

**COLONISATION BY THE WOOD-BORING BIVALVE  
*XYLOPHAGA WASHINGTONA***

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## **Abstract**

Wood falls represent a rare and ephemeral, yet important source of food in the deep-sea. Wood-boring bivalves of the family Xylophaginae have high reproduction and growth rates, allowing them to rapidly colonise and exploit these resources. While efforts have focussed on the taxonomy of this group, there has been less research carried out on reproduction and larval recruitment. Specimens of *Xylophaga washingtona* were collected from an experimental wood deployment placed at a depth of 1,605 m for 15 months off the coast of Oregon, USA. Individuals were compared using the mitochondrial 16S rDNA gene to test for the presence of multiple cohorts. The analyses revealed genetic differentiation within the population. This suggests that colonisers are arriving in multiple cohorts, as opposed to one single cohort. However, the sample size was extremely low, with poor data quality, and the results of this study should therefore not be considered reliable or conclusive. Future studies on the population genetics of *X. washingtona* would be valuable in further addressing recruitment of this species.

## **Introduction**

Large food falls in the deep ocean, such as whale carcasses or sunken wood, are rare events on local scales. However, when they do occur, they represent a huge influx of energy in a highly localised area and are quickly colonised and exploited by an opportunistic fauna (Bienhold 2013). Wood falls have been recorded in all oceans and at all depths (Bienhold 2013). At the seafloor, sunken wood is rapidly broken down and eroded by a range of wood-boring organisms (Tyler et al. 2007). Bivalve molluscs of the genus *Xylophaga* play a principle role in this process (Tyler et al.

2007) and belong to the relatively understudied family Xylophaginae (Voight 2007). These bivalves are found in deep waters in all of the world's oceans, with several new species recently described from the eastern Pacific Ocean (Voight 2007) and the Mediterranean (Romano et al. 2014). They are found at depths of 10 to 5,050 m and are restricted to wood falls, on which they feed (Voight 2009). Using toothed ridges of their shells, *Xylophaga* bore into wood and ingest fragments, which are then broken down with the help of symbiotic gill bacteria (Voight 2007). Due to difficulty of sampling in deep waters and patchiness of wood fall habitats on the seafloor, there is limited knowledge on the biology, distribution and dispersal of the Xylophagainids (Voight 2009; Romano et al. 2014).

Biological communities associated with highly productive habitats in the deep sea, such as wood falls, hydrothermal vents or seeps, must overcome several challenges in order to maintain populations (Metaxas & Kelly 2010). This includes coping with the ephemeral nature and patchy distribution of their food source (Metaxas & Kelly 2010). The type of colonisers and their succession on wood falls is also dependent on a number of factors, including geographic location of the wood fall, season, and the size and type of wood (Bienhold et al. 2013) as well as larval dispersal, survival, settlement, growth and recruitment to the adult population (Metaxas & Kelly 2010).

As with other productive deep sea habitats, little is known about the dispersal of organisms between wood falls (Metaxas & Kelly 2010; Bienhold et al. 2013). We know that wood-boring bivalves must be able to rapidly locate and colonise wood falls in order to maintain their populations (Tyler et al. 2007; Bienhold et al. 2013). Some species in the genus *Xylophaga* produce larvae that are able to survive long periods of time in the water column (Tyler et al. 2007; Haga & Kase 2013; Romano et

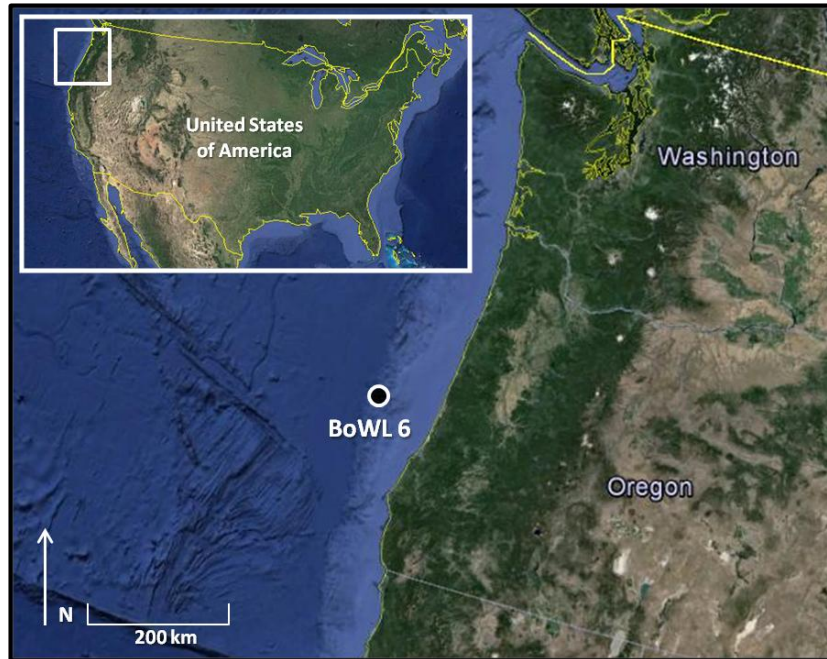
al. 2013), suggesting a capacity for long-distance dispersal (Romano et al. 2013). In hydrothermal vent systems, the larvae of the sessile tubeworm *Riftia pachyptila* have been shown to arrive in discrete larval cohorts of closely related individuals (Shank & Halanych 2007). This suggests that closely related individuals remain together during transport (Shank & Halanych 2007). While we have some limited insight into the reproductive strategies of Xylophagainids, which produce both planktonic and brooded young (Voight 2007), it is still unclear how larvae detect the presence of wood (Bienhold et al. 2013).

This study aimed to investigate recruitment in a species of Xylophagainae, *Xylophaga washingtona*, with a focus on whether *X. washingtona* colonises wood falls in single or multiple cohorts. This was tested by comparing genetic variation at the mitochondrial 16S rDNA gene among specimen collected from an experimental deep-sea wood fall off the coast of Oregon, USA. *X. washingtona* was hypothesised to colonise a given wood fall in multiple cohorts.

## **Materials and Methods**

### *Sampling*

Individuals of *X. washingtona* were collected from an experimental wood deployment placed at a depth of 1,605 m off the coast of Oregon (43° 54.522' N 125° 10.238' W) for 15 months (Figure 1). This wood deployment forms part of the *Bone and Wood Landers* project run by the University of Hawaii in collaboration with Auburn University.



**Figure 1.** Map showing location of the experimental wood deployment off the coast of Oregon, USA. Map generated in Google Earth and modified in Microsoft Office PowerPoint 2007.

Nine individuals were randomly selected from ethanol-preserved samples and sequenced for the 16S genetic marker (n=9). A small piece of siphon from each individual was removed for DNA extraction using a DNeasy<sup>®</sup> kit following the manufacturer's protocols. A section of the mitochondrial 16S rDNA gene was amplified using a PTC-100<sup>™</sup> Programmable Thermal Controller (MJ Research, Inc.) and the following PCR reaction mix: 10  $\mu$ l GoTaq Flexi Buffer, 6  $\mu$ l MgCl<sub>2</sub> solution, 1  $\mu$ l PCR Nucleotide Mix, 0.5  $\mu$ l of each primer, 0.25  $\mu$ l GoTaq G2 Flexi DNA Polymerase, 2  $\mu$ l DNA template, filled to a total volume of 50  $\mu$ l with dH<sub>2</sub>O, under the following PCR conditions: 94°C 2 min (94°C 0:30, 40°C 1:00, 72°C 1:00) 35 times, 72°C 5:00. In order to maximise yield some of the PCR reaction mixes were made using 8  $\mu$ l MgCl<sub>2</sub> and 4  $\mu$ l DNA template. The PCR products were then re-run under the standard conditions after being diluted 1:10 with dH<sub>2</sub>O. DNA was extracted using

either QIAquick<sup>®</sup> PCR Purification kit or QIAquick<sup>®</sup> Gel Extraction kit. All PCR products were sent to GENEWIZ<sup>®</sup> for sequencing.

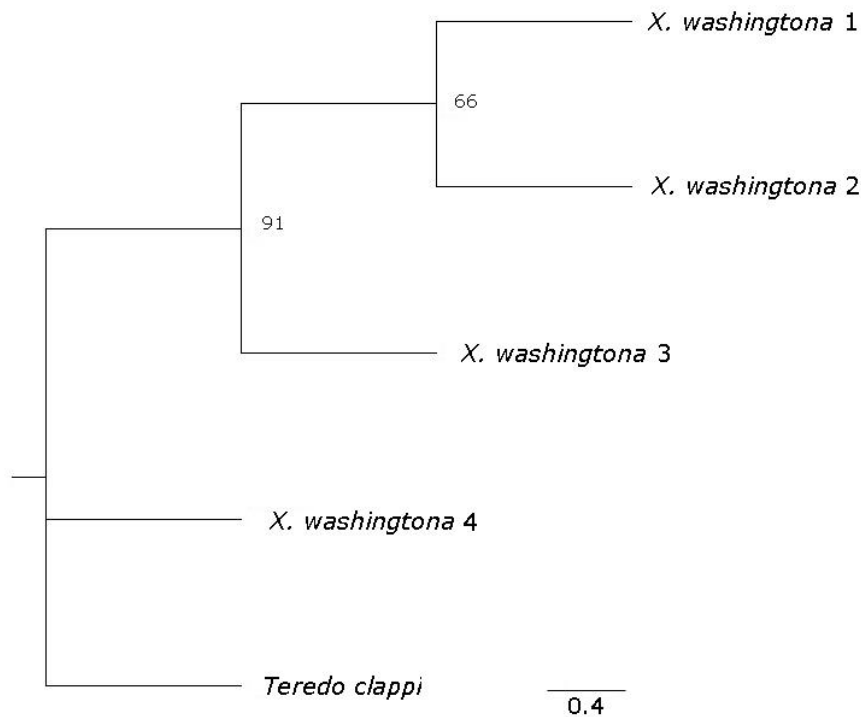
### *Analysis*

Once sequences were obtained from GENEWIZ<sup>®</sup>, consensus sequences for each individual were obtained from forward and reverse sequences. The sequences were trimmed in Geneious R7 (v7.1.7), aligned using a De Novo Assemble and a consensus sequence was generated. Each sequence was submitted to BLAST (Madden 2002) nucleotide and blastx searches in the GenBank<sup>®</sup> data base to verify lack of contamination. Several of the sequences showed signs of contamination and were discarded, leaving a total of 4 sequences. A mitochondrial 16S rDNA sequence of the Teredinid *Teredo clappi* was added to the generated dataset from GenBank<sup>®</sup> to act as an outgroup for the analysis. The sequences were multi-aligned in Geneious R7 using MUSCLE (Edgar 2004), with a final sequence length of 246 bp after trimming and alignment. A Maximum Likelihood tree was generated in RAxML-HPC Blackbox v8.0.24 (Stamatakis et al. 2008) to show phylogenetic relatedness among individuals and nodal support was assessed by 1000 bootstrap replicates. The tree was exported to Geneious R7 for editing.

### **Results**

Four of nine specimens of *X. washingtona* from the wood deployment were successfully sequenced. Due to contamination, five specimens were discarded and sequence quality of the four successful specimens was poor. A Maximum Likelihood tree was therefore constructed using mitochondrial 16S rDNA sequences obtained for four individuals of *X. washingtona* and one *Teredo clappi* (Figure 2).

Some variation in the level of relatedness among individuals was recorded. The most closely related individuals were *X. washingtona* 1 and *X. washingtona* 2. These individuals were separated by a branch length of 2.00 (Table 2) and bootstrap values show 66% nodal support. *X. washingtona* 1 and *X. washingtona* 2 were separated from *X. washingtona* 3 in a well-supported clade (91% bootstrap support) with a branch length of 3.00 separating these specimens. *X. washingtona* 4 was found to be more closely related to *Teredo clappi* than to other specimens of *X. washingtona*. This is likely related to the low quality of sequence data. *X. washingtona* 1, 2 and 3 were most distantly related to *Teredo clappi*. The variation in genetic make-up suggests that these specimens are not from the same cohort.



**Figure 2.** Maximum Likelihood tree generated from sequences of the mitochondrial 16S rDNA gene of *Xylophaga washingtona* and *Teredo clappi*, with bootstrap supports on the nodes.

**Table 2.** Distance between species, measured as branch length.

	<i>X. washingtona</i> 1	<i>X. washingtona</i> 2	<i>X. washingtona</i> 3	<i>X. washingtona</i> 4	<i>Teredo clappi</i>
<i>X. washingtona</i> 1		2.00	3.00	4.00	4.00
<i>X. washingtona</i> 2	2.00		3.00	4.00	4.00
<i>X. washingtona</i> 3	3.00	3.00		3.00	3.00
<i>X. washingtona</i> 4	4.00	4.00	3.00		2.00
<i>Teredo clappi</i>	4.00	4.00	3.00	2.00	

## Discussion

BLAST (Madden 2002) nucleotide and blastx searches in GenBank revealed several sequences that had been contaminated and were therefore excluded from the analysis. This resulted in a very low sample size (n=4) as time constraints prohibited further DNA extraction. In addition, the quality of the data that was used was poor. This could be due to a number of factors including poor extraction technique, due to inexperience, or unsuitable annealing temperature during sequencing.

Despite these limitations in the data set, it is still valuable to discuss the results obtained. While *X. washingtona* 1 and *X. washingtona* 2 were more closely related than the rest of the group (branch length = 2.00), it is clear that genetic variation exists in the sampled population of *X. washingtona*. The minimum distance between individuals was 2.00, with a maximum of 4.00. One individual was found to be more closely related to *Teredo clappi*, in the family Teredinidae, than to the other individuals of *X. washingtona*, although this is likely a result of poor data quality. Bootstrap values show moderate nodal support for the split between *X. washingtona* 1 and *X. washingtona* 2 and high support for their relationship to *X. washingtona* 3.

The observed genetic variation indicates that individuals in the sampled population were not closely related and therefore, despite occurring on the same wood fall, do not arise from a single cohort. This supports the hypothesis and suggests that *X. washingtona* colonises wood not in a single cohort from the same source, but rather in multiple cohorts. These cohorts could be arriving at wood falls from different individuals in the same source population, or from different individuals and different sources.

Depending on the hydrodynamics of a system and the life history characteristics of an organism, some recruitment can result from retention of larvae

produced by the colonisers, as found by Shank & Halanych (2007). In a study on the deep-sea wood-dwelling sipunculan *Phascolosoma turnerae*, Rice et al. (2012) concluded that retention of larvae was unlikely to act as a source population as the larvae are planktotrophic, with a life span greater than 7 months. The size distribution of colonising populations can also be used to shed light on the number of recruitment events (Tyler 2007; Romano et al. 2013).

Future studies on the population genetics of *X. washingtona* would be valuable in further addressing recruitment of this species. Research on population connectivity and genetic variation within and among populations should be prioritised. It would be useful to repeat this study using a larger sample size, as well as other genetic markers, and to complement it with size-frequency analyses. By understanding the genetic structure of potential source populations, questions on recruitment in this species could be more adequately addressed. Recruitment studies using genetic techniques should also be coupled with *in-situ* or laboratory based ecological experiments.

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