direct comparisons between samples [D]. Within species sequence diversity is < 2% for ~ 85% of the sequenced samples. The remaining ~ 15% have sequence divergence of > 40% (Figure 19-A*). This high intraspecific sequence divergence stems from examples of high sequence divergence among certain samples (Figure 19-D). For example (Figure 19-D*), the ranked specimen *Xylopholas sp.* (Taxon X) with the Process ID WB089-06 is 6.96% different from specimen ranked *Xylopholas sp.* (Taxon X) with the Process ID WB088-06. Other examples have sequence divergences up to a 182.37% (Figure 19-D**). When comparing Process IDs, I noticed that certain individuals (e.g. WB091-06; WB034-06, WB021-06) are responsible for this high divergence. I will discuss this later.

Figure 19: Genetic distance (%) within species [A], genus [B] and family [C]. Direct comparison between two samples and the resulting genetic distance [D] (see text on page 80f for details and explanation of '*").

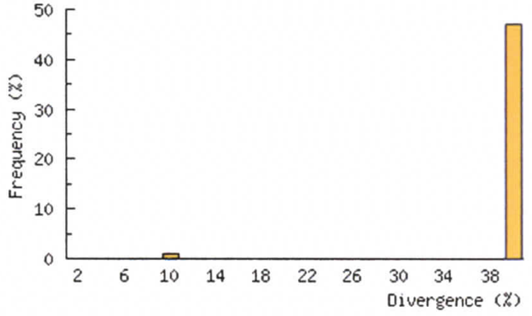
A



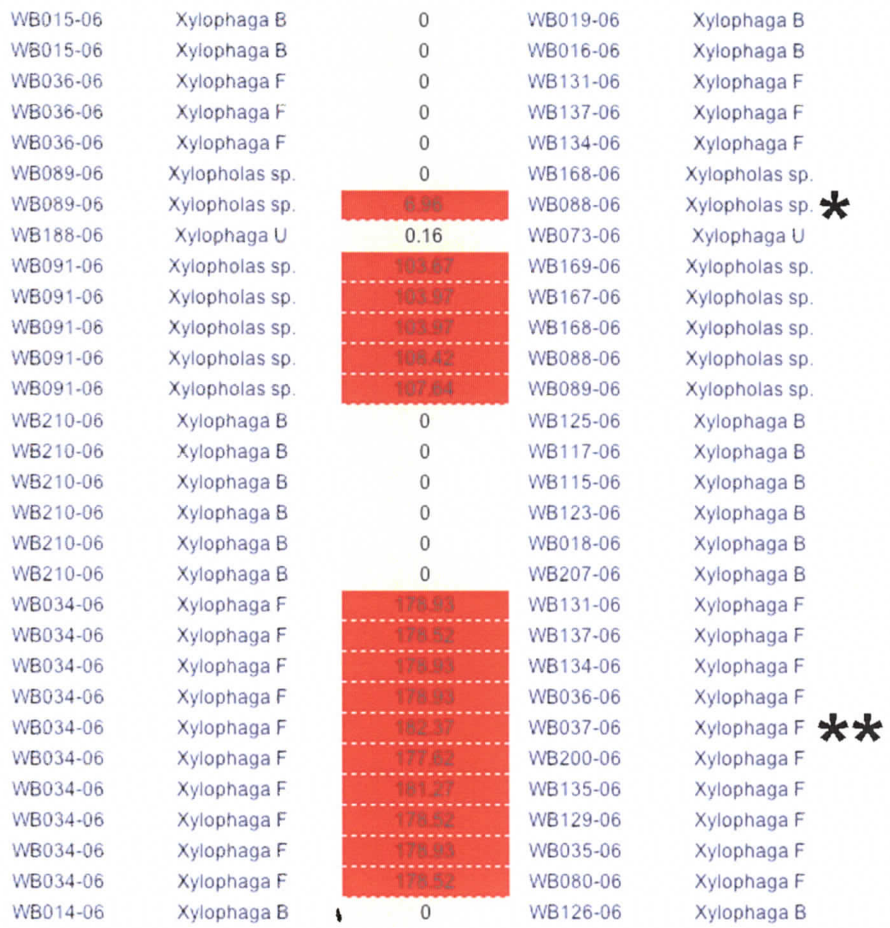
B



C



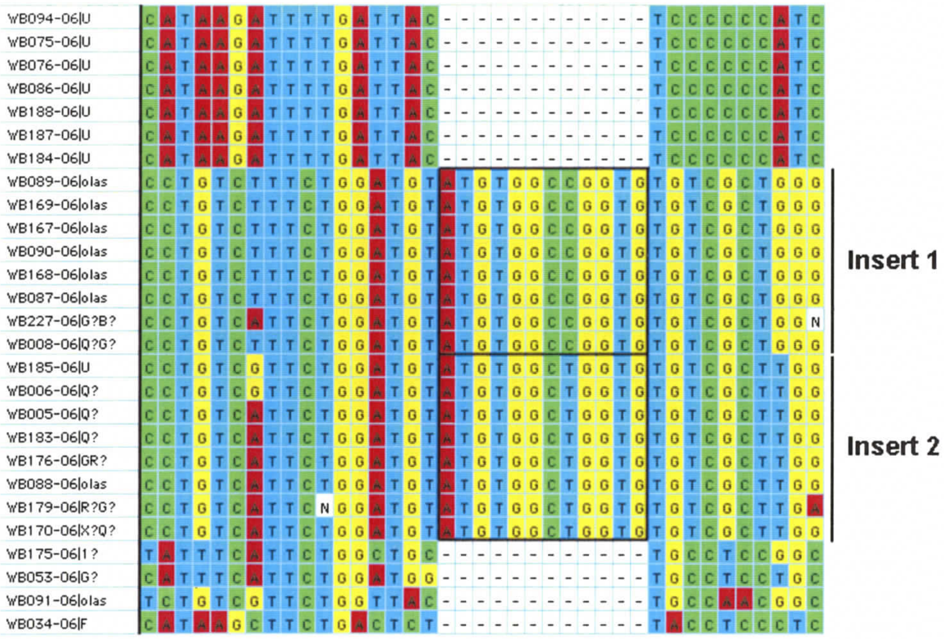
D



| | | | | |
|---|---|---|---|---|
| WB015-06 | Xylophaga B | 0 | WB019-06 | Xylophaga B |
| WB015-06 | Xylophaga B | 0 | WB016-06 | Xylophaga B |
| WB036-06 | Xylophaga F | 0 | WB131-06 | Xylophaga F |
| WB036-06 | Xylophaga F | 0 | WB137-06 | Xylophaga F |
| WB036-06 | Xylophaga F | 0 | WB134-06 | Xylophaga F |
| WB089-06 | Xylopholas sp. | 0 | WB168-06 | Xylopholas sp. |
| WB089-06 | Xylopholas sp. | 6.96 | WB088-06 | Xylopholas sp. * |
| WB188-06 | Xylophaga U | 0.16 | WB073-06 | Xylophaga U |
| WB091-06 | Xylopholas sp. | 103.67 | WB169-06 | Xylopholas sp. |
| WB091-06 | Xylopholas sp. | 103.97 | WB167-06 | Xylopholas sp. |
| WB091-06 | Xylopholas sp. | 103.97 | WB168-06 | Xylopholas sp. |
| WB091-06 | Xylopholas sp. | 108.42 | WB088-06 | Xylopholas sp. |
| WB091-06 | Xylopholas sp. | 107.64 | WB089-06 | Xylopholas sp. |
| WB210-06 | Xylophaga B | 0 | WB125-06 | Xylophaga B |
| WB210-06 | Xylophaga B | 0 | WB117-06 | Xylophaga B |
| WB210-06 | Xylophaga B | 0 | WB115-06 | Xylophaga B |
| WB210-06 | Xylophaga B | 0 | WB123-06 | Xylophaga B |
| WB210-06 | Xylophaga B | 0 | WB018-06 | Xylophaga B |
| WB210-06 | Xylophaga B | 0 | WB207-06 | Xylophaga B |
| WB034-06 | Xylophaga F | 178.93 | WB131-06 | Xylophaga F |
| WB034-06 | Xylophaga F | 178.52 | WB137-06 | Xylophaga F |
| WB034-06 | Xylophaga F | 178.93 | WB134-06 | Xylophaga F |
| WB034-06 | Xylophaga F | 178.93 | WB036-06 | Xylophaga F |
| WB034-06 | Xylophaga F | 182.37 | WB037-06 | Xylophaga F ** |
| WB034-06 | Xylophaga F | 177.62 | WB200-06 | Xylophaga F |
| WB034-06 | Xylophaga F | 181.27 | WB135-06 | Xylophaga F |
| WB034-06 | Xylophaga F | 178.52 | WB129-06 | Xylophaga F |
| WB034-06 | Xylophaga F | 178.93 | WB035-06 | Xylophaga F |
| WB034-06 | Xylophaga F | 178.52 | WB080-06 | Xylophaga F |
| WB014-06 | Xylophaga B | 0 | WB126-06 | Xylophaga B |

#### 3.3.1.1 CO1 Alignment

The sequence alignment of 116 CO1 sequences shows a 12 bp insert in 16 of the 116 sequenced specimens (Figure 20). Two variations of the insert are present, with only one bp difference between the two. 43.75 % of the specimens containing the insert are representatives of Taxon X. The remaining specimens are Taxon U (6.25%), Taxon Q? (25%), Taxon G? (12.5%), Taxon B? (6.25%) and Taxon R? (6.25%). Specimens with insert 1 are highlighted with one asterisk on Figure 21, specimens with insert 2 are highlighted with two asterisks on Figure 21. With the exception of two examples in each insert category, 'insert 1' and 'insert 2' specimens group apart from each other. See discussion.



```
WB094-06|U        CATAAGATTTTGATTAC------------TCCCCCCATC
WB075-06|U        CATAAGATTTTGATTAC------------TCCCCCCATC
WB076-06|U        CATAAGATTTTGATTAC------------TCCCCCCATC
WB086-06|U        CATAAGATTTTGATTAC------------TCCCCCCATC
WB188-06|U        CATAAGATTTTGATTAC------------TCCCCCCATC
WB187-06|U        CATAAGATTTTGATTAC------------TCCCCCCATC
WB184-06|U        CATAAGATTTTGATTAC------------TCCCCCCATC
WB089-06|olas     CCTGTCTTTCTGGATGTATGTGGCCGGTGTGTCGCTGGG
WB169-06|olas     CCTGTCTTTCTGGATGTATGTGGCCGGTGTGTCGCTGGG
WB167-06|olas     CCTGTCTTTCTGGATGTATGTGGCCGGTGTGTCGCTGGG
WB090-06|olas     CCTGTCTTTCTGGATGTATGTGGCCGGTGTGTCGCTGGG
WB168-06|olas     CCTGTCTTTCTGGATGTATGTGGCCGGTGTGTCGCTGGG
WB087-06|olas     CCTGTCTTTCTGGATGTATGTGGCCGGTGTGTCGCTGGG
WB227-06|G?B?     CCTGTCATTCTGGATGTATGTGGCCGGTGTGTCGCTGGN
WB008-06|Q?G?     CCTGTCTTTCTGGATGTATGTGGCCGGTGTGTCGCTGGG
WB185-06|U        CCTGTCGTTCTGGATGTATGTGGCTGGTGTGTCGCTTGG
WB006-06|Q?       CCTGTCGTTCTGGATGTATGTGGCTGGTGTGTCGCTTGG
WB005-06|Q?       CCTGTCATTCTGGATGTATGTGGCTGGTGTGTCGCTTGG
WB183-06|Q?       CCTGTCATTCTGGATGTATGTGGCTGGTGTGTCGCTTGG
WB176-06|GR?      CCTGTCATTCTGGATGTATGTGGCTGGTGTGTCGCTTGG
WB088-06|olas     CCTGTCATTCTGGATGTATGTGGCTGGTGTGTCGCTTGG
WB179-06|R?G?     CCTGTCATTCNGGATGTATGTGGCTGGTGTGTCGCTTGA
WB170-06|X?Q?     CCTGTCATTCTGGATGTATGTGGCTGGTGTGTCGCTTGG
WB175-06|1?       TATTTCATTCTGGCTGC------------TGCCTCCGGC
WB053-06|G?       CATTTCATTCTGGATGG------------TGCCTCCTGC
WB091-06|olas     TCTGTCGTTCTGGTTAC------------TGCCAACGGC
WB034-06|F        CATAAGCTTCTGACTCT------------TACCTCCCTC
```

Figure 20: Alignment of CO1 sequences, highlighting the 12 bp insert found in 16 specimens.

### 3.3.1.2 Neighbour-Joining Tree (NJ)

The neighbour-joining tree (Figure 21) shows the grouping of *Bankia setacea* and the morphological Taxa B, F, G, U and X. I will refer to these clades as ‘tight clades’. Following Hebert et al. (2002) and the parameters used by CBOL, I define tight clades as clades with more than 2 members and less than 2% mean sequence divergence. At the time of writing this paragraph, the voucher specimens of Taxon R and M had not been sequenced. A clear association of the ambiguous specimens (R?, M?) is not possible at this point. Temporary Taxon Q forms a tight clade that consist of three Q?.

Fourty of the 116 sequences did not fall out within a clade. I will refer to these sequences as outliers. Their morphological associations are: one F, one B, two *Bankia setacea*, three X, two U and representatives of the ambiguous morphological taxa: 10 F?, five G?, two U?, five Q?, one T?, two R?,wit two M? and four B?. The R?s are outliers, however two M?s fall out within the F-tight clade. I will discuss possible explanations for this later.

Some morphologically ambiguous specimens, marked with a ‘?’, fell out within tight clades (Table 12). The association of morphologically ambiguous samples (marked with ‘?’ following the taxon letter) is hence confirmed by the genetic data. For example, I ranked WB-6-133 a F?. It falls within the F-tight clade. I consequently consider WB-6-133 to be a member of Taxon F.

Table 12: Species identification success rates. Morphological taxa names with number of specimens (see also Table 9). '% agreement' signifies the number of sequenced specimens of the taxon, that fall out within the same 'tight clade'. 'CO1 designation' indicates position of remaining sequences. A '?' indicates interpretation not possible at the time. A '-' indicates unavailability of information to date.

| **Morphological Taxon** | **# morphological specimens** | **% not sequenced** | **% agreement** | **CO1 designation** |
|---|---|---|---|---|
| **B** | 26 | 7.7 (2) | 95.8 (23) | 1 as outlier |
| **F** | 21 | 4.8 (1) | 95 (19) | 1 as outlier |
| **G** | 7 | 0 | 100 (7) | - |
| **M** | 1 | 100 | - | - |
| **Q** | 1 | 100 | - | - |
| **R** | 10 | 100 | - | - |
| **T** | 6 | 100 | - | - |
| **U** | 13 | 7.7 (1) | 75 (9) | 1 within F tight clade<br>2 as outliers |
| **X** | 8 | 0 | 62.5 (5) | 3 as outliers |
| **B?** | 33 | 91 (30) | 0 | 3 as outliers |
| **F?** | 31 | 42 (13) | 33.3 (6) | 12 as outliers |
| **G?** | 14 | 71.4 (10) | 0 | 4 outliers |
| **M?** | 6 | 83.3 (5) | ? | ? |
| **Q?** | 37 | 86.5 (32) | 40 (2) | 3 as outliers |
| **R?** | 4 | 75 (3) | ? | ? |
| **T?** | 4 | 75 (3) | ? | ? |
| **U?** | 20 | 85 (17) | 0 | 1 within G tight clade<br>2 as outliers |
| **X?** | 6 | 83.3 (5) | 0 | 1 within Q tight clade |
| ***Bankia setacea*** | 6 | 0 | 66.7 (4) | 2 as outliers |
| **Xylophagainae (1)** | 6 | 66.7 (4) | ? | 1 within Q tight clade<br>1 as outlier |

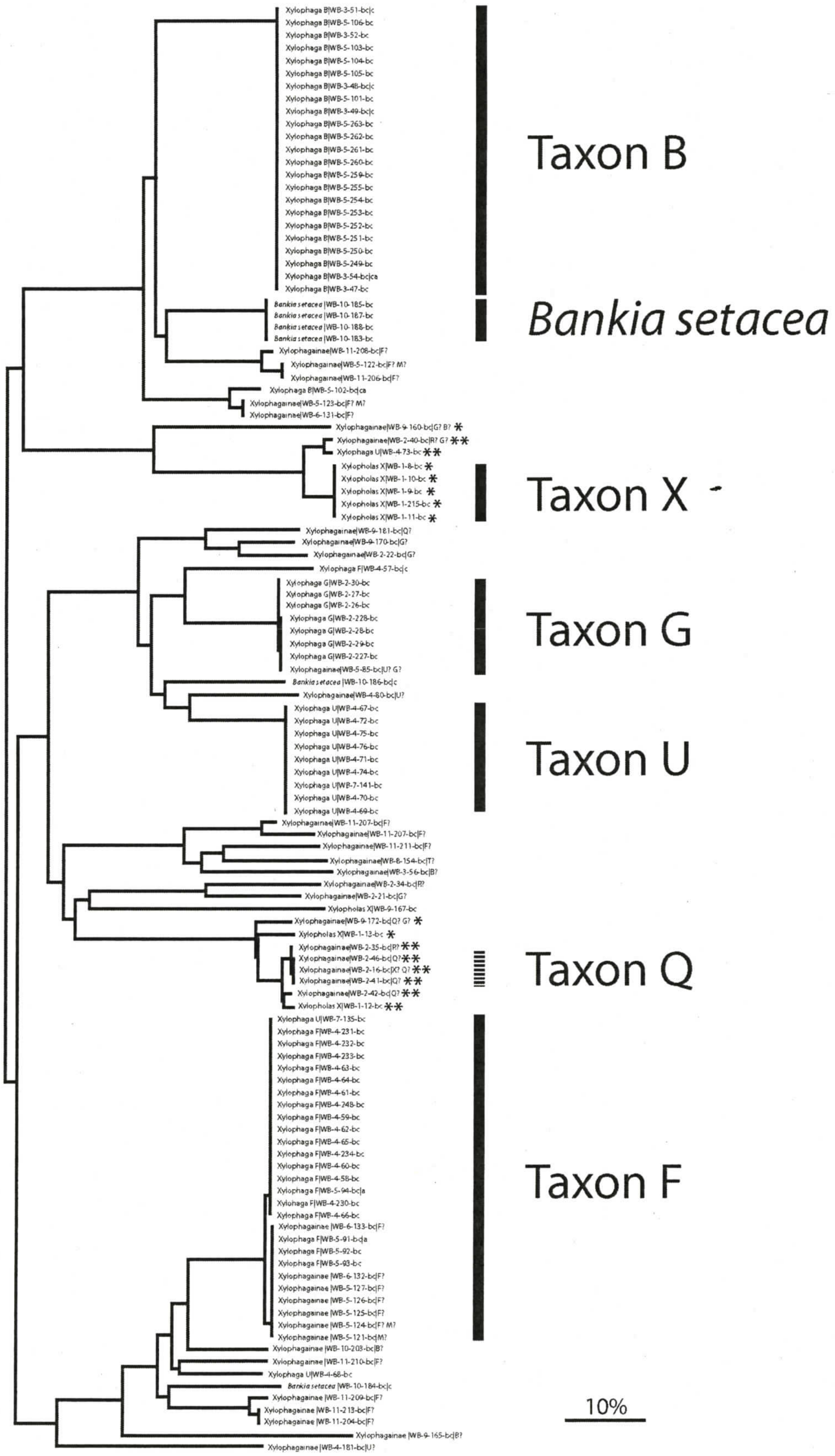
Within tight clades, sequence divergence is ~ 0.08% (tight clades averaged individually, then averaged over all 6 tight clades).

shows sequence divergences within and among tight clades. Percent distance among morphological Taxa is higher than 42.4% in all cases. BOLD calculates these values based on properly ranked specimens only. This means, only specimens that have an assigned species rank (e.g. Xylophaga F) are considered. Specimens designated Xylophagainae – M? are not taken into consideration.

Table 13: Tight clades (< 2% sequence divergence) as depicted in Figure 21. Number of sequences per location, percent divergence among the sequenced specimens as well as percent divergence within tight clade. SE = Standrad Error

| Tight Clade | # Sequences | Locations | Mean % Divergence | % Min | % Max | SE | Overall % divergence |
|---|---|---|---|---|---|---|---|
| B | 6 | Axial | 0 | 0 | 0 | 0 | 0 |
| | 17 | Endeavour | 0 | 0 | 0 | 0 | |
| Bankia setacea | 4 | Hecate Strait | 0 | 0 | 0 | 0 | 0 |
| X | 5 | Escanaba | 0 | 0 | 0 | 0 | 0 |
| G | 7 | GR-14 | 0.088 | 0 | 0.155 | 0.076 | 0.088 |
| | 1 | Endeavour | 0 | 0 | 0 | 0 | |
| U | 8 | Cleft | 0.033 | 0 | 0.155 | 0.064 | 0.033 |
| | 1 | ODP1026 | 0 | 0 | 0 | 0 | |
| F | 15 | Cleft | 0.083 | 0 | 0.574 | 0.126 | 0.363 |
| | 9 | Endeavour | 0.487 | 0 | 0.974 | 0.487 | |
| | 2 | Baby Bare | 0 | 0 | 0 | 0 | |
| | 1 | ODP1026 | 0 | 0 | 0 | 0 | |

Figure 21: Neighbour-Joining Tree of 116 individual CO1 sequences. The labels consist of the BOLD process ID, the specimen ID and 'extra info'. The scale bar represents percent sequence divergence. Extra info: c = re-checked morphology, confirmed; ca = checked but ambiguous. '*' = 12 bp insert 1, '**' = 12-bp insert 2


Xylophaga B|WB-3-51-bc|c
Xylophaga B|WB-5-106-bc
Xylophaga B|WB-3-52-bc
Xylophaga B|WB-5-103-bc
Xylophaga B|WB-5-104-bc
Xylophaga B|WB-5-105-bc
Xylophaga B|WB-3-48-bc|c
Xylophaga B|WB-5-101-bc
Xylophaga B|WB-3-49-bc|c
Xylophaga B|WB-5-263-bc
Xylophaga B|WB-5-262-bc
Xylophaga B|WB-5-261-bc
Xylophaga B|WB-5-260-bc
Xylophaga B|WB-5-259-bc
Xylophaga B|WB-5-255-bc
Xylophaga B|WB-5-254-bc
Xylophaga B|WB-5-253-bc
Xylophaga B|WB-5-252-bc
Xylophaga B|WB-5-251-bc
Xylophaga B|WB-5-250-bc
Xylophaga B|WB-5-249-bc
Xylophaga B|WB-3-54-bc|ca
Xylophaga B|WB-3-47-bc
Taxon B
*Bankia setacea* |WB-10-185-bc
*Bankia setacea* |WB-10-187-bc
*Bankia setacea* |WB-10-188-bc
*Bankia setacea* |WB-10-183-bc
*Bankia setacea*
Xylophagainae|WB-11-208-bc|F?
Xylophagainae|WB-5-122-bc|F? M?
Xylophagainae|WB-11-206-bc|F?
Xylophaga B|WB-5-102-bc|ca
Xylophagainae|WB-5-123-bc|F? M?
Xylophagainae|WB-6-131-bc|F?
Xylophagainae|WB-9-160-bc|G? B? *
Xylophagainae|WB-2-40-bc|R? G? **
Xylophaga U|WB-4-73-bc **
Xylopholas X|WB-1-8-bc *
Xylopholas X|WB-1-10-bc *
Xylopholas X|WB-1-9-bc *
Xylopholas X|WB-1-215-bc *
Xylopholas X|WB-1-11-bc *
Taxon X
Xylophagainae|WB-9-181-bc|Q?
Xylophagainae|WB-9-170-bc|G?
Xylophagainae|WB-2-22-bc|G?
Xylophaga F|WB-4-57-bc|c
Xylophaga G|WB-2-30-bc
Xylophaga G|WB-2-27-bc
Xylophaga G|WB-2-26-bc
Xylophaga G|WB-2-228-bc
Xylophaga G|WB-2-28-bc
Xylophaga G|WB-2-29-bc
Xylophaga G|WB-2-227-bc
Xylophagainae|WB-5-85-bc|U? G?
Taxon G
*Bankia setacea* |WB-10-186-bc|c
Xylophagainae|WB-4-80-bc|U?
Xylophaga U|WB-4-67-bc
Xylophaga U|WB-4-72-bc
Xylophaga U|WB-4-75-bc
Xylophaga U|WB-4-76-bc
Xylophaga U|WB-4-71-bc
Xylophaga U|WB-4-74-bc
Xylophaga U|WB-7-141-bc
Xylophaga U|WB-4-70-bc
Xylophaga U|WB-4-69-bc
Taxon U
Xylophagainae|WB-11-207-bc|F?
Xylophagainae|WB-11-207-bc|F?
Xylophagainae|WB-11-211-bc|F?
Xylophagainae|WB-8-154-bc|T?
Xylophagainae|WB-3-56-bc|B?
Xylophagainae|WB-2-34-bc|R?
Xylophagainae|WB-2-21-bc|G?
Xylopholas X|WB-9-167-bc
Xylophagainae|WB-9-172-bc|Q? G? *
Xylopholas X|WB-1-13-bc *
Xylophagainae|WB-2-35-bc|R? **
Xylophagainae|WB-2-46-bc|Q? **
Xylophagainae|WB-2-16-bc|X? Q? **
Xylophagainae|WB-2-41-bc|Q? **
Xylophagainae|WB-2-42-bc|Q? **
Xylopholas X|WB-1-12-bc **
Taxon Q
Xylophaga U|WB-7-135-bc
Xylophaga F|WB-4-231-bc
Xylophaga F|WB-4-232-bc
Xylophaga F|WB-4-233-bc
Xylophaga F|WB-4-63-bc
Xylophaga F|WB-4-64-bc
Xylophaga F|WB-4-61-bc
Xylophaga F|WB-4-248-bc
Xylophaga F|WB-4-59-bc
Xylophaga F|WB-4-62-bc
Xylophaga F|WB-4-65-bc
Xylophaga F|WB-4-234-bc
Xylophaga F|WB-4-60-bc
Xylophaga F|WB-4-58-bc
Xylophaga F|WB-5-94-bc|a
Xylohaga F|WB-4-230-bc
Xylophaga F|WB-4-66-bc
Xylophagainae |WB-6-133-bc|F?
Xylophaga F|WB-5-91-bc|a
Xylophaga F|WB-5-92-bc
Xylophaga F|WB-5-93-bc
Xylophagainae |WB-6-132-bc|F?
Xylophagainae |WB-5-127-bc|F?
Xylophagainae |WB-5-126-bc|F?
Xylophagainae |WB-5-125-bc|F?
Xylophagainae |WB-5-124-bc|F? M?
Xylophagainae |WB-5-121-bc|M?
Taxon F
Xylophagainae |WB-10-203-bc|B?
Xylophagainae |WB-11-210-bc|F?
Xylophaga U|WB-4-68-bc
*Bankia setacea* |WB-10-184-bc|c
Xylophagainae |WB-11-209-bc|F?
Xylophagainae |WB-11-213-bc|F?
Xylophagainae |WB-11-204-bc|F?
10%
Xylophagainae |WB-9-165-bc|B?
Xylophagainae |WB-4-181-bc|U?

The 'tight clades' of taxa B, F, G, U, X and *Bankia setacea* all contain distinct CO1 sequences, with less than 2% sequence divergence within clade. Figure 22 compares the morphological classification (top row) to the CO1 classification (bottom row). The results for taxa M, R and T remain inconclusive. Too few specimens were sequenced. B1 constitutes specimens from Endeavour and Axial. Specimens from Hecate Strait (B2) remain to be sequenced. The 'Q tight clade' consists of four morphologically ambiguous specimens. I cannot confirm its status as a true tight clade until further sequences are analyzed. It is not included in Figure 22.

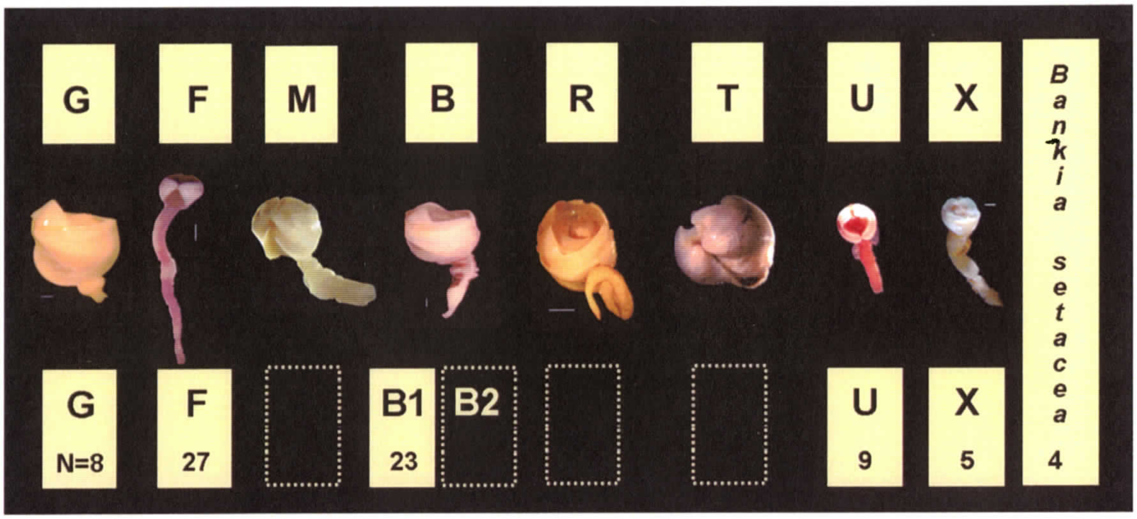


Figure 22: Morphological taxa versus CO1 sequences (< 2% divergence). Top row = morphological taxa. Bottom row = CO1 sequences. N = number of sequenced individuals per morphological taxon. Empty boxes indicate taxa of which too few specimens were sequenced.

### 3.3.2 18S rDNA

The sequencing of the 18S rDNA gene from 39 specimens of six morphological taxa yielded sequence lengths ranging between 1837 and 1843 basepairs. Sequences from the same morphological taxon showed no variation. All sequencing products were weak, resulting in low trace peaks. Upon alignment of the replicates, I was able to identify the correct base for any given position (R. Haesevoets, personal communication). Table 14 summarizes my sequencing attempts. Note that it took multiple successful amplifications, to obtain a single full 18S sequence. The individual sequences can be found in the Appendix.

Table 14: Summary of taxa sampled along with number of extractions, success rate and number (N) of resulting, full-length, sequences.

| Taxon | # Extractions | # Amplifications | # Successful Amplifications | Success Rate [%] | N |
|---|---|---|---|---|---|
| R | 11 | 94 | 56 | 60 | 8 |
| G | 7 | 78 | 41 | 53 | 6 |
| F | 13 | 89 | 24 | 27 | 5 |
| M | 3 | 12 | 12 | 100 | 3 |
| T | 16 | 67 | 41 | 61 | 7 |
| X | 18 | 6 | 0 | 0 | 0 |
| U | 1 | 13 | 0 | 0 | 0 |
| B | 26 | 133 | 76 | 57 | 10 |
| Q | 17 | 50 | 12 | 24 | 0 |
| *X.washingtona* | 3 | 3 | 0 | 0 | 0 |

The 39 18S sequences grouped into six distinctive clusters. The individual clusters displayed at least 5 bp differences (e.g. R and F, Table 15), reaching as high as 151 bp differences (e.g. B1 and *P. pholadiformis*, Table 15). The morphological taxon T split into T1 and T2, both occurring at the same location and B split into B1 (Endeavour) and B2 (Hecate Strait and Santa Cruz). Taxon R was unambiguously separated by > 4 bp differences from all other taxa (Table 15). Taxa F, G and M share 100% 18S sequence identity (Table 15). Taxon B2 and *Xylophaga washingtona* have zero bp differences. See discussion for further explanation.

Table 15: Above diagonal: number of basepair differences: Below the diagonal: ratio between number of differences and total number of common bases. [1 and 5] are sequences downloaded from Genbank. [2,3 and 4] are sequences loaned from a collaborator.

| | **R** | **B1** | **B2** | **F** | **G** | **M** | **T1** | **T2** | **[1]** | **[2]** | **[3]** | **[4]** | **[5]** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **R (n=8)** | - | 49 | 55 | 5 | 5 | 5 | 14 | 37 | 19 | 55 | 13 | 28 | 128 |
| **B1 (n=4)** | 0.03 | - | 16 | 47 | 47 | 47 | 41 | 57 | 52 | 16 | 53 | 59 | 151 |
| **B2 (n=6)** | 0.03 | 0.01 | - | 53 | 53 | 53 | 48 | 62 | 58 | 0 | 59 | 68 | 151 |
| **F (n=5)** | 0.00 | 0.03 | 0.03 | - | 0 | 0 | 15 | 39 | 53 | 53 | 18 | 29 | 128 |
| **G (n=6)** | 0.00 | 0.03 | 0.03 | 0.00 | - | 0 | 15 | 39 | 20 | 53 | 18 | 29 | 128 |
| **M (n=3)** | 0.00 | 0.03 | 0.03 | 0.00 | 0.00 | - | 15 | 39 | 20 | 53 | 18 | 29 | 128 |
| **T1 (n=2)** | 0.01 | 0.02 | 0.03 | 0.01 | 0.01 | 0.01 | - | 32 | 19 | 48 | 21 | 26 | 128 |
| **T2 (n=5)** | 0.02 | 0.03 | 0.04 | 0.02 | 0.02 | 0.02 | 0.02 | - | 62 | 62 | 40 | 49 | 140 |
| **[1] *X. atlantica* (AY070132)** | 0.01 | 0.03 | 0.03 | 0.03 | 0.01 | 0.01 | 0.01 | 0.04 | - | 58 | 28 | 37 | 130 |
| **[2] *X. washingtona*** | 0.03 | 0.01 | 0.00 | 0.03 | 0.03 | 0.03 | 0.03 | 0.04 | 0.03 | - | 59 | 68 | 151 |
| **[3] *Xylopholas sp.* (Atlantic)** | 0.01 | 0.03 | 0.03 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.02 | 0.03 | - | 35 | 135 |
| **[4] *Xyleredo sp.* (Atlantic)** | 0.02 | 0.04 | 0.04 | 0.02 | 0.02 | 0.02 | 0.02 | 0.03 | 0.02 | 0.04 | 0.02 | - | 139 |
| **[5] *P. pholadiformis* (AY070120)** | 0.07 | 0.09 | 0.09 | 0.07 | 0.07 | 0.07 | 0.07 | 0.08 | 0.08 | 0.09 | 0.08 | 0.08 | - |

The morphological taxa do not match up with the 18S data in all cases (Figure 23). DNA from the morphological Taxa U and X was extracted, however, would not amplify despite numerous adjustments to the protocols and primer combinations. The Taxa remain unsequenced (empty boxes in Figure 23).

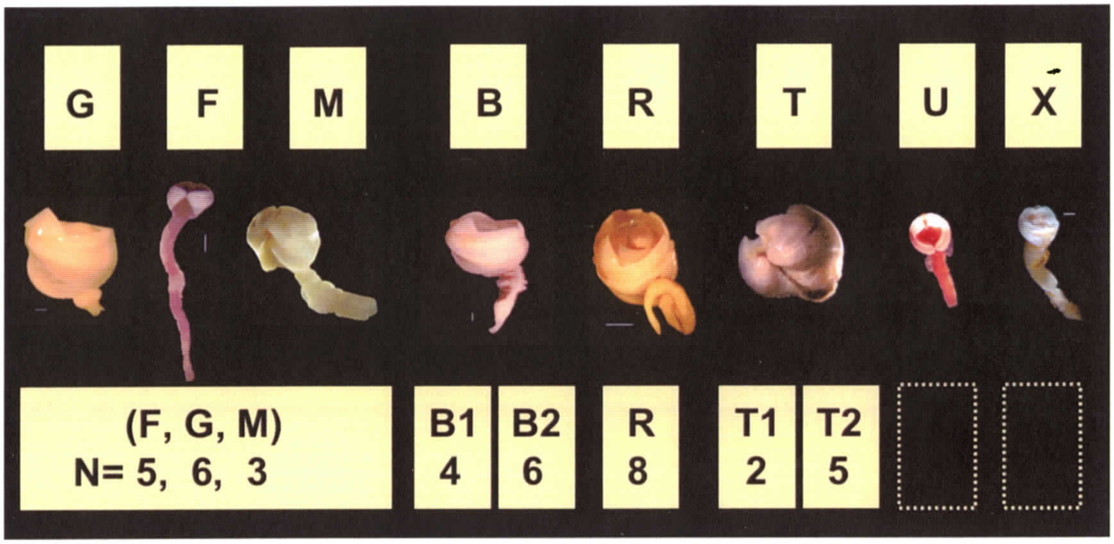


Figure 23: Morphological taxa versus unique 18S sequences. Top row = morphological taxa. Bottom row = 18S sequences. N = number of sequenced individuals per morphological taxon. Non-sequenced morphological taxa represented by empty boxes. B1 from Axial and Endeavour; B2 from Hecate Strait and Santa Cruz; T1 and T2 from Ucluelet.

### 3.3.3 Phylogenetic Analysis (18S)

#### 3.3.3.1 Neighbour-Joining Tree (NJ)

The NJ-tree supports the Xylophagainae clade, placing *Pholas dactylus* (Linnaeus 1758), a member of the family Pholadidae, on the outside. Taxa F, G and M form a clade and fall out together with R. Together, their sequences are closest to *Xylopholas* (Atlantic). *Xyloredo* (Atlantic) falls out with T1. A second major clade comprises B2 and *Xylophaga washingtona*, as well as B1 and T2. (Figure 24-A).

#### 3.3.3.2 Maximum Parsimony Tree (MP)

There are a total of 1839 characters (bp). 1591 are constant, 169 are variable but parsimony-uninformative and 79 are parsimony informative. The maximum parsimony analysis of the 18S alignment produced six equally likely MP-trees. The 50%-majority-consensus tree is shown in Figure 24-B. Bootstrap values from 1000 replicates indicate the strength of the nodes. Values < 50 were omitted. *P. dactylus* is separated from the clade of the subfamily Xylophagainae. F, G and M are grouped together (bs 97) and fall out with R (bs 59). Together they are in a clade with *Xylopholas sp.* from the Atlantic (bs 63). The relationship of the latter clade with *Xyloredo sp.* (Atlantic), T1 and *X. atlantica* is not resolved. Taxon B2 and *X. washingtona* fall out in a second major clade, with a bootstrap value of 100. B1 is related to the latter clade (bs 100) and groups together with T2 (bs 76).

#### 3.3.3.3 Maximum Likelihood Tree (ML)

The topology of the ML-tree is similar to that of the MP tree described above, however it provides more resolution. The clade for the subfamily Xylophagainae is clearly visible, with *X. atlantica* at its base. The *Xylopholas* (Atlantic), R, F, G, M clade is the same as in the MP-tree. However, here, *Xyloredo sp.* (Atlantic) groups with T1, T2, B1, B2 and *X. washingtona* (Figure 24-C).

#### 3.3.3.4 Bayesian Inference Tree (B)

The B-tree was constructed after 104000 generations with the standard deviation of the split frequencies below 0.01 (0.009875). The tree topology is similar to the MP and

ML trees. Posterior probability values are high for the Xylophagainae clade (1.00). F, G and M have a posterior probability of 1.00 and group with R by 0.78. *Xylopholas* (Atlantic) joins them with a high posterior probability of 0.98. The second major clade consists of B2 and *X. washingtona* (1.00) together with B1 (1.00), joined with T2 (0.92) and T1 (0.69) (Figure 24-D).

### 3.3.4 Combined Phylogenetic Analysis (18S and CO1)

To increase the number of characters in the genetic analysis, I concatenated the 18S and matching CO1 sequences.

#### 3.3.4.1 Neighbour-Joining Tree (NJ)

By adding characters to the sequence alignment, I increased the genetic distances among sequences, which resulted in a more drawn-out tree (Figure 25-A). *Pholas dactylus* falls out within the Xylophagainae-clade. *Xylopholas* (Atlantic) groups with R and *Xyloredo* (Atlantic) groups with G. F and M fall out together and group with a T1, T2 clade. Together they group with *Xylophaga atlantica*. This clade groups with a *Xylophaga washingtona*, B1 clade. The NJ-tree is rooted with *Petricola pholadiformis*.

#### 3.3.4.2 Maximum Parsimony Tree (MP)

Adding the CO1 sequences increased the total number of characters (bps) to 2556. Of these, 1769 are constant, 516 are variable but parsimony-uninformative and 271 are parsimony informative. The maximum parsimony analysis of the combined alignment produced three equally parsimonious trees. The 50%-majority-consensus tree is shown in Figure 25-B. Bootstrap values from 1000 replicates indicate the strength of the nodes. Values < 50 were omitted. *P. dactylus* is separated from the clade of the subfamily Xylophagainae. *Xylophaga washingtona* falls out with B1 (bs 100) and groups with *Xylopholas* (Atlantic). Together, this clade groups with a R,G (bs 75) clade. F and M group together (bs 100). T1 and T2 fall out together with no bootstrap support. The clade groups with *Xyloredo* (Atlantic). *Xylophaga atlantica* is at the root of the above-described clades, supported by a bs value of 98.

The addition of the CO1 data to the 18S data increased the resolution on the MP tree. F, G and M are now separated into G and R (bs 75) and F and M (bs 100). *Xyloredo* (Atlantic) now groups with T1 and T2 with a bs value of 55.

### 3.3.4.3 Maximum Likelihood Tree (ML)

The ML-tree depicts *Xylophaga atlantica* at the base of the Xylophagainae-clade. Two main clades are: G,R grouping with F,M and *Xylopholas* (Atlantic); and *Xyloredo* (Atlantic) with T1,T2 and *Xylophaga washingtona*, B1 (Figure 25-C).

### 3.3.4.4 Bayesian Inference Tree (B)

I aborted the B-tree inference algorithm (MCMC) after 112000 generations when the standard deviation of the split frequency reached a minimum of 0.019640 before increasing again (< 0.01 = significant) (Figure 25-D). *Xylopholas* (Atlantic) falls out with R and groups with G. *Xyloredo* (Atlantic) groups with a T1,T2 clade and together falls out with a *Xylophaga washingtona*, B1 clade. F falls out with M. Their association to the other two clades is unresolved. *Xylophaga atlantica* falls out at the base of the Xylophagainae clade.

Figure 24: 18S rDNA Data [A] Neighbour-joining tree. Scalebar indicates percent sequence divergence (0.01 = 1 %). [B] Consensus Maximum Parsimony tree of six equally parsimonious trees. Bootstrap values indicated below nodes. 1000 replicates. Bootstrap values < 50 were omitted. [C] Maximum Likelihood tree. Scale bar indicates percent (0.01 = 1%) sequence divergence. [D] Bayesian Inference tree. Posterior probability values (clade credibility) indicated at nodes. Scalebar indicates percent expected sequence divergence (0.01 = 1 %).

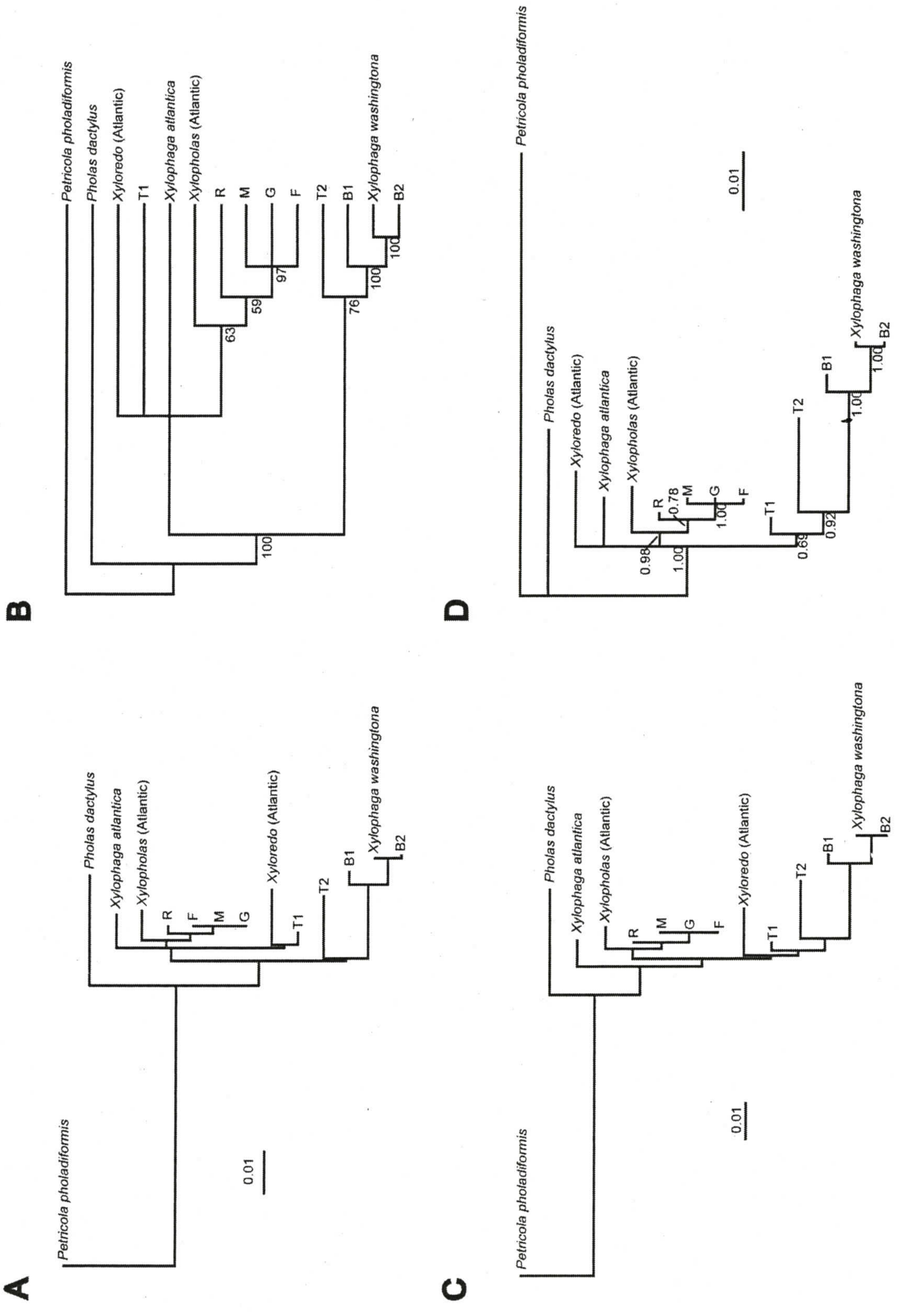
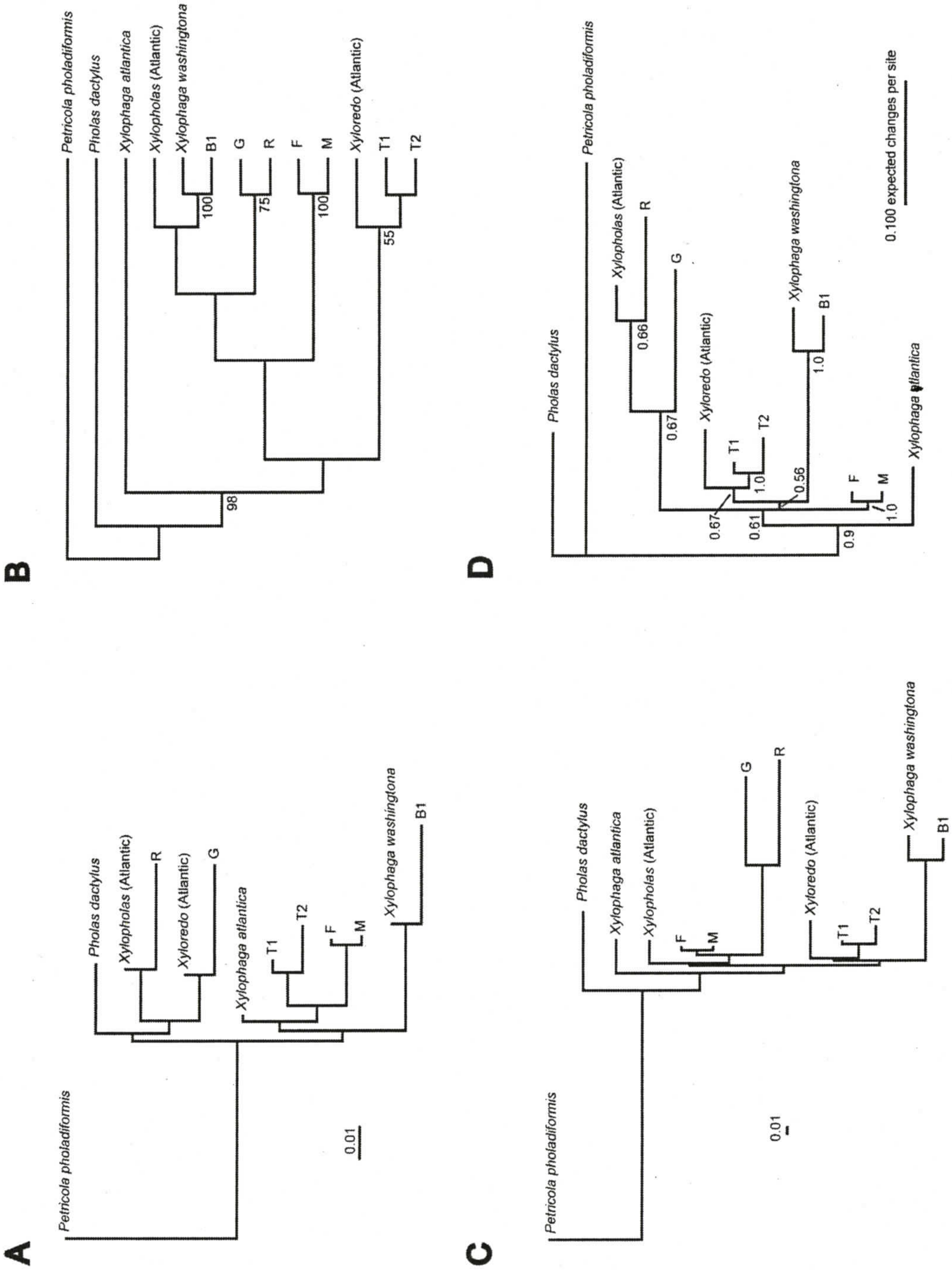

A
Petricola pholadiformis
Pholas dactylus
Xylophaga atlantica
Xylopholas (Atlantic)
R
F
M
G
Xyloredo (Atlantic)
T1
T2
B1
Xylophaga washingtona
B2
0.01
B
Petricola pholadiformis
Pholas dactylus
Xyloredo (Atlantic)
T1
Xylophaga atlantica
Xylopholas (Atlantic)
R
M
G
F
T2
B1
Xylophaga washingtona
B2
100
63
59
97
76
100
100
C
Petricola pholadiformis
Pholas dactylus
Xylophaga atlantica
Xylopholas (Atlantic)
R
M
G
F
Xyloredo (Atlantic)
T1
T2
B1
Xylophaga washingtona
B2
0.01
D
Petricola pholadiformis
Pholas dactylus
Xyloredo (Atlantic)
Xylophaga atlantica
Xylopholas (Atlantic)
R
0.78
M
G
F
1.00
0.98
1.00
T1
0.69
T2
0.92
B1
1.00
Xylophaga washingtona
B2
1.00
0.01

Figure 25: 18S rDNA and CO1 Data [A] Neighbour-joining tree. Scalebar indicates percent sequence divergence (0.01 = 1 %). [B] Consensus Maximum Parsimony tree of six equally parsimonious trees. Bootstrap values indicated below nodes. 1000 replicates. Bootstrap values < 50 were omitted. [C] Maximum Likelihood tree. Scale bar indicates percent (0.01 = 1%) sequence divergence. [D] Bayesian Inference tree. Posterior probability values (clade credibility) indicated at nodes. Scalebar indicates percent expected sequence divergence (0.1 = 10 %).


A
Petricola pholadiformis
Pholas dactylus
Xylopholas (Atlantic)
R
Xyloredo (Atlantic)
G
Xylophaga atlantica
T1
T2
F
M
Xylophaga washingtona
B1
0.01
B
Petricola pholadiformis
Pholas dactylus
Xylophaga atlantica
Xylopholas (Atlantic)
Xylophaga washingtona
B1
G
R
F
M
Xyloredo (Atlantic)
T1
T2
100
75
100
55
98
C
Petricola pholadiformis
Pholas dactylus
Xylophaga atlantica
Xylopholas (Atlantic)
F
M
G
R
Xyloredo (Atlantic)
T1
T2
Xylophaga washingtona
B1
0.01
D
Pholas dactylus
Petricola pholadiformis
Xylopholas (Atlantic)
R
G
0.66
0.67
Xyloredo (Atlantic)
T1
T2
1.0
0.67
Xylophaga washingtona
B1
1.0
0.56
0.61
F
M
1.0
0.9
Xylophaga atlantica
0.100 expected changes per site

## 3.4 Discussion

### 3.4.1 Sequence Quality and BOLD Results

The sequence quality of most samples, both the 18S as well as the CO1 samples, was low, resulting in low trace peaks. Low trace peaks render the sequencing data interpretation more difficult, since background noise can mask the actual signal. This phenomenon has been known from the Mollusca and is attributed to the presence of polysaccharides in the mucus secreted by these animals (Distel D, Steinke D, personal communications). Ambiguous bases can pose a problem during sequence interpretation.

I encountered this problem quite frequently during the analysis of my 18S data. Due to the number of replicates from multiple samples from any given location as well as the number of replicates due to the usage of multiple forward and reverse primers, I have confidence in my sequences.

I obtained the best results when using the QIAGEN Tissue extraction kit. I did not notice any difference in genomic DNA yield between the two tissue types used (siphon and foot). What did make a difference was the preservation of the specimen. Extractions from frozen material always yielded high DNA concentrations. I obtained lower, but sufficient, DNA concentrations from samples fixed in 95% EtOH. Poor yields resulted from 70% EtOH fixations and I did not obtain any DNA from 7%-buffered Formalin fixations. *Xylophaga washingtona* from the Royal BC Museum were stored in 100% EtOH, but was originally fixed in 7%-buffered Formalin. I was not able to extract DNA from these samples. Also, Taxon U samples from WB-B, had been fixed in 7%-buffered Formalin and did not amplify. I extracted genomic DNA from Taxon X, however the 18S sequence would not amplify.

Specimens WB091-06, WB034-06 and WB021-06 (Figure 19) are responsible for high intra-specific sequence divergences. I checked their morphological characters, suspecting a false morphological assessment, but was not able to identify these specimens with certainty, due to the missing tissue that I sent to CBOL for sequencing. Based on the high sequence divergences however I conclude that their morphological assessment was wrong. These specimens should be re-assigned for future analyses.

Species F displayed one order of magnitude higher intra-specific species divergences (Table 13). The specimens from Endeavour are responsible for this variation as they display a high diversity within this location. I do not know for certain why this is the case, but suspect poor sequence quality or faulty morphological assessments on my part. Again, I was not able to re-confirm the morphological attributes because of the tissue sample sent to CBOL.

### 3.4.2 Species status revealed by morphology and genetic markers

Today most scientists work within the paradigm of 'descent with modification'. The pre-Darwinian paradigms invoking the creation of diversity without diverging lineages, have been mostly abandoned (Mayden 2002). So, why are there at least 24 (Bock 2004) different conceptualizations of species? Theories and concepts are the fundamental link between pattern and process and aid in our understanding and interpretation of nature (Bock 2004). No single species concept turned out to be applicable to any given situation. Consequently workers tend to either adapt the species boundaries of a given concept to their organisms or formulate species concepts that fit their organisms (Mayden 2002).

A unifying advance in recent years has been the changing perception of the word 'species' from a group of similar organisms to a distinct kind of biological individuality (akin to 'organism' or 'cell'). Despite this trend, some authors state: "For the time being, the adoption of a universal concept for all taxonomies appears to be a utopia." (Mayden 2002, p.326), call it "simply impossible" (Bock 2004), while other authors propose a single concept solution (Hebert et al. 2003).

Mayden (Hebert et al. 2003) proposes a hierarchical system where a primary theoretical concept, the Evolutionary Species Concept (ESC), encompasses the secondary operational concepts. The operational concepts serve as tools for discovering natural entities consistent with the primary concept. The ESC does not prevent the existence of independent lineages thought to be species because of operational or pragmatic concerns for particular types of traits. According to Mayden it is the only concept currently available to serve as an over-arching idea as to the notion of biological species. The ESC's dominant feature is its freedom from baggage encountered in all other concepts, which require particular qualities before the species status can be assigned. The problem with a non-operational concept such as the ESC is the difficulty to find anything in nature that fits the concept without prior knowledge or guidelines or tools to use, in the discovery phase. Mayden's solution for tracking entities that lack definition is to employ the secondary concepts, which mostly are consistent with the primary concept and outline minimal standards for species recognition (Hebert et al. 2004). Researchers can use these concepts as surrogate concepts to the ESC. Species can be identified based on criteria of homologous divergence in morphology, ecology, behaviour, genetics or any other quality supporting the hypothesis of lineage independence.

Several of the 24 species concepts violate the basic principle of evolutionary theory that evolutionary change is gradual and hence that no boundary can be drawn between successive species along a phyletic lineage, by being based on the premise that different species can be distinguished from one another along a phyletic lineage (Ward et al. 2005).

Bock argues that these 'species concepts' could in fact be definitions of the species taxon, but in no way can they be considered a concept The large number of species concepts is a result of a failure to separate the species and the phyletic lineage as well as a confusion between the species concept and the species taxon (Hogg and Hebert 2004).

In this thesis I am using a Cohesion Species Concept for the morphological work and a Phylogenetic Species Concept for my genetic work. Both serve as proxies for the definition of a species taxon.

**Morphological Taxon B**: The CO1 data confirm the existence of Taxon B (Figure 21). To date, only specimens from Axial and Endeavour have been sequenced. Hecate Strait and Santa Cruz remain to be sequenced. There is no sequence divergence among the samples from Endeavour and Axial (Table 12).

The 18S sequence data split morphological Taxon B into the genetic Taxa B1 and B2. B1 is only found at the Endeavour collection site whereas I found B2 at the collection site furthest to the Noth (Hecate Strait) and furthest to the South (Santa Cruz).

There are no basepair differences between an unpublished *Xylophaga washingtona* sequence, obtained from a collaborator, other than three inserts in the B2 sequence of 9, 37 and 30 bps. Given the published geographic distribution of *X. washingtona*, the location and depth it had been found, I am tempted to conclude that B2 in fact is *X. washingtona*. The missing CO1 data could resolve this question.

**Morphological Taxon F, G and M**: The CO1 data support Taxon F and Taxon G as separate entities (Figure 21). Taxon F samples from Cleft have less than 0.1% sequence divergence, those from Endeavour less than 0.5%. The overall sequence divergence between the two locations is less than 0.4% (Table 12). The divergence between the two sampling sites is illustrated in Figure 21;'tight clade' F displays a gap between specimens from Cleft (location 4) and Endeavour (location 5), with one exception (WB-5-94) (Figure 21). Taxon F, from Baby Bare, groups with specimens from Endeavour.

Taxon M is undersampled at this point. Two 'M?' fall out within the Taxon F tight clade. Two additional 'M?' however fall out together, separated from any tight clade. At this point, I have too few CO1 sequences from this morphological Taxon to clearly state, whether the morphological Taxon M is supported by CO1 or not. Dr. Voight had suggested that Taxon M could represent highly stressed individuals of Taxon F. I originally disagreed on the basis of my morphological classification. My 18S results however show that morphological Taxa, F, G and M share 100% 18S-sequence identity. Given the CO1 data for Taxa F and G, I am concluding that the phylogenetic resolution of the 18S rDNA gene is not high enough to resolve the F, G, M clade.

**Morphological Taxon R**: CO1 data are available. The 18S data however separate this taxon from the other taxa.

**Morphological Taxon T**: I did not obtain enough CO1 sequences from Taxon T. The single sequence of a 'T?' does, however, not associate with any of the reported tight clades. The 18S data does not support the morphological classification entirely. Morphological Taxon T is genetically split into T1 and T2. The T samples from Ucluelet were among the poorest preserved in this study. They were collected as by-catch during a bottom trawl and degraded on deck before being subjected to a series of freezing and thawing events. Not a single siphon was present that would have allowed for a more detailed morphological analysis of the specimens. It is hence possible that the Ucluelet collection does contain two different morphological taxa but, due to the lack of characters, I was not able to discern those.

**Morphological Taxa U**: The CO1 data support the status of Taxon U (Figure 21). Individuals from Cleft display less than 0.04% sequence divergence. The 18S gene of Taxa U did not amplify.

**Morphological Taxa X**: The CO1 data support the status of Taxon X (Figure 21). Individuals from Escanaba display 0% sequence divergence. The 18S gene of Taxon X did not amplify.

**Temporary Taxon Q**: This Taxon appears to form its own tight clade (Figure 21, dashed line). At present it contains three Q?s. Additional sequences are necessary to confirm the status of this Taxon.

***Bankia setacea***: The CO1 data confirm this Taxon (Figure 21). Four individuals from Hecate Strait display 0% sequence divergence.

I detected a 12-bp insert in 16 of the 116 sequenced specimens. Two types of the insert are present (Insert 1 and 2; Figure 20, Figure 21 *,**), corresponding with 'tight clade' X and 'tight clade' Q. Although the pattern is not 100% consistent (* and ** mix in both 'tight clades'), I believe it is possible that the insert is a genus specific marker for *Xylopholas*. The addition of CO1 sequences from Taxa X and Q could resolve this pattern further.

The application of the barcoding system was first demonstrated by Hebert et al. (2005). The authors created CO1 profiles on three different taxonomic levels. The first was a compilation of 100 CO1 sequences obtained from the GenBank database, each from a different family, representing all available classes. The second was a compilation of 100 CO1 sequences from eight of the largest insect orders, each representing a different family. The third was a collection of 200 closely related moths, covering five families, collected around Guelph, Ontario. The analysis of these studies revealed 96% accuracy in assigning the species to a phylum and 100% accuracy in assigning insects to their order. 150 additional moth CO1 DNA sequences could be correctly identified based on the database established in the level three study.

It became clear that a low intraspecific variation and a high interspecific variation were crucial for the success of this initiative. In 2003, Hebert et al. carried out a follow-up study examining 13000 pairwise comparisons of 2238 animal species from 447 genera in 11 phyla. 80% of such pairwise comparisons showed > 8% sequence divergence and more than 98% of pairs showed > 2% sequence divergence. Individuals from the same species showed sequence variation averaging less than 0.3% (Hebert et al. 2003). This pattern was since confirmed in birds (Hebert et al. 2004), fish (Hillis and Dixon 1991) and various insect groups (Hillis and Dixon 1991).

Species delineation in under-sampled groups relies on threshold values to separate intra- from interspecific variation (Turner 2002). The NJ-tree (Figure 21) shows six tight clades representing Taxa B, X, G, U, F and *Bankia setacea*. All the groupings show < 2 % sequence divergence within tight clade and > 40 % sequence divergence among tight clades, supporting (Culliney and Turner 1976) my morphological classifications, set forth in Chapter 2. Dr. Voight (personal communication) arrived at the same conclusion after extensive morphological analysis of specimens from the same collections.

The barcoding methodology is however not only successful in associating the unknown to the known, but also in detecting so far unknown cryptic species. A study of the neotropic skipper butterfly (*Astraptes fulgerator*) revealed ten species within a collection of what was previously thought to be one species. Although the adults are morphologically indistinguishable, subsequent studies revealed major differences in caterpillar morphology, as well as food plant preferences (Culliney and Turner 1976).

The 18S sequencing revealed two genetic lineages each in the morphological Taxa B (B1, B2) and T (T1, T2). To date, no Ts and B2s were analyzed (CO1). It is possible that the CO1 sequences of B2 could form a separate tight clade from B1. A re-assessment of the morphological characters and character states would be necessary. Perhaps a higher resolution (more character states) is necessary.

### 3.4.3 Overall Success Rate

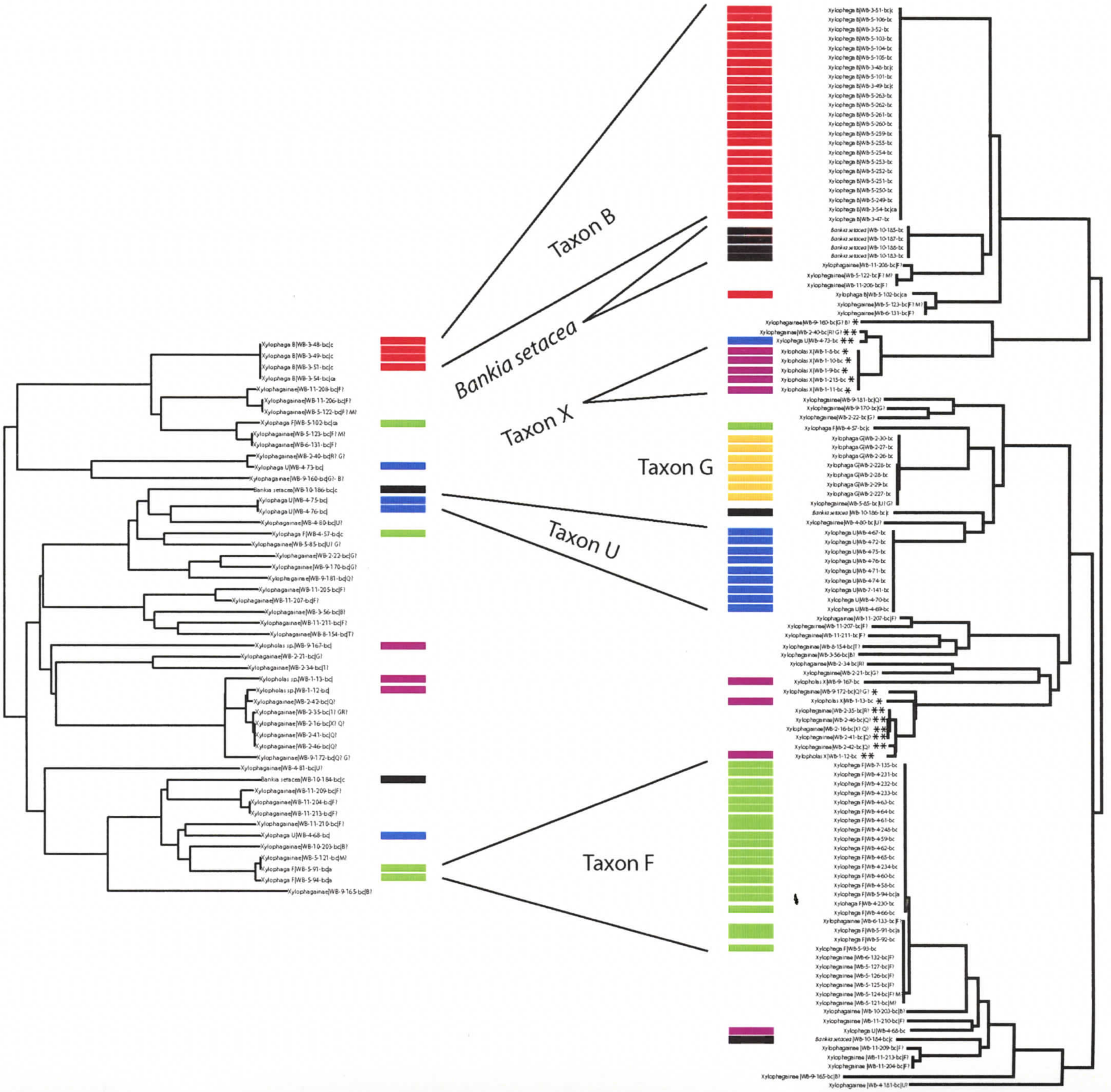
My morphological assessment of the samples was correct in 98.5% of the cases. 68 morphologically unambiguous specimens grouped into six 'tight clades'. Only one single specimen of Taxon U grouped with 'tight clade' F. I re-analyzed the morphology of that particular specimen and concluded that it was in fact a member of Taxon F, and consequently changed its taxonomic rank in BOLD.

Furthermore, a morphologically ambiguous specimen (G?) grouped within the 'tight clade' G and five morphologically ambiguous specimens (F?) grouped within the 'tight clade' F. I consider this corroboration for the morphological ambiguity and further proof for the practicality of the approach.

Figure 26 illustrates how the pattern emerged upon adding 66 sequences to the initial dataset of only 50 sequences. I am confident that adding the remaining 134 sequences will further clarify the pattern, as many of the outliers will group within their respective 'tight clades'.

In a few instances I obtained within species sequence divergence rates that are mis-representative (Figure 19). It is important to note that BOLD uses the user specified designations (aka. my taxon names) as the operating taxonomic unit. If I make a morphologically wrong assessment of a certain specimen, BOLD will still calculate the intraspecific distances and report them as too high. The idea is to go back to the vouchered specimen and check the morphology. In my case, due to the small nature of the specimens, I could not verify the morphology of the specimens that created the high sequence divergences (Figure 19-D), as their morphology is too compromised due to tissue extractions.

Figure 26: Pattern emergence with increased sample size (50 sequences versus 116 sequences). Red = Taxon B, Black = *Bankia setacea*, Purple = Taxon X, Orange = Taxon G, Blue = Taxon U, Green = Taxon F


Taxon B
Bankia setacea
Taxon X
Taxon G
Taxon U
Taxon F

### 3.4.4 Phylogenetic Relationships

"The process of choosing a region that is likely to be appropriate for a particular systematic question is perhaps the most critical step in any phylogenetic analysis. If the region chosen is evolutionarily too conserved (i.e. the sequences are nearly the same in all study taxa), then considerable time will be wasted collecting invariant data. On the other hand, regions that differ among taxa to the extent that alignments are difficult or questionable also are unlikely to yield robust phylogenies." (Haderlie 1983; Turner).

The phylogenetic analyses of the 18S data showed that it does not provide all the resolution needed to discern the phylogenetic relationships among the newly discovered, morphological taxa. The addition of the CO1 data to the alignment increased the number of informative characters more than threefold, however, many clades are still unresolved or poorly supported. The use of multiple tree inference algorithms is common practice to find congruence amongst and instill confidence in the final phylogeny (Knudsen 1961).

I cannot present a final phylogeny of the woodborers of the Northeast Pacific. I can however state that Taxon B1 is most closely related to *Xylophaga washingtona*, supported by all four analysis (NJ, MP, ML and B) and support values (MP bootstrap 100; B posterior probability = 1.0). These two taxa are also very similar morphologically (Turner 1956). I attempted to sequence a museum deposited *Xylophaga washingtona*, however, I was unsuccessful due to the original preservative (7%-buffered Formalin).

Taxa T1 and T2 form a clade in all four analyses. It receives no bootstrap support in the MP tree, but the highest posterior probability value in the B-tree. It is important to recall that the combined B-tree (Figure 25-D) does not represent statistically significant data.

As in the 18S data analysis alone, F and M group in the combined analysis as well, suggesting a close relationship of the two Taxa, if not that these two taxa represent the same species (Voight, personal communication).

Culliney and Turner (1955) state that *X. atlantica* is most closely related to *X. washingtona*. In the combined analysis, *Xylophaga atlantica* falls out at the base of the subfamily clade and *X. washingtona* deep within the clade. I first explained the basal position of *X. atlantica* with the fact that I am comparing Atlantic specimens with specimens from the Northeast Pacific. The presence of *Xylopholas* and *Xyloredo*, both from the Atlantic, however, render this explanation false.

At this point, I cannot make any further, concrete, statements regarding the phylogenetic relationships among the deep-sea wood-boring clams from the Northeast Pacific. Further sampling of already collected specimens and, ideally, of additional collections is necessary.

In summary, I conclude that a combination of the morphological characters and the two genetic markers yield sufficient support for the classification of a new species. Given the limited samples obtained to date, I conclude that the morphological Taxa F, G, U and X as well as the genetic Taxon B1 represent new 'species'.

# Chapter 4: Conclusions and Future Directions

## 4.1 Summary

The Xylophagainae is globally distributed with representatives in all world oceans. A planktonic larval phase appears to be the best means of dispersal for an organism living on a patchily distributed, ephemeral habitat such as waterlogged driftwood. Published records, however, show that a free-swimming larval stage is rare among the deep-sea woodborers. Only two confirmed incidents are known (Turner 1956; Turner 1972), in contrast to multiple incidents of brood sheltering, where the young can be found attached to the adult (Turner 1972; Turner 2002). Consequently, based on published records, the subfamily shows a high degree of localized provinciality. A single species, *Xylophaga washingtona*, is known from the Northeast Pacific (Johnson et al. 2006).

My goal was to assess the morphological diversity found within the collection area and to form morphological taxa, based on accessible characters and character states. My data support $H_A$ (page 24) by distinguishing eight new morphological taxa from 11 locations (plus one interim Taxon, Taxon Q). I distinguished the morphological taxa based on a subset of characters, used by other deep-sea wood-boring clam researchers, to describe new species (e.g. Pechenik et al. 1979; Hoagland and Turner 1981; Maser and Sedell 1994).

I tested the validity of the morphological taxa by sequencing the CO1 and 18S rDNA genes of a subsample of the specimens. The results support $H_{A2}$ (page 82) and indicate that Taxa B1, F, G, R, U, and X are distinct species. Taxon M is most likely a morphological variation of Taxon F (stressed individuals, Voight, personal communication). Even though the CO1 sequence of the morphological Taxon R is not yet available, the morphological characteristics in combination with the distinguishing 18S sequence lead me to assign the species status to this taxon. CO1 sequences for Taxon T are not available at this time. Since their morphological distinction was ambiguous due to missing or damaged characters, I will refrain from assigning the species status to this taxon, despite the distinctive 18S sequences.

Through corroboration from two genetic markers, I have proven that my morphological distinction method succeeds in identifying new species of deep-sea wood-boring clams. Thus, Figure 27 shows the locations at which the new species were found (adapted from Figure 10). consider Taxa B1, F, G, R, U and X as new species. Taxa B2, T1 and T2 are putative species (Table 16). More work is needed to confirm their status. I will continue to treat them as putative species. I cannot confirm the status of morphological Taxa M and Q and will continue to refer to them as taxa.

Table 16: Summary of newly discovered species and remaining taxa. '*' independently identified by Dr. Voight. '**' Hypothesis put forward by Dr. Voight

| Morphology | CO1 | 18S | Status | Species Comments | Distribution Comments |
|---|---|---|---|---|---|
| Taxon B | B1 | B1 | species | * | |
| | ? | B2 | putative | Could be *Xylophaga washingtona*, if not I think it would be a new species | Overlaps with distribution of *X. washingtona.* |
| Taxon F | F | FGM | species | * | |
| Taxon G | G | FGM | species | * | |
| Taxon R | ? | R | species | * | Only present on Gorda Ridge. |
| | | | | Morphology and 18S convincing. I believe it is a new species. | |
| Taxon U | U | ? | species | * | |
| Taxon X | X | ? | species | * | |
| Taxon T | ? | T1 | putative | Specimens degraded, morphology ambiguous but I believe they | |
| | ? | T2 | putative | could be new species, based on the 18S data. | |
| Taxon M | ? | FGM | unconfirmed | Probably stressed individual of Species F, ** | |
| Taxon Q | ? | ? | unconfirmed | Possibly another new species | |

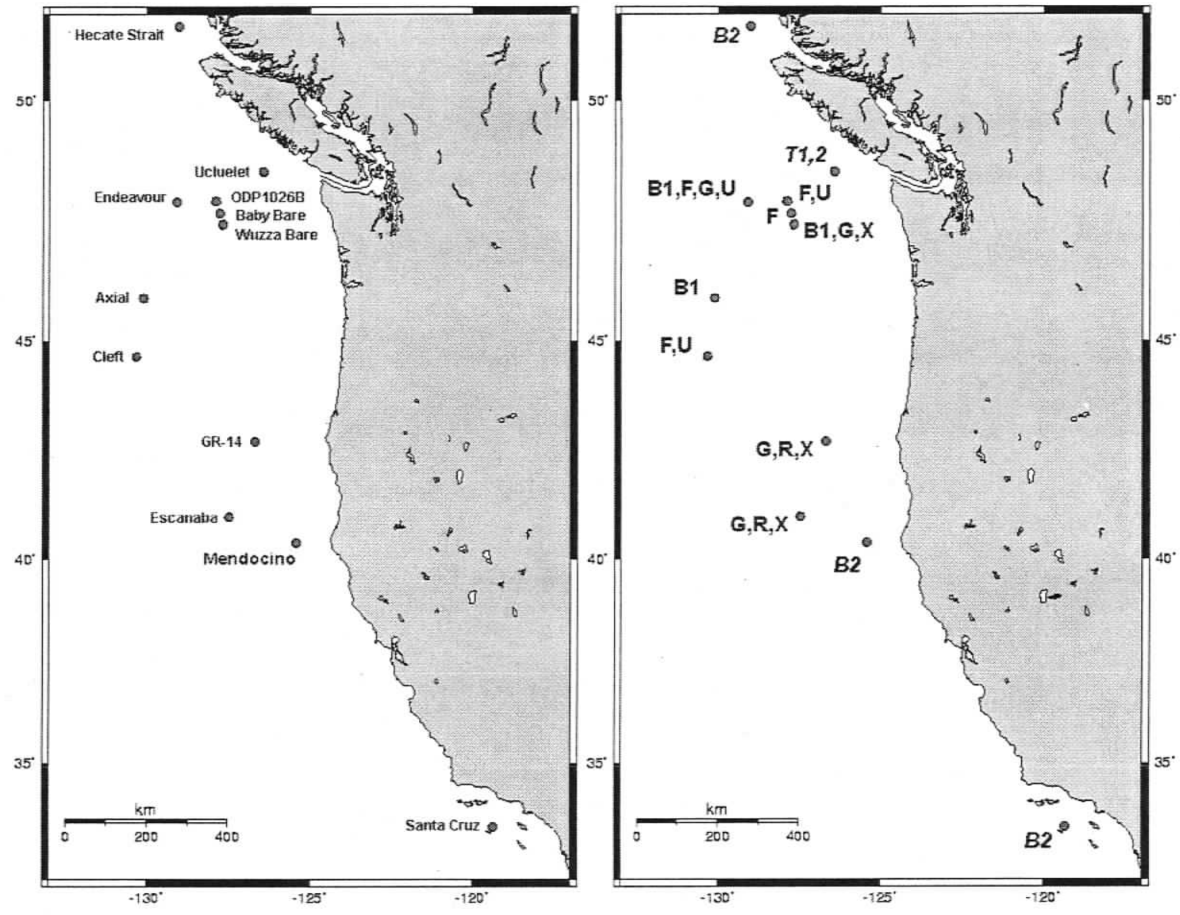


Figure 27: Geographic location of new species of wood-boring clams (Xylophagainae) from the Northeast Pacific. Confirmed species in 'bold', putative species in 'italics'. Unconfirmed Taxa M and Q not plotted.

**Morphological Assessment:** Even though I was not using all available characters for species descriptions, in this case, the characters I did use were sufficient for the separation of species. The most distinguishing characters were the shape and build of the mesoplax as well as the siphon characteristics (i.e. cirri presence or absence at incurrent and excurrent siphons). This was previously reported by Turner (1927). It appears that every new species displays one character that demarcates it from any of the other taxa (Figure 28).

| Species | Character | |
|---|---|---|
| B | triangular mesoplax |  |
| F | long excurrent cirri |  |
| G | short siphon (overall length) |  |
| R | flat triangular mesplax |  |
| U | flowerlike ring of cirri (siphon) |  |
| X | siphonal plates |  |

Figure 28: New species and their single most defining character.

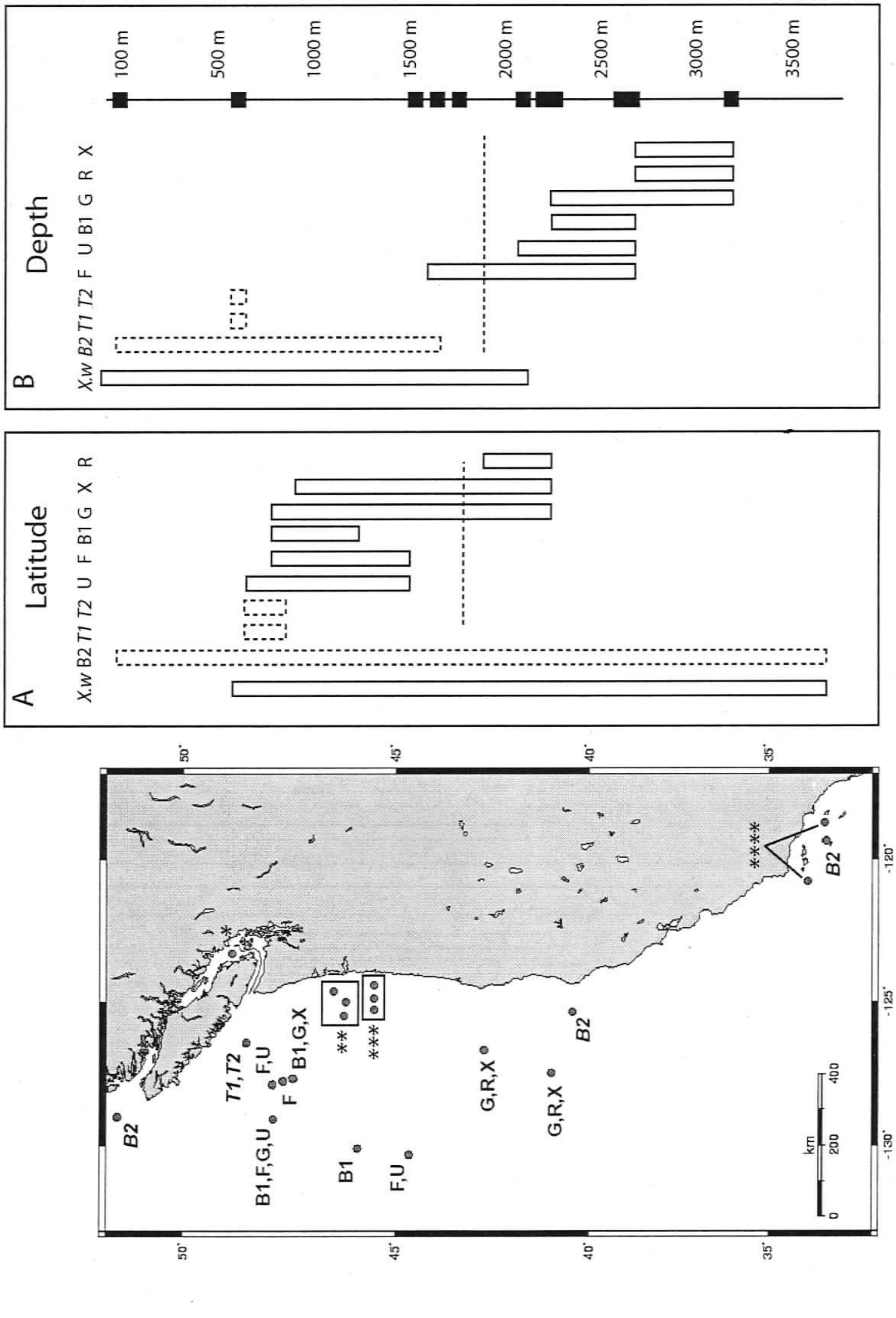
### 4.2 Biogeographic Patterns

The study of biogeography reveals distribution patterns of species in relation to their environment. I mapped the new species against their location (lat / long) and their depth to reveal species ranges from North to South in the Northeast Pacific as well in depth, from shallow to deep (Figure 29).

1. Geographic Location. Figure 29-A maps the latitudinal, North to South, distribution of the new species in the Northeast Pacific. There appears to be a latitudinal divide at the approximate location of the Blanco transform fault, separating the Juan de Fuca and Gorda Ridges (dotted line). A recent study on *Lepetodrilus fucensis*, a vent limpet from the Northeast Pacific, showed that the Blanco transform fault does pose a gene-flow barrier in this taxon (Distel and Roberts 1997). Although the Xylophagainae are not midocean-ridge-specific animals and the life history strategies of the newly discovered species are not known at the time, it is possible that the dispersing propagules of species R and X are not able to cross the fault due to ocean current properties. I did find shell fragments of a specimen at 'Ucluelet' that displayed the species R typical mesoplax. I furthermore have morphological evidence for the presence of species X at Wuzza Bare. I do not have any genetic corroboration for these morphological

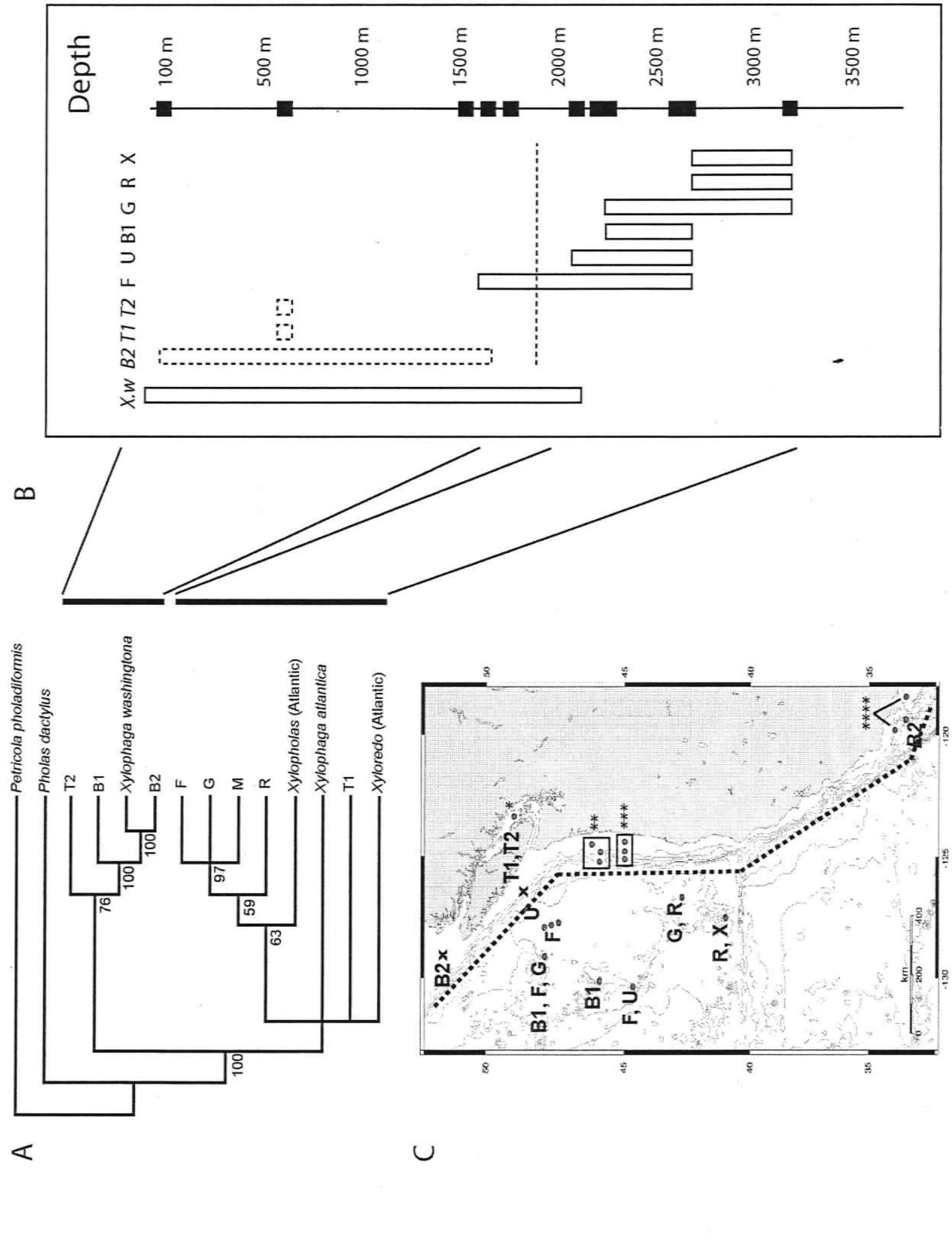
assessments. Their confirmation however, would extend species X and R's ranges to the North, by almost 2 degrees, voiding the gap in Figure 29-A (dotted line).

Figure 29: Geographic location of new species mapped against latitudinal distribution [A] and depth [B]. '*' *X. washingtona;* C.Carl '**' *X. washingtona;* Oregon State University '***' *X. washingtona;* R. Tipper '****' *X. washingtona;* Alan Hancock Foundation (Turner 2002). *X.w* = *Xylophaga washingtona*. Black squares on depth line indicate collection depths.


A
Latitude
X.w B2 T1 T2 U F B1 G X R
B
Depth
X.w B2 T1 T2 F U B1 G R X
100 m
500 m
1000 m
1500 m
2000 m
2500 m
3000 m
3500 m
50°
45°
40°
35°
-130°
-125°
-120°
B2
B1,F,G,U
T1,T2
F,U
F
B1,G,X
B1
F,U
**
***
G,R,X
G,R,X
B2
****
B2
*
km
0
200
400

2. Depth. Figure 29-B maps the depths (shallow to deep) of the new species in the Northeast Pacific. The dotted line indicates a break in the depth distribution of the new species. The black squares on the depth-line indicate the depths of the sampling sites. Putative species B2, T1 and T2 appear to favour a shallower niche (< 1800 m), while species B1, G, R, U and X favour depths below 2000 m. Species F bridges the gap, living between ~1500 and ~ 2600 m.
   The divide between these two species groups is supported by the phylogenetic analysis of the 18S rDNA gene (Figure 30-A). The NJ and MP-trees display a similar pattern, although T1 falls out in the 'deep' clade (Chapter 3, Figure 7-A,B). The position of T1, however, is unresolved in the MP-tree (Chapter 3, Figure 7-B). Furthermore, the assessment of the morphological Taxon T was most problematic of all taxa. Together with missing CO1 data, that would help interpret the species status, this could explain the discrepancies seen in the phylogenies.
   It appears that the 'shallow' clade, for the most part, corresponds with the area covered by the continental shelf. The location 'Santa Cruz' however is ~ 1700 m deep and not on the continental shelf (Figure 30-C) Proximity to land could be more important than depth per se. Distance to shore would have effects such as: variable supply of wood (Newell 1979), different current exposure (North Pacific Gyre, Equatorial Counter Current, near-shore counter currents, etc.) that alter variables like oxygen, temperature and nutrient content of the water.

Figure 30: Relationship between species and depth. [A] 18S rDNA MP-tree (Note that I changed the layout of the tree by swiveling branches around their nodes.) Two branches supported by a bs value of 100, highlighted by black bars, correspond with species living shallower and deeper than 1800 m respectively [B] Species specific depth ranges. Black squares on depth bar indicate sampling location depths. [C] Map of collection area. The shallow clade appears to correspond with samples collected on the continental shelf or slope. The deep clade corresponds with samples from the abyssal plain and mid-ocean ridges. '*' *X. washingtona;* C.Carl '**' *X. washingtona;* Oregon State University '***' *X. washingtona;* R. Tipper '****' *X. washingtona;* Alan Hancock Foundation (Turner 2002)


A
Petricola pholadiformis
Pholas dactylus
T2
B1
Xylophaga washingtona
B2
F
G
M
R
Xylopholas (Atlantic)
Xylophaga atlantica
T1
Xyloredo (Atlantic)
100
76
100
100
97
59
63
B
Depth
100 m
500 m
1000 m
1500 m
2000 m
2500 m
3000 m
3500 m
X.w
B2
T1
T2
F
U
B1
G
R
X
C
B2
T1,T2
U
F
B1, F, G
B1
F, U
G, R
R, X
B2
*
**
***
****
50
45
40
35
-130
-125
-120
km
0
200
400

## 4.3 Niche Separation

Several species ccur at the same location. This leads me to suspect that, to avoid competition, these species inhabit different micro-niches within the wood blocks. The nature of these micro-niches was not the focus of this study and remains to be investigated. Based on circumstantial data however, I propose the following micro-niches:

1. Sympatric species utilize different food sources.
   a. "Wood" via the cellulolytic activity of bacterial endosymbionts, housed in the gills.
   b. "Suspension-Feeding": Cirri on the incurrent siphon allow for selection of suitable food material from the pelagos.
   c. "Deposit-Feeding": Cirri on the incurrent siphon allow for selection of suitable food material from fallen detritus.
   d. "Bacteria-Harvesting": Bacteria are grown externally and harvested via the siphon.

   Although Hoagland and Turner suggested a cessation of wood-boring for filter-feeding upon crowding of the wood block, no data exist that would confirm these feeding habits.

   The data supporting the utilization of wood as a food source remain circumstantial. The Teredinidae display this particular symbiosis, where bacteria in the gills digest the wood . In the Xylophagainae, bacteria, similar in their morphology and anatomical location within the gills, were found in *X. atlantica* and *X. washingtona* . Yet, to date, the connection between the cellulolytic bacteria in the gills and the wood-chip containing caecum has not been proven experimentally.

   The idea of external bacterial harvesting has not been proposed in the Xylophagainae. This feeding mode is known from other molluscs .

2. Seasonal Succession Niche. I observed likely succession phenomena on two occasions. The first incident of a succession was at Cleft, where we retrieved two sets of wood blocks. WB-A is the 12-month retrieval, WB-B is the 24-month retrieval. Species F is the dominant species in WB-A. The wood blocks of WB-B are heavily deteriorated. I found Species U in small numbers deep within the wood blocks. I did not find any intact specimens of Species F. The only remnants of Species F are empty shells, displaying the species-specific characters. The second incident of a possible succession occurred at Ucluelet, the location of an opportunistic, deep-sea, wood find. Only morphological Taxon T is present at this location. However, I found empty shells of what might be Species R, deeper in the wood. Species R is so far only known from the Gorda Ridge.
   Succession presents an opportunity for deep-sea wood-boring clams to exploit a niche, unoccupied by other species. Species F (at Cleft) appears to be an early successional species, aka. it arrives early and grows to high numbers. As the wood deteriorates (due to boring), Species F might succumb to predation. Species U makes use of the wood remains by hiding deep within the sponge-like structure.

3. Boring-Depth. Different species might occupy different depths in the woodblocks. I observed species (F, B), which appear to favour deep positions. Largest animals reside deepest in the wood block. Other species (G) reside directly below the wood surface. Unfortunately F and G never co-occurred in one block.

### 4.4 Methods

Conducting rigorous scientific experiments in the deep sea is inherently difficult. Accessing remote study sites and handling scientific contraptions continue to pose difficulties for research submersibles as well as funding agencies. Using experimental wood blocks facilitates aspects of the research, yet I encountered difficulties:

a) # of sample locations
b) Regular sample location visits
c) Specimen extraction (from wood)
d) Fixation and preservation

**Sample Locations:** Due to financial restrictions, accessing sampling sites at a specific point in time was not possible. My research depended on the opportunistic usage of collaborative research cruises. To analyze patterns displayed by the Xylophagainae, more rigorous sampling of the Northeast Pacific will be necessary. I discuss an observed pattern (e.g. the latitudinal gap, Figure 29) in my samples. This pattern might, however, change with increased sampling sites. Furthermore, accessing sampling sites at regular time intervals is crucial for the study of seasonality and succession phenomena.

Figure 31 shows a suggested experimental design for a settlement plate setup that can be used in areas where regular visits are guaranteed (submersible or SCUBA). The device consists of a main baseplate, to which ten wood blocks can be attached magnetically. Four of these devices are currently deployed in Saanich Inlet, BC, Canada at approximately 100 m, as well as in the Strait of Georgia, at approximately 200 m. Upon wood block exchange, the research submersible ROPOS would detach specific wood blocks and replace them with 'fresh' ones. Initial results from recovered wood blocks have shown that no members of the Xylophagainae inhabit Saanich Inlet. *Bankia setacea*, a member of the sister family Teredinidae (ship-worms), are present in high numbers.

An alternative to regular access via submersible or SCUBA is to construct a benthic lander that exposes woodblocks to the deep sea at regular intervals, governed by a pre-programmed control unit (Figure 32).

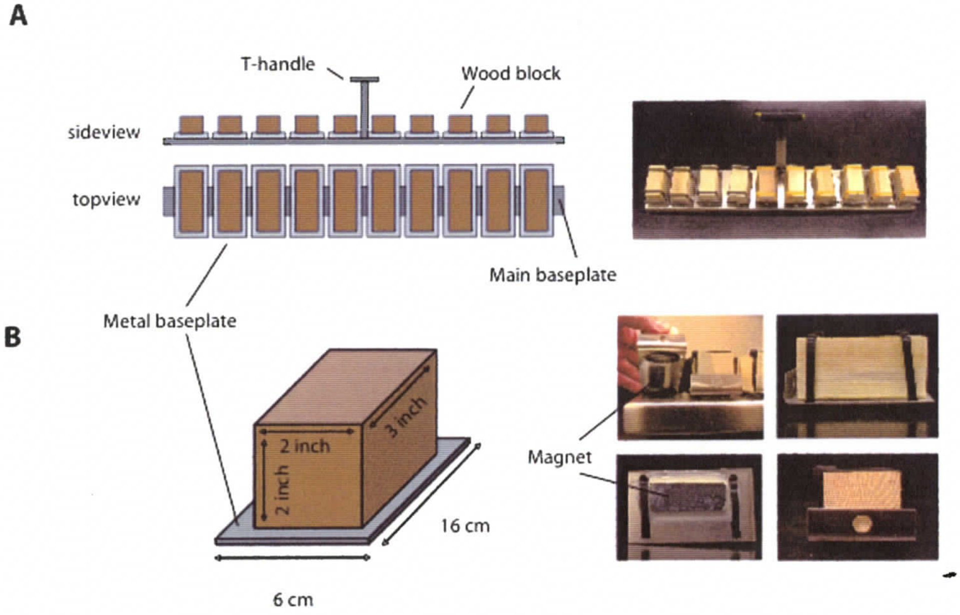


Figure 31: Settlement Rig. [A] Rig overview. [B] Wood block on baseplate with magnets.

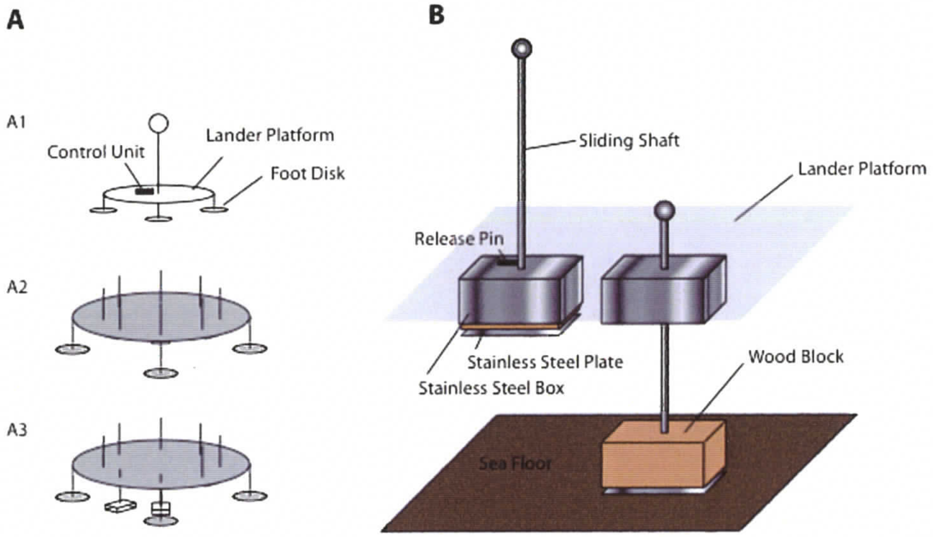


Figure 32: Benthic lander - seasonality and succession. [A] Overview [A1] Benthic lander, consisting of lander platform with feet and control unit, responsible for pulling release pins [A2] Deployed lander [A3] Deployed lander with two released wood blocks [B] Detail view of retracted wood block and released wood block.

**Specimen Extraction:** The Xylophagainae presumably bore into wood, at least in part, to protect themselves from predators. Manually extracting the specimens from a solid piece of fir demonstrated the degree of protection wood can provide. I had to use power-tools and a hammer and chisel to split the wood blocks, to gain access to the specimens. This procedure damaged many of the samples, leading to difficulties during the morphological assessment process. I have constructed and deployed 'sliced' and spring-loaded wood blocks (superblocks) that, upon recovery, allow for a quick access to the specimens (Figure 33). I chose marine grade stainless steel for all metal parts to avoid corrosion. The entire contraption was placed in mesh-bags as described in Chapter 2. I have not recovered such a block to date. Upon removal of the springs and metal brackets, the wood slats fan out, yielding access to a much higher percentage of the contained fauna.

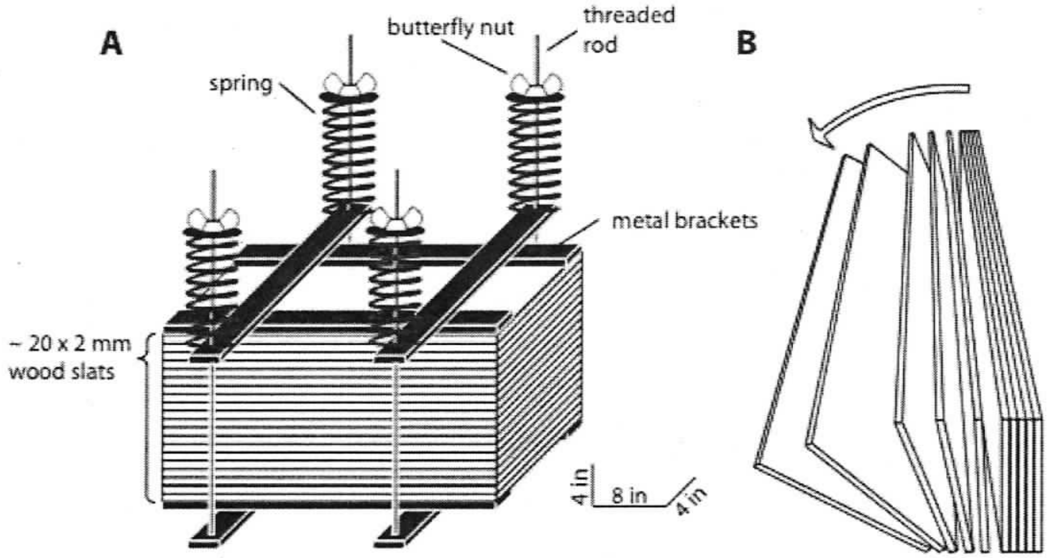


Figure 33: [A] Superblock ready for deployment. Two metal brackets are connected via four threaded rods. Together they press approximately 20 2 mm wide wood slats together. Upon recovery and removal of the springs and brackets, the wood slats fan out for easy access to the specimes [B].

**Fixation and Preservation:** Fixation and preservation are both crucial steps when attempting to perform a morphological and/or genetic study. The superblocks (Figure 33) can be fanned while submerged in a fixative, allowing for a faster fixation and preservation compared to an intact wood block (see Chapter 2).

Initially, my study was not designed with genetic research in mind. Many specimens were fixed in 70% EtOH or 7% buffered Formalin. Extraction of mollusk DNA is difficult per se (Chapter 3). Specimens preserved in EtOH < 90% render DNA extraction difficult and tend to produce low DNA yields. Freezing the samples leads to tissue lysation, which destroys the morphological characters.

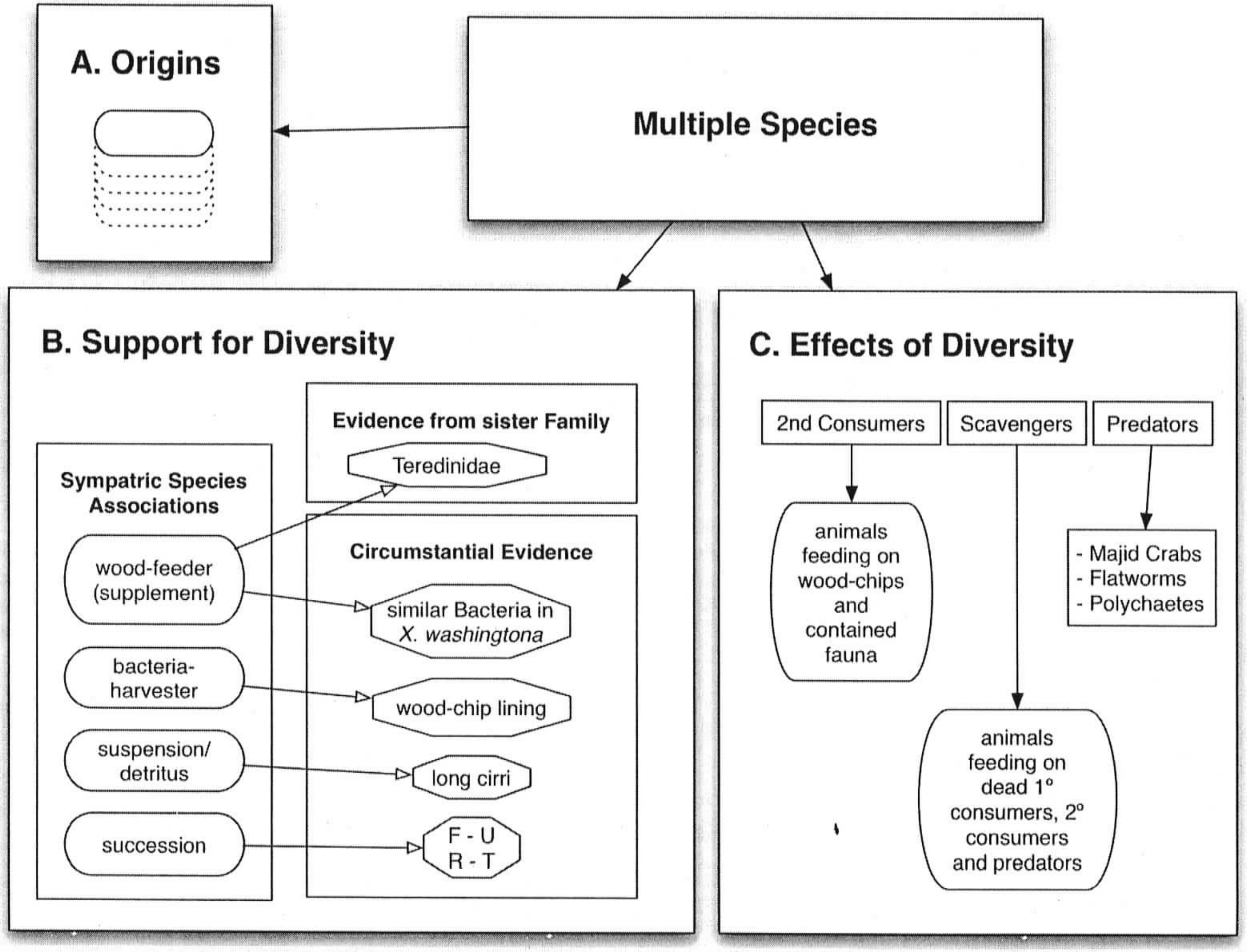
### 4.5 Future Directions

I have provided evidence for the existence of multiple species in the Northeast Pacific (Chapter 2-3). Circumstantial evidence from other molluscan systems, leads me to suggest sympatric species associations based on different feeding strategies. Figure 34 illustrates the function of the Xylophagainae as 'termites' of the deepsea, facilitating access to carbon, otherwise locked in cellulose, via their boring and symbiotic wood-digestion activities. I propose three future research directions: A) The evolutionary origin of the multiple species in the Northeast Pacific. Is there a single ancestor that gave rise to the diversity through adaptive radiation? Are there multiple independent immigrations and adaptive radiations? B) The analysis of the micro-niches inhabited by the wood borers is crucial for the understanding of the community dynamics of the subfamily. Basic biological data such as the type of life history strategy, the main food source, mating and dispersal behaviour need to be addressed in future studies. C) To date, no one has published a report on the community structure of wood-associated fauna. We do know that newly deployed wood blocks attract macro-predators such as the deep-sea spider crab *Macroregonia macrochira*. Smaller predators such as flatworms (Dr. Voight, personal communication) and an unknown polychaete (personal observation) exist as well. It is fair to assume that the clams as 1° consumers would also attract, not only top predators, but also 2nd consumers and scavengers, feeding on expelled wood-chips and the fauna (probably bacteria) contained therein as well as deceased members of the community.

Finally, more samples are needed to investigate further the species richness, not only in the Northeast Pacific, but globally. Only when we can compare multiple samples from multiple oceans, and regions within those, can we begin to elucidate the phylogenetic relationships within the Xylophagainae and make statements about phylogenetic descent, adaptive radiations and speciation.

I regard the Consortium for Barcoding of Life as instrumental in such an endeavour as its main goal is to facilitate the comprehension of what is where. Increasingly large, online databases are going to have a snowballing effect, where access to information facilitates the generation of more data.

Figure 34: Future directions flow chart. Evolutionary precursor(s) are unknown to date. Speculative information is displayed in rounded squares. Facts are displayed in squares and evidence is displayed in octagons.


A. Origins
Multiple Species
B. Support for Diversity
Sympatric Species Associations
wood-feeder (supplement)
bacteria-harvester
suspension/detritus
succession
Evidence from sister Family
Teredinidae
Circumstantial Evidence
similar Bacteria in *X. washingtona*
wood-chip lining
long cirri
F - U
R - T
C. Effects of Diversity
2nd Consumers
Scavengers
Predators
animals feeding on wood-chips and contained fauna
animals feeding on dead 1° consumers, 2° consumers and predators
- Majid Crabs
- Flatworms
- Polychaetes

### 4.6 Conclusions

My results indicate that the species richness of deep-sea wood-boring clams in the Northeast Pacific has been underestimated. With the discovery of at least six (B1, F, G, R, U, and X) new species, corroborated both by morphology and genetics, we increased the total species count in the Northeast Pacific from one to at least seven. My results furthermore suggest that the 41 known species of deep-sea woodborers (Chapter 1) are an underestimate of the global species richness. The most likely cause for this underestimation must lie in the scarce number of specifically targeted samples in the world's oceans. A multi-disciplinary approach such as the one displayed in this study is crucial.

## Bibliography

Avise, J. C. (2000). Phylogeography - The history and formation of species. Cambridge, MA, Harvard University Press.

Baco, A. R. and C. R. Smith (2003). "High species richness in deep-sea chemoautotrophic whale skeleton communities." Marine Ecology-Progress Series **260**: 109-114.

Baker, A., F. Rowe, et al. (1986). "A new class of Echinodermata from New Zealand." Nature **321**: 862-864.

Bartsch, P. (1921). "A new classification of the shipworms and description of some new wood boring mollusks." Proceedings of the Biological Society of Washington **34**: 25-32.

Baverstock, P. and C. Moritz (1990). Sampling design. Molecular Systematics. D. M. Hillis and C. Moritz. Sunderland, Sinauer Associates: 13-24.

Beaulieu, S. E. (2001). "Colonization of habitat islands in the deep sea: recruitment to glass sponge stalks." Deep-Sea Research Part I-Oceanographic Research Papers **48**(4): 1121-1137.

Bock, W. J. (1986). Species concepts, speciation and macroevolution. Modern aspects of species. K. Iwatsuki, P. Raven and W. J. Bock. Tokyo, University of Tokyo Press: 31-57.

Bock, W. J. (2004). "Species: the concept, category and taxon." Journal of Zoological Systematics and Evolutionary Research **42**(3): 178-190.

Boynton, L. C. and R. C. Miller (1927). "The occurrence of a cellulase in the ship-worm." The Journal of Biological Chemistry **75**: 613-618.

Brown, D., P. Wensink, et al. (1972). "Comparison of the ribosomal dna's of Xenopus laevis and Xenopus mulleri: the evolution of tandem genes." Journal of Molecular Biology **63**: 57-73.

Burland, T. (2000). DNASTAR's Lasergene sequence analysis software. Methods in Molecular Biology. Madison, WI, USA, DNASTAR. **132:** 71-91.

Cavalli-Sforza, L. and A. Edwards (1967). "Phylogenetic analysis: Models and estimation procedures." American Journal of Human Genetics **19**: 233-257.

Chen, Z., W. Yan, et al. (2006). "Discovery of seep carbonate nodules as new evidence for gas venting on the northern continental slope of South China Sea." Chinese Science Bulletin **51**(10): 1228-1237.

Chomczynski, P. and N. Sacchi (1987). "Single-Step Method of Rna Isolation by Acid Guanidinium Thiocyanate Phenol Chloroform Extraction." Analytical Biochemistry **162**(1): 156-159.

Coan, E. V., P. Valentich Scott, et al. (2000). Bivalve seashells of western North America. Santa Barbara, Museum of Natural History.

Culliney, J. and R. Turner (1976). "Larval Development of the Deep-Water Wood Boring Bivalve, *Xylophaga atlantica* Richards (Mollusca, Bivalvia, Pholadidae)." Ophelia **15**: 149-161.

de Ronde, C. E. J., P. Stoffers, et al. (2002). "Discovery of active hydrothermal venting in Lake Taupo, New Zealand." Journal of Volcanology and Geothermal Research **115**(3-4): 257-275.

Deburgh, M. E. and C. L. Singla (1984). "Bacterial-Colonization and Endocytosis on the Gill of a New Limpet Species from a Hydrothermal Vent." Marine Biology **84**(1): 1-6.

Desbruyeres, D. and L. Laubier (1988). "Exploitation of a Concentrated Organic-Matter Source in the Deep-Sea - Role of a New Polychaetous Annelid." Comptes Rendus De L Academie Des Sciences Serie Iii-Sciences De La Vie-Life Sciences **307**(6): 329-335.

Distel, D. L., A. R. Baco, et al. (2000). "Marine ecology - Do mussels take wooden steps to deep-sea vents?" Nature **403**(6771): 725-726.

Distel, D. L., D. L. Beaudoin, et al. (2002). "Coexistence of Multiple Proteobacterial Endosymbionts in the Gills of the Wood-Boring Bivalve *Lyrodus pedicellatus* (Bivaliva: Teredinidae)." Applied and Environmental Microbiology **68**(12): 6292-6299.

Distel, D. L. and S. J. Roberts (1997). "Bacterial endosymbionts in the gills of the deep-sea wood-boring bivalves Xylophaga atlantica and Xylophaga washingtona." Biological Bulletin **192**(2): 253-261.

Dobzhansky, T. (1937). Genetics and the origin of species. New York, Columbia University Press.

Fisher, C. R. (1990). "Chemoautotrophic and Methanotrophic Symbioses in Marine-Invertebrates." Reviews in Aquatic Sciences **2**(3-4): 399-436.

Fitch, W. (1977). "On the problem of discovering the most parsimonious tree." The American Naturalist **111**: 223-257.

Folmer, O. (1994). DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. Biology. New Brunswick, State University of New Jersey: 38.

Gage, J. D. (1996). "Why are there so many species in deep-sea sediments?" Journal of Experimental Marine Biology and Ecology **200**(1-2): 257-286.

Giribet, G., S. Carranza, et al. (1996). "First molecular evidence for the existence of a Tardigrada + Arthropoda clade." Molecular Biology and Evolution **13**(1): 76-84.

Giribet, G. and D. L. Distel (2003). Bivalve phylogeny and molecular data. Molecular Systematics and Phylogeography of Mollusks. C. Lydeard and D. Lindberg**:** 45-90.

Grassle, F. J. and N. Maciolek (1992). "Deep-sea species richness: regional and local diversity estimates from quantitative bottom samples." The American Naturalist **139**: 313-341.

Graur, D. and W.-H. Li (2000). Fundamentals of molecular evolution. Sunderland, Sinauer Associates Inc.

Gray, J. S. (2002). "Species richness of marine soft sediments." Marine Ecology-Progress Series **244**: 285-297.

Haderlie, E. C. (1983). "Depth Distribution and Settlement Times of the Molluscan Wood Borers Bankia-Setacea (Tryon, 1863) and Xylophaga-Washingtona Bartsch, 1921, in Monterey Bay." Veliger **25**(4): 339-&.

Hajibabaei, M., D. H. Janzen, et al. (2006). "DNA barcodes distinguish species of tropical Lepidoptera." PNAS **103**(4): 968-971.

Harvey, R. (1996). "Deep water Xylophagaidae (Pelecypoda:Pholadacea) from the North Atlantic with descriptions of three new species." Journal of Conchology **35**: 473-481.

Hashimoto, J., S. Ohta, et al. (2001). "First hydrothermal vent communities from the Indian Ocean discovered." Zoological Science **18**(5): 717-721.

Hebert, P. D., A. Cywinska, et al. (2003). "Biological identifications through DNA barcodes." Procedings of the Royal Society of London **270**: 313-321.

Hebert, P. D., A. Cywinska, et al. (2003). "Biological identifications through DNA barcodes." Proc. R. Soc. Lond. **270**: 313-321.

Hebert, P. D. N., E. H. Penton, et al. (2004). "Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator." Proceedings of the National Academy of Sciences of the United States of America **101**(41): 14812-14817.

Hebert, P. D. N., M. Y. Stoeckle, et al. (2004). "Identification of birds through DNA barcodes." Public Library of Science Biology **2**(10): 1657-1663.

Hecker, B. (1985). "Fauna from a cold sulfur-seep in the Gulf of Mexico: comparison with hydrothermal vent communities and evolutionary implications." Bulletin of the Biological Society of Washington **6**: 465-473.

Hillis, D. M. and S. K. Davis (1988). "Ribosomal DNA - Intraspecific Polymorphism, Concerted Evolution, and Phylogeny Reconstruction." Systematic Zoology **37**(1): 63-66.

Hillis, D. M. and M. T. Dixon (1991). "Ribosomal DNA - Molecular Evolution and Phylogenetic Inference." Quarterly Review of Biology **66**(4): 410-453.

Hoagland, K. E. and R. D. Turner (1981). "Evolution and Adaptive Radiation of Wood-Boring Bivalves (Pholadacea)." Malacologia **21**(1-2): 111-148.

Hogg, I. D. and P. D. N. Hebert (2004). "Biological identification of springtails (Hexapoda : Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes." Canadian Journal of Zoology-Revue Canadienne De Zoologie **82**(5): 749-754.

Huelsenbeck, J. P. and F. Ronquist (2001). "MRBAYES: Bayesian inference of phylogenetic trees." Bioinformatics **17**(8): 754-755.

Huelsenbeck, J. P., F. Ronquist, et al. (2001). "Evolution - Bayesian inference of phylogeny and its impact on evolutionary biology." Science **294**(5550): 2310-2314.

Hyland, J., E. Baptiste, et al. (1991). "Macroinfaunal Communities of the Santa-Maria Basin on the California Outer Continental-Shelf and Slope." Marine Ecology-Progress Series **78**(2): 147-161.

Ivanova, N., J. R. deWaard, et al. (2006). "An inexpensive, automation-friendly protocol for recovering high-quality DNA." Molecular Ecology Notes **(in press)**.

Johnson, S. B., C. R. Young, et al. (2006). "Migration, isolation, and speciation of hydrothermal vent limpets (Gastropoda ; Lepetodrilidae) across the Blanco Transform Fault." Biological Bulletin **210**(2): 140-157.

Jones, W. J., Y. J. Won, et al. (2006). "Evolution of habitat use by deep-sea mussels." Marine Biology **148**(4): 841-851.

Juniper, K. (2003). Deep-sea hydrothermal vent and seep habitats and related governance issues. Workshop on the Governance of high seas biodiversity conservation, Cairns, Australia, highseasconservation.org.

Karl, D. M. (1999). "A sea of change: biogeochemical variability in the north Pacific subtropical gyre." Ecosystems **2**: 181-214.

Klinkhammer, G. P., C. S. Chin, et al. (2001). "Discovery of new hydrothermal vent sites in Bransfield Strait, Antarctica." Earth and Planetary Science Letters **193**(3-4): 395-407.

Knudsen, J. (1961). The bathyal and abyssal *Xylophaga*. Galathea Report, Volume 5. T. Wolff. Copenhagen, Danish Science Press, LTD.**:** The bathyal and abyssal Xylophaga. Galathea Report, Volume 5:162-209.

Lee, M. S. Y. (2003). "Species concepts and species reality: salvaging a Linnaean rank." Journal of Evolutionary Biology **16**(2): 179-188.

Levin, L. A. (2005). Ecology of cold seep sediments: Interactions of fauna with flow, chemistry and microbes. Oceanography and Marine Biology - an Annual Review, Vol. 43. **43:** 1-46.

Lissner, A. L., G. L. Taghon, et al. (1991). "Recolonization of Deep-Water Hard-Substrate Communities - Potential Impacts from Oil and Gas Development." Ecological Applications **1**(3): 258-267.

Lutz, R. A. (1988). "Dispersal of organisms at deep-sea hydrothermal vents: a review." Oceanologica Acta: 23.29.

Maddison, D. R. and W. P. Maddison (2003). MacClade Version 4.06 for OS X. Sunderland, Massachusetts, Sinauer Associates, Inc.

Maddocks, R. F. and P. L. Steineck (1987). "Ostracoda from Experimental Wood-Island Habitats in the Deep-Sea." Micropaleontology **33**(4): 318-355.

Mallet, J. (1995). "A species definition for the modern synthesis." Trends in Ecology & Evolution **10**: 294-299.

Maser, C. and J. Sedell (1994). From the forest to the sea. The ecology of wood in streams, rivers, estuaries and oceans. Delray Beach, FL, St. Lucie Press.

May, R. M. (1992). "Bottoms up for the oceans." Nature **357**: 278-279.

May, R. M. (1993). "Marine Species Richness - Reply." Nature **361**(6413): 598-598.

Mayden, R. (2002). "On biological species, species concepts and individuation in the natural world." Fish and Fisheries **3**(3): 171-196.

Mayden, R. and R. Wood (1995). "Systematics, species concepts, and the evolutionary significant unit in biodiversity and conservation biology." American Fisheries Society Symposium.

Mayr, E. (1940). "Speciation phenomena in birds." Amer. Nat. **74**: 249-278.

Mayr, E. (1942). Systematics and the origin of species. New York, Columbia University Press.

McLean, J. H. (1993). "New Species and Records of Lepetodrilus (Vetigastropoda, Lepetodrilidae) from Hydrothermal Vents." Veliger **36**(1): 27-35.

Menzies, R. (1965). "Conditions for existence of life on the abyssal sea floor." Oceanography and Marine Biology Annual Review **3**: 195-210.

Meyer, C. P. and G. Paulay (2005). "DNA barcoding: Error rates based on comprehensive sampling." Public Library of Science Biology **3**(12): 2229-2238.

Mishler, B. D. and R. Brandon (1987). "Individuality, pluralism and the phylogenetic species concept." Biol. Phil. **2**: 397-414.

Miyazaki, J. I., M. Shintaku, et al. (2004). "Phylogenetic relationships of deep-sea mussels of the genus Bathymodiolus (Bivalvia : Mytilidae)." Marine Biology **144**(3): 527-535.

Mullineaux, L. S. (1987). "Organisms Living on Manganese Nodules and Crusts - Distribution and Abundance at 3 North Pacific Sites." Deep-Sea Research Part a-Oceanographic Research Papers **34**(2): 165-184.

Mullineaux, L. S. (1994). Implications of mesoscale flows for dispersal of deep-sea larvae. Reproduction, larval biology and recruitment of the deep-sea benthos. C. Young and K. Eckelbarger. New York, Columbia University Press: 201-222.

Nelson, D. and C. Fisher (1995). Chemoautotrophic and methanotrophic endosymbiotic bacteria at deep-sea vents and seeps. The Microbiology of Deep-Sea Hydrothermal Vents. D. M. Karl. Boca Raton, CRC Press.

Newell, R. C. (1979). Biology of intertidal animals. Faversham, Kent, Marine Ecological Surveys Ltd.

Ohmoto, H. (1996). "Evidence in pre-2.2 Ga paleosols for the early evolution of atmospheric oxygen and terrestrial biota." Geology **24**(12): 1135-1138.

Parker, P. G., A. A. Snow, et al. (1998). "What molecules can tell us about populations: Choosing and using a molecular marker." Ecology **79**(2): 361-382.

Parker, T. and V. Tunnicliffe (1994). "Dispersal Strategies of the Biota on an Oceanic Seamount: Implications for Ecology and Biogeography." Biological Bulletin **187**: 336-345.

Paull, C. K., B. Schlining, et al. (2005a). "Distribution of chemosynthetic biological communities in Monterey Bay, California." Geology **33**(2): 85-88.

Paull, C. K., W. Ussler, et al. (2005b). "Bioerosion by chemosynthetic biological communities on Holocene submarine slide scars." Geo-Marine Letters **25**(1): 11-19.

Pechenik, J. A., F. E. Perron, et al. (1979). "The role of phytoplankton in the diets of adult larval shipworms, Lyrodus pedicillatus (Bivalvia: Teredinidae)." Estuaries **2**(1).

Pei-Ning, X. and D. L. Distel (2004). "Purification and characterization of an endo-1, 4-$\beta$-D glucanase from the cellulolytic system of the wood-boring marine mollusk *Lyrodus pedicellatus* (Bivalvia: Teredinidae)." Marine Biology **144**: 947-953.

Poore, G. C. B. and G. D. F. Wilson (1993). "Marine Species Richness." Nature **361**(6413): 597-598.

Posada, D. and K. Crandall (1998). "Modeltest: testing the model of DNA substitution." Bioinformatics **14**(9): 817-818.

Purchon, R. (1941). "On the biology and relationships of the lamellibranch *Xylophaga dorsalis* (Turton)." Journal of the Marine Biological Association of the United Kingdom **25**(1): 1-39.

Rannala, B. and Z. H. Yang (1996). "Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference." Journal of Molecular Evolution **43**(3): 304-311.

Romey, W. L., R. C. Bullock, et al. (1994). "Rapid Growth of a Deep-Sea Wood-Boring Bivalve." Continental Shelf Research **14**(12): 1349-&.

Romey, W. L., K. M. Castro, et al. (1991). "Recruitment in the Deep-Sea Wood-Boring Bivalve Xylophaga-Atlantica Richards." Veliger **34**(1): 14-20.

Ronquist, F. and J. P. Huelsenbeck (2003). "MrBayes 3: Bayesian phylogenetic inference under mixed models." Bioinformatics **19**(12): 1572-1574.

Rosenzweig, M. (1995). Species diversity in space and time. Cambridge, Cambridge University Press: 436 pp.

Rossello-Mora, R. (2003). "Opinion: The species problem, can we achieve a universal concept?" Systematic and Applied Microbiology **26**(3): 323-326.

Roy, K., D. Jablonski, et al. (1994). "Eastern Pacific Molluscan Provinces and Latitudinal Diversity Gradient - No Evidence for Rapoports Rule." Proceedings of the National Academy of Sciences of the United States of America **91**(19): 8871-8874.

Saitou, N. and N. Masatoshi (1987). "The neighbor-joining method: a new method for reconstructing phylogenetic trees[1]." Molecular Biology and Evolution **4**(4): 406-425.

Santhakumaran, L. (1980). "Two new species of *Xylophaga* from Trondheimsfjorden, western Norway (Mollusca, Pelecypoda)." Sarsia **65**(3-4): 269-272.

Santhakumaran, L. (1984). "Settlement and boring activity of *Xylophaga dorsalis* (Mollusca: Xilophagainae) in different species of timber exposed in Trondheimsfjorden (western Norway)." Oebalia **X**: 59-67.

Schoener, A. (1988). Experimental island biogeography. Analytical Biogeography: an integrated approach to the study of animal and plant distributions. A. A. Myers and P. S. Giller. London, Chapman & Hall**:** 483-512.

Shank, T. M. (2004). "The evolutionary puzzle of seafloor life." Oceanus **42**(2): 1-8.

Shank, T. M., M. B. Black, et al. (1999). "Miocene radiation of deep-sea hydrothermal vent shrimp (Caridea : Bresiliidae): Evidence from mitochondrial cytochrome oxidase subunit I." Molecular Phylogenetics and Evolution **13**(2): 244-254.

Sibuet, M. and K. Olu (1998). "Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins." Deep-Sea Research Part Ii-Topical Studies in Oceanography **45**(1-3): 517-+.

Simpson, G. (1961). Principles of animal taxonomy. New York, Columbia University Press. **xiii:** 247 pp.

Smith, C. R. and A. R. Baco (1998). "Phylogenetic and functional affinities between whale-falls, seep and vent communities." Cahiers de Biologie Marine **39**: 345-346.

Smith, C. R. and A. R. Baco (2003). "Ecology of whale falls at the deep-sea floor." Oceanography and Marine Biology: an Annual Review **41**: 311-354.

Smith, C. R., H. Kukert, et al. (1989). "Vent Fauna on Whale Remains." Nature **341**(6237): 27-28.

Sokal, R. R. and T. J. Crovello (1970). "Biological Species Concept - a Critical Evaluation." American Naturalist **104**(936): 127-&.

Southward, E. C., V. Tunnicliffe, et al. (1995). "Revision of the Species of Ridgeia from Northeast Pacific Hydrothermal Vents, with a Redescription of Ridgeia-Piscesae Jones (Pogonophora, Obturata Equals Vestimentifera)." Canadian Journal of Zoology-Revue Canadienne De Zoologie **73**(2): 282-295.

Stecher, J., V. Tunnicliffe, et al. (2003). "Population characteristics of abundant bivalves (Mollusca, Vesicomyidae) at a sulphide-rich seafloor site near Lihir Island, Papua New Guinea." Canadian Journal of Zoology **81**: 1815-1824.

Stockton, W. L. and T. E. DeLaca (1982). "Food Falls in the Deep-Sea - Occurrence, Quality, and Significance." Deep-Sea Research Part a-Oceanographic Research Papers **29**(2): 157-169.

Sverdrup, H., M. Johnson, et al. (1946). The oceans, their physics, chemistry and general biology. New York, Prentice-Hall.

Swofford, D. L. (2002). PAUP* Version 4.0b10 for Macintosh. Sunderland, Massachusetts, Sinauer Associates.

Templeton, A. R. (1989). The meaning of species and speciation. Speciation and its consequences. D. Otte and J. Endler. Sunderland, MA, Sinauer**:** 3-27.

Thompson, J., D. Higgins, et al. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighing, position specific gap penalties and weight matrix choice. Nucleic Acids Research. **22:** 4673-4680.

Tipper, R. C. (1968). Ecological aspects of two wood-boring molluscs from the continental terrace off Oregon. Oceanography. Corvallis, Oregon State University**:** 137.

Tunnicliffe, V. (1991). "The Biology of Hydrothermal Vents - Ecology and Evolution." Oceanography and Marine Biology **29**: 319-407.

Tunnicliffe, V., S. Juniper, et al. (2003). Reducing Environments of the Deep-Sea Floor. Ecosystems of the World: The Deep Sea. P. Tyler, Elsevier Press.

Tunnicliffe, V., A. G. McArthur, et al. (1998). "A biogeographical perspective of the deep-sea hydrothermal vent fauna." Advances in Marine Biology **34**: 353-442.

Turner, R. (1954). "The family Pholadidae in the Western Atlantic and the Eastern Pacific, Part I - Pholadinae." Johnsonia **3**(33): 1-62.

Turner, R. (1955). "The family Pholadidae in the western Atlantic and eastern Pacific. Part II - Martesiinae, Jouannetiinae and Xylophaginae." Johansonia **3**(34): 65-160.

Turner, R. (1956). "Notes on *Xylophaga washingtona* BARTSCH and on the genus." the Nautilus **70**(1): 10-13.

Turner, R. (1973). "Wood-Boring Bivalves, Opportunistic Species in the Deep Sea." Science **180**: 1377-1379.

Turner, R. (1981). "Wood islands and thermal vents as centers of diverse communications in the deep sea." Biologia Morya **1**: 3-10.

Turner, R. D. (1972). "A new genus and species of deep water wood-boring bivalve (Mollusca, Pholadidae, Xylophagainae)." Basteria **36**(2-5): 97-104.

Turner, R. D. (1972). "*Xyleredo*, a new teredinid-like abyssal wood-borer (Mollusca, Pholadidae, Xylophagainae)." Breviora **397**: 1-19.

Turner, R. D. (1978). "Wood mollusks and deep-sea food chains." Bulletin of the American Malacological Union for 1977: 13-19.

Turner, R. D. (2002). "On the Subfamily Xylophagainae (family Pholadidae, Bivalvia, Mollusca)." Bulletin of the Museum of Comparative Zoology **157**(4): 223-307.

Turner, R. D. and Y. Yakovlev (1983). "Dwarf males in the Teredinidae (Bivalvia, Pholadacea)." Science **219**(4588): 1077-1078.

Valentine, J. (1966). "Numerical Analysis of Marine Molluscan Ranges on Extratropical Northeastern Pacific Shelf." Limnology and Oceanography **11**(2): 198-&.

Van Dover, C. (2000). The ecology of deep-sea hydrothermal vents. Princeton, New Jersey, Princeton University Press**:** 424 pp.

Van Dover, C., C. German, et al. (2002). "Evolution and Biogeography of Deep-Sea Vent and Seep Invertebrates." Science **295**: 1253-1257.

Van Halen, L. (1976). "Ecological species, multispecies and oaks." Taxon **25**: 233-239.

Voight, J. (2005). "First report of the enigmatic echinoderm Xyloplax from the North Pacific." Biological Bulletin **208**: 77-80.

Voight, J. (2006). Biotic interactions define the temporal stability of deep-sea wood-falls in the North Pacific. 11th International Deep-Sea Biology Symposium, South Hampton.

Ward, R. D., T. S. Zemlak, et al. (2005). "DNA barcoding Australia's fish species." Philosophical Transactions of the Royal Society B-Biological Sciences **360**(1462): 1847-1857.

Waren, A. and P. Bouchet (1993). "New Records, Species, Genera, and a New Family of Gastropods from Hydrothermal Vents and Hydrocarbon Seeps." Zoologica Scripta **22**(1): 1-90.

Waterbury, J. B., C. B. Calloway, et al. (1983). "A Cellulolytic Nitrogen-Fixing Bacterium Cultured from the Gland of Deshayes in Shipworms (Bivalvia, Teredinidae)." Science **221**(4618): 1401-1403.

Whiting, M. F., J. Carpenter, et al. (1997). "The strepsiptera problem: Phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology." Systematic Biology **46**: 1-68.

Wiley, E. and R. Mayden (2000). The evolutionary species concept. Species concepts and phylogenetic theory: a debate. Q. Wheeler and R. Meier. New York, Columbia University Press: 70-89.

Wolff, T. (1979). "Macrofaunal utilization of plant remains in the deep sea." Sarsia **1**(2): 117-136.

Won, Y., P. A. Y. Maas, et al. (2002). "Habitat reversal in vent and seep mussels: seep species, Bathymodiolus heckerae, derived from vent ancestors." Cahiers de Biologie Marine **34**: 387-390.

# Appendix A: Materials

**Chemicals:**

- EMD - OmniPur Agarose (Order# 2120)
- Sigma-Aldrich - Polyvinylpyrrolidone (PVP) (Order# P5288-100G)
- Invitrogen - SYBR Safe DNA Gel Stain (Order# S33102)
- Caledon - Dimethyl-Sulphoxide (DMSO) (Order# 4100-1)
- $MgCl_2$
- Roche - Tris(hydroxymethyl)aminomethane (Tris Base) (Order# 604 205)
- Fisher Biotech - Sodium Dodecyl Sulfate (SDS) (Order# BP166-100)

**Solutions:**

- Extraction buffer used for Phenol-Chloroform and CTAB extractions (1 litre):
  - 12.1g 100mM Tris base
  - 37.2g 100mM EDTA
  - 5g SDS
  - 0.03l NaOH
  - 0.95l H2O
- Gel running buffer (TAE)
  - 50x Stock:
    - 242g Tris base
    - 57.1ml glacial acetic acid
    - 100ml 0.5M EDTA (ph 8.0)
  - 1x Solution:
    - 40mM Tris-acetate
    - 1mM EDTA
- EDTA (1 litre)
  - 186.1g disodium EDTA-2H2O to 800ml H2O
  - stir vigorously and adjust pH to 8.0 with NaOH pellets (~20)
  - aliquot and autoclave

**Kits:**

- TAQ PCR Ready Mix
- Tissue Extraction Kit (Catalogue # 69504)
- Purification Kit (Catalogue # 28104)

# Appendix B: Methods

## Genetic Methodology:

### Genomic DNA Extraction Protocols

1. Phenol-Chloroform Extraction

1. Add 700 µl extraction buffer (12.1 g 100mM Tris, 37.2 g 100 mM EDTA, 5 g SDS, 0.95 l H2O, 0.3 l NaOH) to ~25 mg tissue.
2. Use micro pestle to grind up tissue.
3. Add 25 µl proteinase K, shake manually and incubate at 55°C for 15 min.
4. Transfer to ice. Add 500 µl buffer-saturated phenol.
5. Shake manually followed by a 5 min centrifugation at 13'000 rpm.
6. Transfer supernatant to new 1.5 ml micro centrifuge tube repeat 4. and 5.
7. Transfer supernatant to new 1.5 ml micro centrifuge tube and add 500 µl chloroform. Vortex for a few seconds.
8. Centrifuge for 5 min at 13'000 rpm.
9. Transfer supernatant to new 1.5 ml micro centrifuge tube add 1 ml cold 100% EtOH.
10. Shake manually and centrifuge at 13'000 rpm for 10 min.
11. Add 40 µl sodium acetate (3M, ph 5.2). Vortex.
12. Place at –20°C for at least 1 h.
13. Centrifuge at 13'000 rpm for 10 min.
14. Decant 100% EtOH, add 1 ml 70% EtOH slowly.
15. Decant 70% EtOH, add 1 ml 70% EtOH slowly.
16. Decant 70% EtOH. Let air dry for ~30 min.
17. Resuspend pellet in ddH2O (30 – 50 µl)

2. CTAB Extraction

Prepare PVP spin columns:

a) Load 900 µl PVP into a 2 ml spin column and centrifuge at 2900 rpm for 3 min.
b) Disard flow through and set aside for air-drying.

1. Cut up to 25 mg tissue into small pieces, place in a 1.5 ml micro centrifuge tube, and add 500 µl extraction buffer.
2. Use micro pestle to grind up tissue.
3. Add 10 µl proteinase K and incubate over night at 60°C.
4. Centrifuge for ~ 30 s.
5. Transfer supernatant into a prepared PVP spin column. Centrifuge at 2900 rpm for 3 min.
6. Add 1 volume chloroform-isoamyl alcohol (24:1) and mix slowly for 2 min.
7. Centrifuge at 13'000 rpm for 10 min.
8. Transfer supernatant into a fresh 1.5 micro centrifuge tube and add 2/3 volume isopropanol.
9. Put at –20°C for at least 1 h.
10. Centrifuge at 13'000 rpm for 10 min.

11. Eliminate supernatant and add 500 µl 70-75% EtOH.
12. Centrifuge at 13'000 rpm for 10 min.
13. Eliminate supernatant and let air-dry for 30 min.
14. Resuspend in 25-150 µl TE-buffer (10 mM Tris, 1 mM EDTA)

3. Qiagen DNeasy Tissue Extraction Kit (Catalogue # 69504)

1. Cut up to 25 mg tissue into small pieces, place in a 1.5 ml micro centrifuge tube, and add 180 µl Buffer ATL.
2. Use micro pestle to grind up tissue.
3. Add 20 µl proteinase K, mix by vortexing, and incubate at 55°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample. Lysing times varied between 1 and 3 hours.
4. Vortex for 15s. Add 200 µl Buffer AL to the sample, mix thoroughly by vortexing, and incubate at 70°C for 10 min.
5. Add 200 µl ethanol (100%) to the sample and mix thoroughly by vortexing.
6. Pipette the mixture from step 5 into the DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at 8000 rpm for 1 min. Discard flow-through and collection tube.
7. Place the DNeasy Mini spin column in a new 2 ml collection tube and add 500 µl Buffer AW1. Centrifuge for 1 min at 8000 rpm. Discard flow-through and collection tube.
8. Place the DNeasy Mini spin column in a new 2 ml collection tube and add 500 µl Buffer AW2. Centrifuge for 3 min at 13'000 rpm to dry the DNeasy membrane. Discard flow-through and collection tube.
9. Place the DNeasy Mini spin column in a clean 1.5 ml micro centrifuge tube and pipette 200 µl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min and centrifuge for 1 min at 8000 rpm to elute.

### DNA Purification (Qiagen Purification Kit; Catalogue # 28104)

The following protocol was used to purify DNA fragments from the PCR reaction. Fragments are purified from primers, nucleotides, polymerases and salts.

1. Add 5 volumes of Buffer PB to 1 volume of the PCR sample mix.
2. Place QIAquick spin column in a 2 ml collection tube.
3. To bind DNA, apply the sample to the QIAquick spin column and centrifuge for 60 s.
4. Discard flow-through. Place spin column back into the same tube.
5. To wash, add 0.75 ml Buffer PE to the QIAquick column and centrifuge for 60 s.
6. Discard flow-through and place QIAquick column back the same tube. Centrifuge for an additional 60 s to remove all ethanol.
7. Place QIAquick column in a clean 1.5 ml micro centrifuge tube.
8. To elute add 30 µl EB buffer (elution buffer) to the centre of the QIAquick membrane. Let stand for 1 min then centrifuge.

# Appendix C: Data

## Continental Margin Washington

| Sample No. | What | Dimensions | Who | Date | Time | Common Name | Depth | Lat | Lon | Comments: |
|---|---|---|---|---|---|---|---|---|---|---|
| unassigned | 4 sliced fir woodblocks roped together with float '48' | sliced 4x4x8in | Paul Johnson | 6-Oct-05 | 23.38 | Washington Margin | 200 | 46 53.5111 N | 124 51.0283 W | GMT |
| unassigned | 4 sliced fir woodblocks roped together with float '48' | sliced 4x4x8in | Paul Johnson | 6-Oct-05 | 23.37 | Washington Margin | 200 | 46 53.5129 N | 124 51.0308 W | GMT |

## Saanich Inlet, BC, Canada

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| R909-INST-2-0002 | Fir wood block | 4x4x8 | VT | 7/14/05 | 20.53.04 | | | 48 39.169 | 123 29.188 | Quadrant F |
| R909-INST-2-0003 | Fir wood block | 4x4x8 | VT | 7/14/05 | 20.55.39 | | | 48 39.170 | 123 29.188 | Quadrant F |
| R909-INST-2-0004 | Fir wood block | 4x4x8 | VT | 7/14/05 | 21.09.47 | | | 48 39.170 | 123 29.186 | Quadrant F |
| R909-INST-CH7-0005 | Fir wood block | 4x4x8 | Paul S. | 7/14/05 | 21.52.27 | | | 48 39.312 | 123 29.272 | North of cage 1, in new cage area |

## Fraser River, BC, Canada

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| R911-INST-WB1-0002 | block | | | 7/15/09 | 8:17:57 PM | V-traps | 165.5 | 49° 9.511' E | 123° 23.140' S | Deployed first wood block. |
| R911-INST-WB2-0003 | block | | | 7/15/09 | 8:18:34 PM | V-traps | 165.6 | 49° 9.511' E | 123° 23.140' S | Deploying 2nd wood block |
| R911-INST-WB3-0005 | block | | | 7/15/09 | 8:20:57 PM | V-traps | 165.5 | 49° 9.512' E | 123° 23.141' S | Deploying 3rd wood block. |
| R911-INST-WB4-0006 | block | | | 7/15/09 | 8:21:46 PM | V-traps | 165.5 | 49° 9.511' E | 123° 23.140' S | Deploying final wood block. |
| R912-Inst-0003 | 2 blocks | | | 7/16/09 | 1:16:02 AM | N Reef 75 | 184.9 | 49.16 | -123.38 | Deployment of 2 wood blocks - 4x4x8 size. |
| R912-0005 | block | | | 7/16/09 | 1:19:33 AM | N Reef 75 | 184.9 | 49.16 | -123.38 | Deployment of wood block. |
| R912-0007 | block | | | 7/16/09 | 1:22:02 AM | N Reef 75 | 185 | 49.16 | -123.38 | Putting down last wood block, number 4. |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| R915-INST-WC-0001 | tray | | | 7/17/09 | 6:16:37 AM | Happy Valley | 202.1 | 49° 9.479' E | 123° 22.443' S | One-stripe wood chip tray deployed - furthest North, Four-stripe wood chip tray deployed; Problems with computer. Unable to enter long sample entries. Times of entries not in sync with deployment. |
| R915-INST-WC-0002 | tray | | | 7/17/09 | 6:26:52 AM | Happy Valley | 197.4 | 49° 9.485' E | 123° 22.439' S | Three-stripe wood chip tray deployed. (Poor visibility is natural, not caused by ROPOS.); Problems with computer. Unable to enter long sample entries. Times of entries not in sync with deployment. |
| R915-INST-WC-0003 | tray | | | 7/17/09 | 6:27:33 AM | Happy Valley | 201.5 | 49° 9.485' E | 123° 22.439' S | Two-stripe wood chip tray deployed. CONTROL wood chip tray deployed - furthest South.; Problems with computer. Unable to enter long sample entries. Times of entries not in sync with deployment. |
| R915-INST-WB-0012 | block | | | 7/17/09 | 7:22:24 AM | Ridgecrest | 158.7 | 49° 9.326' E | 123° 23.090' S | Wood block is deployed. We are facing south and the sediment traps are arrayed in front of us. Wood block is situated north of traps. |
| ROPOS 915 | wood chip trays x 4 (1-4 stripes) | | | 7/17/09 | | Happy Valley | 201.5 | 49° 9.480' E | 123° 22.444' S | Due East of sponge reef |
| ROPS 911 | Wood blocks x 4 | | | 7/15/09 | | V-traps (near Point 120) | 165.5 | 49° 9.512' E | 123° 23.140' S | Many sponges |
| **ODP 1026B** | | | | | | | | | | |
| unassigned | Fir wood block | 4x4x8 | Earl Davis | 12-Sep-05 | | | 2658 | 47 45.759 N | 127 45.552 W | 20m SW of Hohle 1026b |

Table 17: Summary of outstanding deployments on the continental margin off the coast of Washington, in Saanich Inlet, Strait of Georgia and at ODP 1026B.

## Museum Collections

| Taxonomic Name | Catalog# | Location | # specimen | preservative |
|---|---|---|---|---|
| **Harvard** | Adam Baldinger | abaldinger@oeb.harvard.edu | | |
| Xylophaga abyssorum | 008135 | Western Atlantic | 1 | ? |
| Xylophaga praestans | 228290 | England | 1 | dry |
| Xylophaga robusta | 332002 | Mexico | ? | dry |
| Xylophaga washingtona | 186979 | Washington | 8 | dry |
| Xylophaga washingtona | 341066 | California | ? | dry |
| Xylophaga whoi | 275015 | Cuba | 1 | dry |
| Xylophaga altenai | 279316 | Florida | 5 | ethanol |
| Xylophaga bayeri | 316738 | Florida | 1 | ethanol |
| Xylophaga clenchi | 316745 | Bahamas | 5 | ethanol |
| Xylophaga depalmai | 316735 | Florida | 1 | ethanol |
| Xylophaga erecta | 263603 | Sulu Sea | 1 | ethanol |
| Xylophaga galatheae | 235795 | Tasman Sea | 1 | ethanol |
| Xylophaga gerda | 316742 | Florida | 6 | ethanol |
| Xylophaga grevei | 235794 | Mindanao Sea | 1 | ethanol |
| Xylophaga japonica | 194822 | Japan | 1 | ethanol |
| Xylophaga muraokai | 316747 | California | 22 | ethanol |
| Xylophaga muraokai | 316749 | California | 50 | ethanol |
| Xylophaga profunda | 316751 | Bahamas | 1 | ethanol |
| Xylophaga tipperi | 316737 | Florida | 4 | ethanol |
| Xylophaga lobata | 263601 | Sulu Sea | 4 | ethanol |
| Xyleredo ingolfia | 279637 | Iceland | 30 | dry and ethanol |
| Xyleredo nooi | 279634 | Bahamas | 30 | ethanol |
| **LA County** | Lindsey Groves | lgroves@nhm.org | | |
| Xylophaga washingtona | 171527 | California | 20 | |
| **Smithsonian** | | | | |
| Xylphaga sp. | 832948 | North Atlantik | 9 | ethanol |
| **RBCM** | Kelly Sendall | KSENDALL@royalbcmuseum.bc.ca | | |
| Xylophaga washingtona | 001-351-001 | Queen Charlotte Sound: Goose Bank | 6 | dry |
| Xylophaga washingtona | 001-353-001 | | lots | dry |
| Xylophaga washingtona | 979-03297 | vancouver Isl: Barkley Sound | 26 | dry |
| Xylophaga washingtona | 001-350-001 | | lots | wet |
| Xylophaga washingtona | 975-802-060 | vancouver Isl: Departure Bay | 20 | wet |
| Xylophaga washingtona | 979-06133 | | lots | wet |
| Xylophaga washingtona | 984-386-004 | Vancouver Isl: Port Alberni | lots | wet |

Table 18: List of *Xylophaga* available at Harvard, LA County Museum, Smithsonian and Royal British Columbia Museum.

**Morphological Taxon Sequences (non-truncated)**

Special Characters:

G = Guanosine
A = Adenosine
T = Thymidine
C = Cytidine
B = C, G or T not A
D = A, G or T not C
H = A, C or T not G
K = G or T
M = A or C
N = G, A, T or C
R = G or A
S = C or G
V = A, C or G not T
W = A or T
X = G, A, T or C
Y = C or T

Taxon R

```
TTTTTTACCCGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGT
CTAAGTACACGCCTTTACACGGCTAAACTGCGAATGGCTCATTAAATCAGTTATGGTTCCTTA
GATCGTACAATCCTACTTGGATAACTGTGGCAATTCTAGAGCTAATACATGCAACACAGCTCC
GACCCTCGCGGGGAAGAGCGCTTTTGTTAGTCTAAAACCATCGCGGCGGTCGTTCGCGCGG
CTGTCGCTCAAACATGGTGACTCTGAACAACTTTGTGCCGATCGCACGGCCTTTGCGCCGGC
GACGCATCTTTCAAGTGTCTGCCCTATCAACTTTCGATGGTACGCGCTATGCCTACCATGGTG
ATAACGGGTAACGGGGAATCAGGGTTCGATTCCGGARAGGGAGCATGARATACGGCTACCA
CATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACGA
AAAATAACAATACGGGACTCTTTCGAGGCCCCGTAATTGGAATGAGTACACTTTAAATCCTTT
AACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATA
GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATGTCGGGTGCAGGCTTGCGG
TCCGCCTCGCGGCGGTCACTGCTCGTCCTGACCTCCACGCCGGTGTTACCGTCCCTTGGTGC
TCTTGATTGAGTGTCTCGGGCGGCCGGTACGTTTACTTTGAAGAAATTAGAGTGCTCAAAGC
AGGCCCTAGCCGCCTGAATAATGGTGCATGGAATAATGGAATAGGACCTCGGTTCTATTTTG
TTGGCTCTCGGAGCTCGAGGTAATGATTAATAGGGACTGACGGGGGCATTCGTATTGCGGC
GCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACAGCGAAAGCATTTGCCAAGCAT
GTTTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTCGTAGTTCT
GACCATAAACGATGCCGACCGTCGATCCGCCGGAGTTGCTTCAATGACTCGGCGGGCAGAC
CCCGGGAAACCAAAGTTTCTGGGTTCCGGGGGGAGTATGGTTGCAAAGCTGAAACTTAAAG
GAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAATTTGACTCAACACGGGG
AACCTCACCCGGCCCGGACACTGTAAGGATTGACAGATTGAGAGCTCTTTCTTGATTCGGTG
GGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGA
ACGAGACTCTAGCCTGCTAAATAGTTAGGGGATCCTCTACGCGAGTCCCCGTCAACTTCTTA
GAGGGACAAGTGGCGCTTAGCCACACGAGATTGAGCAATAACAGGTCTGTGATGCCCTTAG
ATGTTCGGGGCCGCACATGCGCTACACTGAATGGATCAGCGTGCGTCTCGCCTGGCCCGAG
AGGGCCGGGAAACCCGCTGAACCCCATTCGTGCTAGGGATTGGGGCTTGCAATTGTTCCCC
ATGAACGAGGAATTCCCAGTAAGCGCGAGTCATCAGCTCGTGTTGATTACGTCCCTGCCCTT
TGTACACACCGCCCGTCGCTACTACCGATCGCTCCAGTTAATGAGCTCTTCGGATTGGTCCC
```

GTAGGCGGGTCTTCGGGCCTGCTCTCGGCGTGCCGAGAAGATGCGCAAATTGACCGGAGTA
GAGGGAGTAAAAGTCGTAACAAGGTATCCGTAGGTGAACCTGCGGAAG

Taxon B1

CCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCTAAGTA
CAAGCCTTTACACGGCTAAACTGCGAATGGCTCATTAAATCAGTTATGGTTCCTTAGATCGTA
CACTATCCTACTTGGATAACTGTGGCAATTCTAGAGCTAATACATGAAACCAAGCTCCGACCC
TTTTCGGGGAAGAGCGCTTTTGTTAGCCCAAAACCAACGCGGCGGTTGCCGCTTGCGGTGG
CCGTCGCTAAAAGAGTGGTGAATCTGAACAACTTTGTGCCGATCGCACGGCCTTTGCGCCG
GCGACATATCTTACAAGTGTCTGCCCTATCAACTGTCGATGGTACGTGCTATGCCTACCATG
GTGATAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCATGAGATACGGCTA
CCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGA
CGAAAAATAACAATACGGGACTCTTTCGAGGCCCCGTAATTGGAATGAGTACACTTTAAATC
CTTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCC
AATAGCGTATATYAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCTCGGGTGCAGGCTT
GCGGTCCGCCTCGCGGCGGTCACTGCTCSTCCTGGCCTCCAGGCCGGTGTCACCTGTTGGT
GCTCTTGATTGAGTGTCTCGGGCGGCCGGTACGTATACTTTGAAGTAATKAGAGTGCTTAAA
GCAGGCTACAACTGCCTGAATAATGGTGCATGGAATAGTGGAATAGGACCTCGGTTCTATTT
TGTTGGCTCTCGGAGCTCGAGGTAATGATTAAGAGGGACAGCCGGGGGCATYCGTATTGCG
GCGCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACAGCGTAAGCATTTGCCAAG
CATGTTTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTCGTAGT
TCTGACCATAAACGATGCCGACCGTCGATCCGCCGGAGTTGCTTCAATGACTCGGCGGGCA
GTCCCCGGGAAACCAAAGTTTCTGGGTTCCGGGGGGAGTATGGTTGCAAAGCTGAAACTTA
AAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAATTTGACTCAACACG
GGGAACCTCACCCGGCCCGGACACTGTAAGGATTGACAGATTGAGAGCTCTTTCTTGATTCG
GTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAA
CGAACGAGACTCTAGCCTGCTAAATAGCTCGGGTATCACTGGCGTAAAGCTAGTGCCCGTTT
AGAACTTCTTAGAGGGACAGGTGGCGCTTAGCCACACGAGATTGAGCAATAACAGGTCTGT
GATGCCCTTAGATGTTCGGGGCCGCACATGCGCTACACTGAATGGTTCAGCGTGAAGGCCT
GGCCCGAGAGGGCTGGGAAACCCGTTGAACCCCATTCGTGCTAGGGATTGGGGCTTGCAAT
TGTTCCCCATGAACGAGGAATTCCCAGTAAGCGCGAGTCATCAGCTCGTGTTGATTACGTCC
CTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATCGCTCCAGTTAATGAGTTCTTCGGAT
TGGTCCCGTAGGCTGGTTTCGGCTGGCTCTCGGCGTGCCGAGAAGATGCGCAAGTTGACCG
GGGTAGAGGTCGTAAAAGTCGTAACAAGGTATCCGTAGGTGAACCTGCGGAAGAATAAAA

Taxon B2

CCTGGTTGACYCTGCCAGTAGTCATAWGCTTKTYTCAAAGATTAAGCCATGCATGTCTAAGTA
CAAGCCTTTACACGGCTAAACTGCGAATGGCTCATTAAATCAGTTATGGTTYCTTAGATYGTA
CACTATYCTACTTGGATAACTGTGGCAATTYTAGAGCTAATACATGAAACCAAGCTCCGACCC
TTTTCGGGGAAGAGCGCTTTTGTTAGCCCAAAACCAAMGCGGCGGTTGCCGCTTGCGGTGS
CCKTYGCTAAAAGAGTGGTGAATYTGAACAACTTTGTGCCGATCGCACGGCCTTKGCGCCGG
CGACATATCTTACAAGTGTYTGCCCTATCAACTGTCGATGGTACGTGCTATGCCTACCATGGT
GAWAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCATGAGATACGGCTAC
CACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGAC
GAAAAATAACAATACGGGACTCTTTCGAGGCCCCGTAATTGGAATGAGTACACTTTAAATCCT
TTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAA
TAGCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCTCGGGTGCAGGCTTGC
GGTCCGCCTCGCGGCGGTCACTGCTCGTCCTGGCCTCCAGGCCGGTGTCACCTGTTGGTGC
TCTTGATTGAGTGTCTCGGGCGGCCGGTACGTTTACTTTGAAGAAATTAGAGTGCTTAAAGC
AGGCTACAACTGCCTGAATAATGGTGCATGGAATAGTGGAATAGGACCTCGGTTCTATTTTG
TTGGCTCTCGGAGCTCGAGGTAATGATTAAGAGGGACAGCCGGGGGCATTCGTATTGCGGC
GCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACAGCGTAAGCATTWGCCAAGCA
TGTTTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTCGTAGTTCT
GACCATAAACGATGCCGACCGTCGATCCGCCGGAGTTGCTTCAATGACTCGGCGGGCAGTC

CCCGGGAAACCAAAGTTTCTGGGTTCCGGGGGGAGTATGGTTGCAAAGCTGAAACTTAAAG
GAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAATTTGACTCAACACGGGG
AACCTCACCCGGCCCGGACACTGTAAGGATTGACAGATTGAGAGYTCTTTYTTGATTCGGTG
GGTGGTGGTGCATGGCCGTTYTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGA
ACGAGACTCTAGCCTGCTAAATAGCTCGGGTATCACTGGCGTAAAGCTAGTGCCCGTTTAGA
ACTTCTTAGAGGGACAGGTGGCGCTTAGCCACACGAGATTGAGCAATAACAGGTCTGTGAT
GCCCTTAGATGTTCGGGGCCGCACATGCGCTACACTGAATGGTTCAGCGTGAAGGCCTGGC
CCGAGAGGGCTGGGAAACCCGTTGAACCCCATTCGTGCTAGGGATTGGGGCTTGCAATTGT
TCCCCATGAACGAGGAATTCCCAGTAAGCGCGAGTCATCAGCTCGTGTTGATTACGTCCCTG
CCCTTTGTACACACCGCCCGTCGCTACTACCGATCGCTCCAGTTAATGAGTTCTTCGGATTG
GTCCCGTAGGCTGGTTTCGGCTGGCTCTCGGCGTGCCGAGAAGATGCGCAAGTTGACCGGG
GTAGAGGTCGTAAAAGTCGTAACAAGGTATCCGTAGGTGAACCTGCGGAAG

Taxon F

CTTTTACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTYTCAAAGATTAAGCCATGCATGTC
TAAGTACACGCCTTTACACGGCTAAACTGCGAATGGCTCATTAAATCAGTTATGGTTCCTTAG
ATCGTACAATCCTACTTGGATAACTGTGGCAATTCTAGAGCTAATACATGCAACACAGCTCCG
ACCCTCGCGGGGAAGAGCGCTTTTGTTAGCCCAAAACCATCGCGGTGGTCGTTCGCGCGGC
CGCCGCTCAAACATGGTGACTCTGAACAACTTTGTGCCGATCGCACGGCCTTTGCGCCGGC
GACGCATCTTTCAAGTGTCTGCCCTATCAACTTTCGATGGTACGCGCTATGCCTACCATGGTG
ATAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCATGAGATACGGCTACCA
CATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACGA
AAAATAACAATACGGRACTCTTTCGAGGCCCCGTAATTGGAATGAGTACACTTTAAATCCTTT
AACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATA
GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATGTCGGGTGCAGGCTTGCGG
TCCGCCTCGCGGCGGTCACTGCTCGTCCTGACCTCCACGCCGGTGTTACCGTCCCTTGGTGC
TCTTGATTGAGTGTCTCGGGCGGCCGGTACGTTTACTTTGAAGAAATTAGAGTGCTCAAAGC
AGGCCCTAGTAGCCTGAATAATGGTGCATGGAATAATGGAATAGGACCTCGGTTCTATTTTG
CTGGCTCTCGGAGCTCGAGGTAATGATTAATAGGGACTGACGGGGGCATTCGTATTGCGGC
GCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACAGCGAAAGCATTTGCCAAGCAT
GTTTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTCGTAGTTCT
GACCATAAACGATGCCGACCGTCGATCCGCCGGAGTTGCTTCAATGACTCGGCGGGCAGAC
CCCGGGAAACCAAAGTTTCTGGGTTCCGGGGGGAGTATGGTTGCAAAGCTGAAACTTAAAG
GAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAATTTGACTCAACACGGGG
AACCTCACCCGGCCCGGACACTGTAAGGATTGACAGATTGAGAGCTCTTTCTTGATTCGGTG
GGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGA
ACGAGACTCTAGCCTGCTAAATAGTTAGGGGATCCTCTACGCGAGTCCCCGCAAACTTCTTA
GAGGGACAAGTGGCGCTTAGCCACACGAGATTGAGCAATAACAGGTCTGTGATGCCCTTAG
ATGTTCGGGGCCGCACATGCGCTACACTGAATGGATCAGCGTGCGTCTCGCCTGGCCCGAG
AGGGCCGGGAAACCCGCTGAACCCCATTCGTGCTAGGGATTGGGGCTTGCAATTGTTCCCC
ATGAACGAGGAATTCCCAGTAAGCGCGAGTCATCAGCTCGTGTTGATTACGTCCCTGCCCTT
TGTACACACCGCCCGTCGCTACTACCGATCGCTCCAGTTAATGAGCTCTTCGGATTGGTCCC
GTAGGCGGGTCTTCGGGCCTGCTCTCGGCGTGCCGAGAAGATGCGCAAATTGACCGGAGTA
GAGGGAGTAAAAGTCGTAACAAGGTATCCGTAGGTGAACCTGCGGAAG

Taxon G

TTTACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCTA
AGTACACGCCTTTACACGGCTAAACTGCGAATGGCTCATTAAATCAGTTATGGTTCCTTAGAT
CGTACAATCCTACTTGGATAACTGTGGCAATTCTAGAGCTAATACATGCAACACAGCTCCGAC
CCTCGCGGGGAAGAGCGCTTTTGTTAGCCCAAAACCATCGCGGTGGTCGTTCGCGCGGCCG
CCGCTCAAACATGGTGACTCTGAACAACTTTGTGCCGATCGCACGGCCTTTGCGCCGGCGAC
GCATCTTTCAAGTGTCTGCCCTATCAACTTTCGATGGTACGCGCTATGCCTACCATGGTGATA
ACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCATGAGATACGGCTACCACAT
CCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACGAAA

AATAACAATACGGGACTCTTTCGAGGCCCCGTAATTGGAATGAGTACACTTTAAATCCTTTAA
CGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGC
GTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATGTCGGGTGCAGGCTTGCGGTC
CGCCTCGCGGCGGTCACTGCTCGTCCTGACCTCCACGCCGGTGTTACCGTCCCTTGGTGCTC
TTGATTGAGTGTCTCGGGCGGCCGGTACGTTTACTTTGAAGAAATTAGAGTGCTCAAAGCAG
GCCCTAGTAGCCTGAATAATGGTGCATGGAATAATGGAATAGGACCTCGGTTCTATTTTGCT
GGCTCTCGGAGCTCGAGGTAATGATTAATAGGGACTGACGGGGGCATTCGTATTGCGGCGC
TAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACAGCGAAAGCATTTGCCAAGCATGT
TTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTCGTAGTTCTGA
CCATAAACGATGCCGACCGTCGATCCGCCGGAGTTGCTTCAATGACTCGGCGGGCAGACCC
CGGGAAACCAAAGTTTCTGGGTTCCGGGGGGAGTATGGTTGCAAAGCTGAAACTTAAAGGA
ATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAATTTGACTCAACACGGGGAA
CCTCACCCGGCCCGGACACTGTAAGGATTGACAGATTGAGAGCTCTTTCTTGATTCGGTGGG
TGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAAC
GAGACTCTAGCCTGCTAAATAGTTAGGGGATCCTCTACGCGAGTCCCCGCAAACTTCTTAGA
GGGACAAGTGGCGCTTAGCCACACGAGATTGAGCAATAACAGGTCTGTGATGCCCTTAGAT
GTTCGGGGCCGCACATGCGCTACACTGAATGGATCAGCGTGCGTCTCGCCTGGCCCGAGAG
GGCCGGGAAACCCGCTGAACCCCATTCGTGCTAGGGATTGGGGCTTGCAATTGTTCCCCAT
GAACGAGGAATTCCCAGTAAGCGCGAGTCATCAGCTCGTGTTGATTACGTCCCTGCCCTTTG
TACACACCGCCCGTCGCTACTACCGATCGCTCCAGTTAATGAGCTCTTCGGATTGGTCCCGT
AGGCGGGTCTTCGGGCCTGCTCTCGGCGTGCCGAGAAGATGCGCAAATTGACCGGAGTAGA
GGGAGTAAAAGTCGTAACAAGGTATCCGTAGGTGAACCTGCGGAAGGATCAAA

Taxon T1

ACTTTTACCYGGTTGATCCTGCCAGTAGTCAWATGCTTKTCTCMAAGATTAAGCCATGCATG
TCTAAGTACACGCCTTTACACGGCTAAACTGCGAATGGCTCATTAAATCAGTTATGGTTCCTT
AGATCGTACAATCCTACTTGGATAACTGTGGCAATTCTAGAGCTAATACATGCAACACAGCTC
CGACCCTTGCGGGGAAGAGCGCTTTTGTTAGCCCAAAACCAACGCGGCGGTCGTTCGCGCG
GCCGTCGCTCACACGTGGCGAATCTGAACAACTTTGTGCCGATCGCACGGCCTTTGCGCCG
GCGACGAATCTTTCAAGTGTCTGCCCTATCAACTTTCGATGGTACGTGCTATGCCTACCATGG
TGATAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCATGAGATACGGCTAC
CACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGAC
GAAAAATAACAATACGGGACTCTTTCGAGGCCCCGTAATTGGAATGAGTACACTTTAAATCCT
TTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAA
TAGCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCTCGGGTGCAGGCTTGC
GGTCCGCCTCGCGGCGGTCACTGCTCGTCCTGACCTCCAGGCCGGTGTTACCGTCCCTTGG
TGCTCTTGATTGAGTGTCTCGGGCGGCCGGTACGTTTACTTTGAAGAAATTAGAGTGCTCAA
AGCAGGCCCTAGCCGCCTGAATAATGGTGCATGGAATAATGGAATAGGACCTCGGTTCTATT
TTGTTGGCTCTCGGAGCTCGAGGTAATGATTAATAGGGACGGACGGGGGCATTCGTATTGC
GGCGCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACAGCGAAAGCATTTGCCAA
GCATGTTTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTCGTAG
TTCTGACCATAAACGATGCCGACCGTCGATCCGCCGGAGTTGCTTCAATGACTCGGCGGGC
AGACCCCGGGAAACCAAAGTTTCTGGGTTCCGGGGGGAGTATGGTTGCAAAGCTGAAACTT
AAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAATTTGACTCAACAC
GGGGAACCTCACCCGGCCCGGACACTGTAAGGATTGACAGATTGAGAGCTCTTTCTTGATTC
GGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATA
ACGAACGAGACTCTAGCCTACTAAATAGCTCGGGGATCCTCTACGTGAGTCCCCGTTAACTT
CTTAGAGGGACAAGTGGCGCTTAGCCACACGAGATTGAGCAATAACAGGTCTGTGATGCCC
TTAGATGTTCGGGGCCGCACATGCGCTACACTGAATGGATCAGCGTGCGTCTCGCCTGGCC
CGAGAGGGCCGGGAAACCCGTTGAACCCCATTCGTGCTaGGGATTGGGGCTTGCAATTGTT
CCCCATGAACGAGGAATTCCCAGTAAGCGCGAGTCATCAGCTCGTGTTGATTACGTCCCTGC
CCTTTGTACACACCGCCCGTCGCTACTACCGATCGCTCCAGTTAATGAGCTCTTCGGATTGG
TCCCGTAGGCGGGTCTTCGGGCCTGCTCTCGGCGTGCCGAGAAGATGCGCAAATTGACCGG
AGTAGAGGGAGTAAAAGTCGTAACAAGGTATCCGTAGGTGAACCTGCGGAGGATC

Taxon T2

TTTTTACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTC
TAAGTACACGCCTTTACACGGCGAAACTGCGAATGGCTCATTAAATCAGTTATGGTTCCTTAG
ATCGTACTATCCTACTTGGATAACTGTGGCAATTCTAGAGCTAATACATGCAACACAGCTCCG
ACCCTCGCGGTAAGAGCGCTTTTGTTAGCCAACCAAAACCAACGCGGCGGTCGTTCGCGGC
CGTCGCTTACACATGATGACTCTGAACAACTTTGTGCTGATCGCACGGCCTTTGCGCCGGCG
ACATATCTTTCAAGTGTCTGCCCTATCAACTTTCGATGGTACGTGCTATGCCTACCATGGTGA
TAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCATGAGATACGGCTACCAC
ATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACGAA
AAATAACAATACGGGACTCTTTCGAGGCCCCGTAATTGGAATGAGTACACTTTAAATCCTTTA
ACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAG
CGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCTCGGGTGCAGGCTAGCGGT
CCGCCTTGCGGCGGTCACTGCTCGTCCCGACCTCCAGGCCGGTGCTCGTCCCTTGGTGCTC
TTGACTGAGTGTCTGGGGCGGCCGGCACGTTTACTTTGAAGAAATTAGAGTGCTCAAAGCAG
GCCATAGCCGCCTGAATAATGGTGCATGGAATAATAGAATAGGACCTCGGTTCTATTTTGTT
GGCTCTCGGAGCTCGAGGTAATGATTAATAGGGACAGACGGGGGCATTCGTATTGCGGCGC
TAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACAGCGAAAGCATTTGCCAAGMATGT
TTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTCGTAGTTCTGA
CCATAAACGATGCCGACCGTCGATCCGCCGGAGTTGCTTCAATGACTCGGCGGGCAGACCC
CGGGAAACCAAAGTGTCTGGGTTCCGGGGGGAGTATGGTTGCAAAGCTGAAACTTAAAGGA
ATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAATTTGACTCAACACGGGGAA
CCTCACCCGGCCCGGACACCGTAAGGATTGACAGATTGAGAGCTCTTTCTTGATTCGGTGGG
TGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAAC
GAGACTCTAGCCTATTAAATAGCTCGGGGATCCTCCACGCGAGACCCCGTTAACTTCTTAGA
GGGACAAGTGGCGCTTAGCCACGCGAGATTGAGCAATAACAGGTCTGTGATGCCCTTAGAT
GTTCGGGGCCGCACATGCGCTACACTGAATGGATCAGCGTGCGTCTCGCCTGGCCCGAGAG
GGCTGGGAAATCACTGAACCCCATTCGTGCTAGGGATTGGGGCTTGCAATTGTTCCCCATCA
ACGAGGAATTCCCAGTAAGCGCGAGTCATCAGCTCGTGTTGATTACGTCCCTGCCCTTTGTA
CACACCGCCCGTCGCTACTACCGATCGCTCCAGTTAATGAGCTCTTCGGATTGGTCCCGTAG
GCGGGTCTTCGGGCCTGCTCTCGGCGTGCCGAGAAGATGCGCAAATTGGCCGGAGTAGAG
GTCGTAAAAGTCGTAACAAGGTATCCGTAGGTGAACCTGCGGAAGGATC

Taxon M

TACCTGGTTGWTCCTGCCAGTAGTMAAAWGCTTKTTTCAAAGATTAAGCCAWGMAWKTTTA
AGWAMAMSCCTTTACACGGCTAAACTGCGAATGGCTCATTAAATCAGTTATGGTTCCTTAGA
TYGTACAATYCTACTTGRATAACTGTGGCAATTCTARAGCTAATACWTGCAACACAGYTCCGA
CCCTCGCGGGGAAGAGCGCTTTTGTTAGCCCAAAACCATCGCGGTGGTCGTTCGCGCGGCC
GCCGCTCAAACATGGTGACTYTGAACAACTTTGTGCCGATCGCACGGCCTTTGCGCCGGCGA
CGCATCTTTCAAGTGTYTGCCCTATCAACTTTCGATGGTACGCGCTATGCCTACCATGGTGAT
AACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCATGAGATACGGCTACCACA
TCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACGAAA
AATAACAATACGGGACTCTTTCGAGGCCCCGTAATTGGAATGAGTACACTTTAAATCCTTTAA
CGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGC
GTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATGTCGGGTGCAGGCTTGCGGTC
CGCCTCGCGGCGGTCACTGCTCGTCCTGACCTCCAMGCCGGTGTTACCGTCCCTTGGTGCT
CTTGATTGAGTGTCTYGGGCGGCCGGTAMGTTTACTTTGAAGAAATTAGAGTGCTCAAAGCA
GGCCCTAGTAGCCTGAATAATGGTGCATGGAATAATGGAATAGGACCTCGGTTCTATTTTGC
TGGCTCTCGGAGCTCGAGGTAATGATTAATAGGGACTGACGGGGGCATTYGTATTGCGGCG
CTAGAGGTGAAATTCTTGGACCKTYGCAAGACGAACTACAGCGAAAGCATTTGCCAAGCAW
GTTTTCATTAATCAAGAACGAAAGTCAGAGGTTCGAAGACGATCAGATACCGTYGTAGTTCT
GACCATAAACGAWGCCGACCGTCGATCCGCCGGAGTTGCTTCAATGACTCGGCGGGCAGAC
CCCGGGAAACCAAAGTTTCTGGGTTCCGGGGGGAGTATGGTTGCAAAGCTGAAACTTAAAG
GAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGTGGCTTAaTTTGACTCAACACGGGG
AACCTCACCCGGCCCGGACACTGTAAGGATTGACAGATTGAGAGCTCTTTCTTGATTCGGTG

```
GGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGA
ACGAGACTCTAGCCTGCTAAATAGTTAGGGGATCCTCTACGCGAGTCCCCGCAAACTTCTTA
GAGGGACAAGTGGCGCTTAGCCACACGAGATTGAGCAATAACAGGTCTGTGATGCCCTTAG
ATGTTCGGGGCCGCACATGCGCTACACTGAATGGATCAGCGTGCGTCTCGCCTGGCCCGAG
AGGGCCGGGAAACCCGCTGAACCCCATTCGTGCTAGGGATTGGGGCTTGCAATTGTTCCCC
ATGAACGAGGAATTCCCAGTAAGCGCGAGTCATCAGCTCGTGTTGATTACGTCCCTGCCCTT
TGTACACACCGCCCGTCGCTACTACCGATCGCTCCAGTTAATGAGCTCTTCGGATTGGTCCC
GTAGGCGGGTCTTCGGGCCTGCTCTCGGCGTGCCGAGAAGATGCGCAAATTGACCGGAGTA
GAGGGAGTAAAAGTCGTAACAAGGTATCCGTAGGTGAACCTGCGGAAGAAT
```

### CO1 Data

Table 19: Summary of samples sent to the Barcode of Life Consortium at the University if Guelph, ON, Canada. 'Code': unique identifier in BOLD (Barcode of Life Database; www.barcodinglife.com). The corresponding sample has the same code without the –bc. 'Taxon': Assigned taxon based on morphological characters. 'Location': Origin of sample with Lat, Long and Depth

| Code | Taxon | Location | Lat | Long | Depth |
|---|---|---|---|---|---|
| WB-1-1-bc | R | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-2-bc | R | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-3-bc | R | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-4-bc | R | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-5-bc | R | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-6-bc | R | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-7-bc | R | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-8-bc | Xylopholas | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-9-bc | Xylopholas | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-10-bc | Xylopholas | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-11-bc | Xylopholas | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-12-bc | Xylopholas | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-13-bc | Xylopholas | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-215-bc | Xylopholas | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-245-bc | R | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-246-bc | R | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-1-247-bc | R | Escanaba, Alvin 4043 | 40.999 | 127. 4934 | -3222 |
| WB-2-14-bc | Xylopholas? | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-15-bc | Xylopholas? | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-16-bc | Xylophaga sp. | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-17-bc | Xylophaga sp. | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-18-bc | Xylophaga sp. | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-19-bc | Xylophaga sp. | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-20-bc | G | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-21-bc | G | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-22-bc | G | GR-14, Alvin 4044 | 42.7559 | -126.7096 | -2721 |
| WB-2-23-bc | G | GR-14, Alvin 4044 | 42.7559 | -126.7096 | -2721 |
| WB-2-24-bc | G | GR-14, Alvin 4044 | 42.7559 | -126.7096 | -2721 |
| WB-2-25-bc | G | GR-14, Alvin 4044 | 42.7559 | -126.7096 | -2721 |
| WB-2-26-bc | G | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-27-bc | G | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-28-bc | G | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-29-bc | G | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-30-bc | G | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-31-bc | Xylophaga sp. | GR-14, Alvin 4044 | 42.7559 | -126.7096 | -2721 |

| | | | | | |
|---|---|---|---|---|---|
| WB-2-32-bc | Xylophaga sp. | GR-14, Alvin 4044 | 42.7559 | -126.7096 | -2721 |
| WB-2-33-bc | 1 | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-34-bc | 1 | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-35-bc | 1 | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-36-bc | 1 | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-37-bc | R? | GR-14, Alvin 4044 | 42.7559 | -126.7096 | -2721 |
| WB-2-38-bc | R? | GR-14, Alvin 4044 | 42.7559 | -126.7096 | -2721 |
| WB-2-39-bc | R | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-40-bc | R | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-41-bc | Q? | GR-14, Alvin 4044 | 42.7559 | -126.7096 | -2721 |
| WB-2-42-bc | Q? | GR-14, Alvin 4044 | 42.7559 | -126.7096 | -2721 |
| WB-2-43-bc | Q | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-44-bc | Q | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-45-bc | Q | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-46-bc | Q | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-227-bc | G | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-2-228-bc | G | GR-14, Alvin 4044 | 42.7559 | 126.7096 | -2721 |
| WB-3-47-bc | B | Axial, ROPOS 740 | 45.9333 | -130.1333 | -1540 |
| WB-3-48-bc | B | Axial, ROPOS 740 | 45.9333 | -130.1333 | -1540 |
| WB-3-49-bc | B | Axial, ROPOS 740 | 45.9333 | -130.1333 | -1540 |
| WB-3-50-bc | B | Axial, ROPOS 740 | 45.9333 | -130.1333 | -1540 |
| WB-3-51-bc | B | Axial, ROPOS 740 | 45.9333 | -130.1333 | -1540 |
| WB-3-52-bc | B | Axial, ROPOS 740 | 45.9333 | -130.1333 | -1540 |
| WB-3-53-bc | B | Axial, ROPOS 740 | 45.9333 | -130.1333 | -1540 |
| WB-3-54-bc | B | Axial, ROPOS 740 | 45.9333 | -130.1333 | -1540 |
| WB-3-55-bc | B | Axial, ROPOS 740 | 45.9333 | -130.1333 | -1540 |
| WB-3-56-bc | B | Axial, ROPOS 740 | 45.9333 | -130.1333 | -1540 |
| WB-4-229-bc | F | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| WB-4-230-bc | F | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| WB-4-231-bc | F | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| WB-4-232-bc | F | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| WB-4-233-bc | F | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| WB-4-234-bc | F | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| WB-4-248-bc | F | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| WB-4-57-bc | F | Cleft, ROPOS 542 | 44.6633 | -130.355 | -2100 |
| WB-4-58-bc | F | Cleft, ROPOS 542 | 44.6633 | -130.355 | -2100 |
| WB-4-59-bc | F | Cleft, ROPOS 542 | 44.6633 | -130.355 | -2100 |
| WB-4-60-bc | F | Cleft, ROPOS 542 | 44.6633 | -130.355 | -2100 |
| WB-4-61-bc | F | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| WB-4-62-bc | F | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| WB-4-63-bc | F | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| WB-4-64-bc | F | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| WB-4-65-bc | F | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| WB-4-66-bc | F | Cleft, ROPOS 542 | 44.6633 | 130.355 | -2100 |
| WB-4-67-bc | U | Cleft, ROPOS 619 | 44.6633 | -130.355 | -2100 |
| WB-4-68-bc | U | Cleft, ROPOS 619 | 44.6633 | -130.355 | -2100 |
| WB-4-69-bc | U | Cleft, ROPOS 619 | 44.6633 | -130.355 | -2100 |
| WB-4-70-bc | U | Cleft, ROPOS 619 | 44.6633 | -130.355 | -2100 |
| WB-4-71-bc | U | Cleft, ROPOS 619 | 44.6633 | -130.355 | -2100 |
| WB-4-72-bc | U | Cleft, ROPOS 619 | 44.6633 | 130.355 | -2100 |
| WB-4-73-bc | U | Cleft, ROPOS 619 | 44.6633 | 130.355 | -2100 |
| WB-4-74-bc | U | Cleft, ROPOS 619 | 44.6633 | 130.355 | -2100 |
| WB-4-75-bc | U | Cleft, ROPOS 619 | 44.6633 | 130.355 | -2100 |
| WB-4-76-bc | U | Cleft, ROPOS 619 | 44.6633 | 130.355 | -2100 |
| WB-4-77-bc | U | Cleft, ROPOS 619 | 44.6633 | 130.355 | -2100 |
| WB-4-78-bc | U | Cleft, ROPOS 619 | 44.6633 | 130.355 | -2100 |
| WB-4-79-bc | U | Cleft, ROPOS 619 | 44.6633 | 130.355 | -2100 |
| WB-4-80-bc | U | Cleft, ROPOS 619 | 44.6633 | 130.355 | -2100 |

| | | | | | |
|---|---|---|---|---|---|
| WB-4-82-bc | F | ODP1026B, JASON 065 | 47.6788 | -127.8555 | -2661 |
| WB-5-100-bc | F | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-101-bc | B | Endeavour, JASON 068 | 47.9472 | -129.096 | -2250 |
| WB-5-102-bc | B | Endeavour, JASON 068 | 47.9472 | -129.096 | -2250 |
| WB-5-103-bc | B | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-104-bc | B | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-105-bc | B | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-106-bc | B | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-107-bc | B | Endeavour, Alvin 4045 | 47.9472 | -129.096 | -2250 |
| WB-5-108-bc | B | Endeavour, Alvin 4045 | 47.9472 | -129.096 | -2250 |
| WB-5-109-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-110-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-111-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-112-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-113-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-114-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-115-bc | M | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-116-bc | M | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-117-bc | M | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-118-bc | M | Endeavour, Alvin 4045 | 47.9472 | -129.096 | -2250 |
| WB-5-119-bc | M | Endeavour, Alvin 4045 | 47.9472 | -129.096 | -2250 |
| WB-5-120-bc | M | Endeavour, Alvin 4045 | 47.9472 | -129.096 | -2250 |
| WB-5-121-bc | M | Endeavour, Alvin 4045 | 47.9472 | -129.096 | -2250 |
| WB-5-122-bc | F? | Endeavour, Alvin 4045 | 47.9472 | -129.096 | -2250 |
| WB-5-123-bc | F? | Endeavour, Alvin 4045 | 47.9472 | -129.096 | -2250 |
| WB-5-124-bc | M | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-125-bc | F | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-126-bc | F | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-127-bc | F | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-249-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-250-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-251-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-252-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-253-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-254-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-255-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-259-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-260-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-261-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-262-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-263-bc | B | Endeavour, Alvin 4045 | 47.9472 | 129.096 | -2250 |
| WB-5-83-bc | U | Endeavour, JASON 068 | 47.9472 | -129.096 | -2250 |
| WB-5-84-bc | U | Endeavour, JASON 068 | 47.9472 | -129.096 | -2250 |
| WB-5-85-bc | U | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-86-bc | U | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-87-bc | U | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-88-bc | U | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-89-bc | F | Endeavour, JASON 068 | 47.9472 | -129.096 | -2250 |
| WB-5-90-bc | F | Endeavour, JASON 068 | 47.9472 | -129.096 | -2250 |
| WB-5-91-bc | F | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-92-bc | F | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-93-bc | F | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-94-bc | F | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-95-bc | F? | Endeavour, JASON 068 | 47.9472 | -129.096 | -2250 |
| WB-5-96-bc | F? | Endeavour, JASON 068 | 47.9472 | -129.096 | -2250 |
| WB-5-97-bc | F | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-98-bc | F | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |
| WB-5-99-bc | F | Endeavour, JASON 068 | 47.9472 | 129.096 | -2250 |

| | | | | | |
|---|---|---|---|---|---|
| WB-6-129-bc | F | Baby Bare, JASON 062 | 47.7105 | -127.7882 | -2641 |
| WB-6-130-bc | F | Baby Bare, JASON 062 | 47.7105 | -127.7882 | -2641 |
| WB-6-131-bc | F | Baby Bare, JASON 062 | 47.7105 | -127.7882 | -2641 |
| WB-6-132-bc | F | Baby Bare, JASON 062 | 47.7105 | 127.7882 | -2641 |
| WB-6-133-bc | F | Baby Bare, JASON 062 | 47.7105 | 127.7882 | -2641 |
| WB-7-134-bc | U | ODP1026B, JASON 065 | 47.6788 | -127.8555 | -2661 |
| WB-7-135-bc | U | ODP1026B, JASON 065 | 47.6788 | -127.8555 | -2661 |
| WB-7-136-bc | U | ODP1026B, JASON 065 | 47.6788 | -127.8555 | -2661 |
| WB-7-137-bc | U | ODP1026B, JASON 065 | 47.6788 | -127.8555 | -2661 |
| WB-7-138-bc | U | ODP1026B, JASON 065 | 47.6788 | -127.8555 | -2661 |
| WB-7-139-bc | U | ODP1026B, JASON 065 | 47.6788 | -127.8555 | -2661 |
| WB-7-140-bc | U | ODP1026B, JASON 065 | 47.6788 | -127.8555 | -2661 |
| WB-7-141-bc | U | ODP1026B, JASON 065 | 47.6788 | -127.8555 | -2661 |
| WB-7-142-bc | U | OPD1026B, JASON 065 | 47.6788 | 127.8555 | -2661 |
| WB-7-143-bc | U | OPD1026B, JASON 065 | 47.6788 | 127.8555 | -2661 |
| WB-7-144-bc | U | OPD1026B, JASON 065 | 47.6788 | 127.8555 | -2661 |
| WB-7-226-bc | U | OPD1026B, JASON 065 | 47.6788 | 127.8555 | -2661 |
| WB-8-145-bc | T | Ucluelet Dredge | 48.556 | 126.436 | -600 |
| WB-8-146-bc | T | Ucluelet Dredge | 48.556 | 126.436 | -600 |
| WB-8-147-bc | T | Ucluelet Dredge | 48.556 | 126.436 | -600 |
| WB-8-148-bc | T | Ucluelet Dredge | 48.556 | 126.436 | -600 |
| WB-8-149-bc | T | Ucluelet Dredge | 48.556 | 126.436 | -600 |
| WB-8-150-bc | T | Ucluelet Dredge | 48.556 | 126.436 | -600 |
| WB-8-151-bc | T | Ucluelet Dredge | 48.556 | 126.436 | -600 |
| WB-8-152-bc | T | Ucluelet Dredge | 48.556 | 126.436 | -600 |
| WB-8-153-bc | T | Ucluelet, bottom trawl | 48.556 | -126.436 | -600 |
| WB-8-154-bc | T | Ucluelet, bottom trawl | 48.556 | -126.436 | -600 |
| WB-9-155-bc | G | Wuzza Bare, Alvin 4046 | 47.4847 | -127.6915 | -2658 |
| WB-9-156-bc | G | Wuzza Bare, Alvin 4046 | 47.4847 | -127.6915 | -2658 |
| WB-9-157-bc | G | Wuzza Bare, Alvin 4046 | 47.4847 | -127.6915 | -2658 |
| WB-9-158-bc | G | Wuzza Bare, Alvin 4046 | 47.4847 | -127.6915 | -2658 |
| WB-9-159-bc | B | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-160-bc | B | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-161-bc | B | Wuzza Bare, Alvin 4046 | 47.4847 | -127.6915 | -2658 |
| WB-9-162-bc | B | Wuzza Bare, Alvin 4046 | 47.4847 | -127.6915 | -2658 |
| WB-9-163-bc | B | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-164-bc | B | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-165-bc | B | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-166-bc | B | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-167-bc | Xylopholas | Wuzza Bare, Alvin 4046 | 47.4847 | -127.6915 | -2658 |
| WB-9-168-bc | G? | Wuzza Bare, Alvin 4046 | 47.4847 | -127.6915 | -2658 |
| WB-9-169-bc | Xylophaga sp. | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-170-bc | Xylophaga sp. | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-171-bc | Q? | Wuzza Bare, Alvin 4046 | 47.4847 | -127.6915 | -2658 |
| WB-9-172-bc | Q? | Wuzza Bare, Alvin 4046 | 47.4847 | -127.6915 | -2658 |
| WB-9-173-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-174-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-175-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-176-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-177-bc | Q? | Wuzza Bare, Alvin 4046 | 47.4847 | -127.6915 | -2658 |
| WB-9-178-bc | Q? | Wuzza Bare, Alvin 4046 | 47.4847 | -127.6915 | -2658 |
| WB-9-179-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-180-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-181-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-182-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-216-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-217-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-218-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |

| | | | | | |
|---|---|---|---|---|---|
| WB-9-220-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-221-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-222-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-223-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-224-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-225-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-235-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-236-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-237-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-238-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-239-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-240-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-241-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-242-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-243-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-9-244-bc | Q | Wuzza Bare, Alvin 4046 | 47.4847 | 127.6915 | -2658 |
| WB-10-183-bc | Bankia sp. | Hecate Strait, dredge | 51.45166 | -129.04 | -100 |
| WB-10-184-bc | Bankia sp. | Hecate Strait, dredge | 51.45166 | -129.04 | -100 |
| WB-10-185-bc | Bankia sp. | Hecate Strait, dredge | 51.45166 | -129.04 | -100 |
| WB-10-186-bc | Bankia sp. | Hecate Strait, dredge | 51.45166 | -129.04 | -100 |
| WB-10-187-bc | Bankia sp. | Hecate Strait, dredge | 51.45166 | 129.04 | -100 |
| WB-10-188-bc | Bankia sp. | Hecate Strait, dredge | 51.45166 | 129.04 | -100 |
| WB-10-189-bc | B | Hecate Strait, dredge | 51.45166 | -129.04 | -100 |
| WB-10-190-bc | B | Hecate Strait, dredge | 51.45166 | -129.04 | -100 |
| WB-10-191-bc | B | Hecate Strait, dredge | 51.45166 | -129.04 | -100 |
| WB-10-192-bc | B | Hecate Strait, dredge | 51.45166 | -129.04 | -100 |
| WB-10-193-bc | B | Hecate Strait, dredge | 51.45166 | 129.04 | -100 |
| WB-10-194-bc | B | Hecate Strait, dredge | 51.45166 | 129.04 | -100 |
| WB-10-195-bc | B | Hecate Strait, dredge | 51.45166 | 129.04 | -100 |
| WB-10-196-bc | B | Hecate Strait, dredge | 51.45166 | 129.04 | -100 |
| WB-10-197-bc | B | Hecate Strait, dredge | 51.45166 | 129.04 | -100 |
| WB-10-198-bc | B | Hecate Strait, dredge | 51.45166 | 129.04 | -100 |
| WB-10-199-bc | B | Hecate Strait, dredge | 51.45166 | 129.04 | -100 |
| WB-10-200-bc | B | Hecate Strait, dredge | 51.45166 | 129.04 | -100 |
| WB-10-201-bc | B | Hecate Strait, dredge | 51.45166 | 129.04 | -100 |
| WB-10-202-bc | B | Hecate Strait, dredge | 51.45166 | 129.04 | -100 |
| WB-10-203-bc | B | Hecate Strait, dredge | 51.45166 | 129.04 | -100 |
| WB-11-204-bc | F? | ODP1026B, Alvin 4151 | 47.76265 | -127.7592 | -2658 |
| WB-11-205-bc | F? | ODP1026B, Alvin 4152 | 47.76265 | -127.7592 | -2658 |
| WB-11-206-bc | F? | ODP1026B, Alvin 4153 | 47.76265 | -127.7592 | -2658 |
| WB-11-207-bc | F? | ODP1026B, Alvin 4154 | 47.76265 | -127.7592 | -2658 |
| WB-11-208-bc | F | OPD1026B, Alvin 4151 | 47.76265 | 127.7592 | -2658 |
| WB-11-209-bc | F | OPD1026B, Alvin 4151 | 47.76265 | 127.7592 | -2658 |
| WB-11-210-bc | F | OPD1026B, Alvin 4151 | 47.76265 | 127.7592 | -2658 |
| WB-11-211-bc | F | OPD1026B, Alvin 4151 | 47.76265 | 127.7592 | -2658 |
| WB-11-212-bc | F | OPD1026B, Alvin 4151 | 47.76265 | 127.7592 | -2658 |
| WB-11-213-bc | F | OPD1026B, Alvin 4151 | 47.76265 | 127.7592 | -2658 |
| WB-12-214-bc | Xylophaga sp. | Mid Gorda, Tiberon 352-J5 | 40.3705 | -125.3806 | -1588 |