UC Berkeley UC Berkeley Electronic Theses and Dissertations

Title

Patterns of specialization in the deep sea at the individual, ecosystem, and evolutionary level

Permalink https://escholarship.org/uc/item/3rv2k6cp

Author Judge, Jenna

Publication Date 2015

Peer reviewed|Thesis/dissertation

Patterns of specialization in the deep sea

at the individual, ecosystem, and evolutionary level

By

Jenna Louise Judge

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Integrative Biology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor David Lindberg, Chair Professor Charles Marshall Professor Rosemary Gillespie Professor Robert Vrijenhoek

Spring 2015

Abstract

Patterns of specialization in the deep sea at the individual, ecosystem, and evolutionary level

by

Jenna Louise Judge

Doctor of Philosophy in Integrative Biology

University of California, Berkeley

Professor David Lindberg, Chair

Most of the deep sea is characterized by low productivity and low biomass, however there is a patchy distribution of unique habitats that support specialist communities and high biomass. These habitats include hydrothermal vents and cold seeps; geological features that provide reduced compounds like sulfide and methane to fuel chemosynthesis, an alternate form of primary production to photosynthesis. Organic falls like whale carcasses and wood support related but distinct communities of scavengers, grazers, filter feeders, and extreme specialists.

This dissertation examines multiple levels of specialization to deep-sea reducing environments to address the following questions:

- 1. Is there a common evolutionary path toward specialization in reducing habitats?
- 2. How have lineages modified their anatomy to specialize in deep-sea habitats?
- 3. How does variation in sunken wood influence community assembly?

To address the first question, I used a supertree of the Gastropoda, one of the most diverse animal groups in reducing habitats, to ask whether lineages followed similar trajectories toward becoming specialists in these ecosystems. For the second question, I focused on a lineage of limpets (Lepetellidae) that has only been found living on empty *Hyalinoecia* polychaete tubes, and made hypotheses about how they utilize their substrate based on a detailed 3D reconstruction of the anatomy of *Lepetella sierra*. The third question focuses on how variation in the substrate can influence diversity and community assembly, particularly in wood fall communities colonizing experimental wood fall substrates of ten different types over the course of two years at 3200m near Monterey, CA. Through the course of exploring these three questions, I have developed collaborations, learned new skills, and contributed new data and perspectives on deepsea reducing environments.

Table of Contents

Introduction	ii
Acknowledgements	vii
Chapter 1: Evolutionary patterns of specialization in deep-sea gastrope	ods: a supertree
perspective	1
Abstract	1
Introduction	1
Methods	
Results	6
Discussion	7
Acknowledgements	9
References	9
Tables	
Figure Legend	
Figures	
Appendices	
Chapter 2: The anatomy of Lepetella sierrai (Vetigastropoda, Lepetelloidea	a):
implications for reproduction, feeding, and symbiosis in lepetellid limpets	
Abstract	
Introduction	
Methods	
Results	
Discussion	42
Acknowledgements	44
References	44
Tables	47
Figure Legend	
Figures	
Chapter 3: Macroinvertebrate Community Assembly on Deep-sea Wood F	alls in Monterey
Bay is Strongly Influenced by Wood Type	60
Abstract	60
Introduction	60
Methods	
Results	
Discussion	64
Acknowledgements	67
References	67
Tables	69
Figure Legend	
Figures	76

Introduction

Deep-sea habitats have many qualities that shape the diversity and abundance of life that have evolved to live there. Of all the factors that affect life, food availability is mostly responsible for limiting the biomass, metabolic rates, and sizes of deep-sea organisms (Somero & Hochachka 1984, Gage & Tyler 1991). Unlike most ecosystems, which are photosythentically driven, some special ecosystems are fueled by chemosynthesis, the process by which microbes utilize energy derived from the oxidation of reduced inorganic molecules to fuel the conversion of inorganic carbon and nutrients to organic matter (Van Dover et al. 2002, Stewart et al. 2005, Dubilier et al. 2008, Kiel 2010). This form of metabolism is generally rare in the deep-sea, but various forms of it are found in habitats such as hydrothermal vents, hydrocarbon seeps, decomposing wood, and sunken carcasses, each having an unique distribution (Wolff 1979, Sibuet & Olu 1998, Tunnicliffe et al. 1998, Van Dover et al. 2002, Smith & Baco 2003).

Hydrothermal vents occur where tectonic plates separate, and volcanic hot spots where pressure from mantle convection produces cracks in the oceanic crust and allows seawater to be heated by the magma below. This heat causes a pressure increase in the water flowing through the cracks, which is then forcefully expelled into the ambient seawater. Low levels of hydrogen sulfide remain in the hydrothermal plume of water and this reduced molecule fuels chemosynthesis at vents (Haymon et al. 1991). Like hydrothermal vents, hydrocarbon seeps also release compounds that act as fuel for chemosynthesis by the action of geologic processes. Seeps typically occur along tectonic margins such as subduction zones and back-arc basins. These active tectonic zones cause petroleum reserves to leak methane, carbon dioxide, and traces of hydrogen sulfide, which fuel chemosynthesis (Barry et al. 1996, Sibuet & Olu 1998). However, not all chemosynthesis is fueled by geology, and much of the sulfide at seeps is a product of anaerobic methane oxidation by microbial symbiotic consortia composed of archaea and sulfate reducing bacteria (Boetius et al. 2000). Sunken whale carcasses and sunken wood represent external organic inputs into the deep-sea that go through stages of decomposition during which sulfidic fuel for chemosynthesis is produced (Smith & Baco 2003, Bienhold et al. 2013). Regardless of how they are fueled, these ecosystems are unique in the deep-sea because of their high productivity, which in turn fuels high biomass in an otherwise food-poor deep sea. In addition to specialist chemosynthesis-associated taxa, opportunistic scavengers, grazers, and predators compose the communities found at these ephemeral and patchy sources of substrate and food in the deep-sea.

Across reducing habitats, there are many aspects that vary, such as available area, energy source and concentration, substrate characteristics, depth, distance from land, and longevity. The organic falls and other chemosynthetic sites form the basis for unique yet overlapping ecosystems that share niches occupied by a range of taxa. The only known primary producers in these ecosystems are the chemoautotrophic bacteria, but on organic falls microbes play an additional decomposition role. The heterotrophic microbes that work to break down wood and lipid-rich whale bones are key to drawing down oxygen and creating a sulfidic environment that supports chemosynthesis in the first place (Bienhold et al. 2013). They also make the organic substrate surface more accessible to animal grazers that consume biofilm, and they make the substrate. For instance, provannid gastropods of the genus *Rubyspira* feed on bone, but they

cannot consume wood (Johnson et al. 2010). Consumers of wood include wood-boring clams like *Xylophaga*, pectinodontid limpets, and the seastar *Xyloplax* (Turner 2002, Voight 2005, Zbinden et al. 2010). Generally, substrate consumers cannot digest bone or wood without help from microbial symbionts that provide necessary enzymes to digest components of bone and cellulose in wood. More diverse than animals with cellulose-degrading symbionts in the deep-sea, are those that have a symbiotic relationship with chemosynthetic bacteria. Because similar chemoautotrophs are found across all reducing habitats, the animal lineages that rely on them, as symbionts, are similarly widespread. However, they have usually diverged, resulting in clades of animals that have diversified to occupy a range of reducing habitats through their relationship with chemosynthetic symbionts. One of the clearest examples of this phenomenon are the bathymodiolin mussels, whose evolutionary history has be traced from sunken wood, to whale falls, and then to vents and seeps, with different lineages specializing to each habitat with corresponding symbiotic bacteria (Distel et al. 2000). Lastly, the least specialized trophic level in these ecosystems are the scavengers and opportunistic predators that are primarily drawn by either carrion or the increased biomass present at organic falls compared to the surrounding area.

Organic material in the form of plants, animals, or their parts has the potential to contribute heterogeneity to the benthic environment as food falls or hard substrates that stand out on the mostly soft sediment of the deep-sea (Stockton & DeLaca 1982, Gooday et al. 1990). For instance, whale carcasses contribute a significant amount of carrion that can be consumed by a range of scavengers, and after the removal of soft tissue the hard skeleton is utilized by other guilds of animals utilizing the structure and associated lipids (Smith & Baco 2003). Even fish carcasses with relatively small skeletons, squid beaks, elasmobranch egg cases, crab carapaces and certain polychaete tubes can provide habitat and possibly food for deep-sea taxa (Haszprunar 1988). The overall rate of deposition and distribution of these organic inputs to the deep-sea is difficult to estimate, but we can be assured it is a significant contribution due to the diversity of organisms that have evolved to take advantage of organic falls. Fossil evidence of deep-sea fauna living on plesiosaur skeletons indicates that vertebrate falls have played a role in shaping deepsea community evolution at least since the Late Cretaceous (Kaim et al. 2008, Danise et al. 2014, Danise & Higgs 2015). Although there is a gap between the disappearance of large marine reptiles and the origination of marine mammals, there is evidence that certain whale-fall specialists can live on fish, turtle and seabird bones, which would have been available as a substitute for larger skeletons (Samadi et al. 2010, Kiel et al. 2011, Rouse et al. 2011). Wood represents a major link between land and the deep-sea, whereas the previously mentioned organic inputs are marine in origin.

Wood has a long history in the ocean, having influences on coastal, surface, and deep-sea communities (Maser & Sedell 1994). Processes on widely varying time scales influence the amount of wood that enters the marine environment from seasonal flooding, stochastic storms and hurricanes, and long-term sea level changes (Savrda 1991, Hyatt & Naiman 2001). Tectonic activity and glaciations have invoked changes in sea level throughout the Phanerozoic. Fossil evidence from the Paleogene suggests that sea level rise in particular was a mechanism through which flooding brought in large amounts of wood and then subsequent ravinement concentrated that wood (Savrda 1991). Additionally, forest composition varies widely across regions and these have also changed drastically through time. From studies on rafting organisms, there are indications that wood and macroalgae can float across ocean basins, potentially bringing plant

material to deep benthic environments far from coastlines (Thiel & Gutow 2005). In the short term, management of forests, dams on rivers, and logging practices influence the source of wood and the likelihood it will be transported to the ocean (Bilby & Ward 1991, Ralph et al. 1994, Montgomery & Piégay 2003). Along coastlines, humans have built many wood structures including houses, piers, and boardwalks. In fact, 44% of the human population lives within 150 km of the ocean and the most common building material is wood (UN Atlas of the Oceans 2004). All of these factors culminate to influence the amount, composition, and distribution of wood on the seafloor that has been available for marine organisms to utilize as substrate.

This dissertation examines multiple levels of specialization to deep-sea reducing environments to address the following questions:

- 1. Is there a common evolutionary path toward specialization in reducing habitats?
- 2. How have lineages modified their anatomy to specialize in deep-sea habitats?
- 3. How does variation in sunken wood influence community assembly?

To address the first question, I used a supertree of the Gastropoda, one of the most diverse animal groups in reducing habitats, to ask whether lineages followed similar trajectories toward becoming specialists in these ecosystems. For the second question, I focused on a lineage of limpets (Lepetellidae) that has only been found living on empty *Hyalinoecia* polychaete tubes, and made hypotheses about how they utilize their substrate based on a detailed 3D reconstruction of the anatomy of *Lepetella sierra*. The third question focuses on how variation in the substrate can influence diversity and community assembly, particularly in wood fall communities colonizing experimental wood fall substrates of ten different types over the course of two years at 3200m near Monterey, CA. Through the course of exploring these three questions, I have developed collaborations, learned new skills, and contributed new data and perspectives on deepsea reducing environments.

References

- Barry J, Garygreene H, Orange D, Baxter C, Robison B, Kochevar R, Nybakken J, R D, & Mchugh C 1996. Biologic and geologic characteristics of cold seeps in Monterey Bay, California. Deep Sea Res. Part I Oceanogr. Res. Pap. 43: 1739–1755.
- Bienhold C, Ristova P, & Wenzhöfer F 2013. How deep-sea wood falls sustain chemosynthetic life. PLoS One 8: 10–15.
- Bilby RE & Ward JW 1991. Characteristics and Function of Large Woody Debris in Streams Draining Old-Growth, Clear-Cut, and Second-Growth Forests in Southwestern Washington. Can. J. Fish. Aquat. Sci. 48: 2499–2508.
- Boetius A, Ravenschlag K, Schubert CJ, Rickert D, Widdel F, Gieseke A, Amann R, Jørgensen BB, Witte U, & Pfannkuche O 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407: 623–626.
- Danise S & Higgs ND 2015. Bone-eating Osedax worms lived on Mesozoic marine reptile deadfalls.
- Danise S, Twitchett RJ, & Matts K 2014. Ecological succession of a Jurassic shallow-water ichthyosaur fall. Nat. Commun. 5: 4789.

- Distel DL, Baco AR, Chuang E, Morrill W, Cavanaugh C, & Smith CR 2000. Do mussels take wooden steps to deep-sea vents? Nature 403: 725–726.
- Dover CL Van, German CR, Speer KG, Parson LM, & Vrijenhoek RC 2002. Evolution and biogeography of deep-sea vent and seep invertebrates. Science 295: 1253–1257.
- Dubilier N, Bergin C, & Lott C 2008. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. Nat. Rev. Microbiol. 6: 725–740.
- Gage JD & Tyler PA 1991. Deep Sea Biology: A Natural History of Organisms at the Deep-Sea Floor. Cambridge University Press, Cambridge.
- Gooday a. J, Turley CM, & Allen J a. 1990. Responses by Benthic Organisms to Inputs of Organic Material to the Ocean Floor: A Review [and Discussion]. Philos. Trans. R. Soc. A Math. Phys. Eng. Sci. 331: 119–138.
- Haszprunar G 1988. Comparative Anatomy of Cocculiniform Gastropods and its Bearing on Archaeogastropod Systematics. Malacol. Rev. 4: 64–84.
- Haymon RM, Fornari DJ, Edwards MH, Carbotte S, Wright D, & Macdonald KC 1991.
 Hydrothermal vent distribution along the East Pacific Rise crest (9°09'–54'N) and its relationship to magmatic and tectonic processes on fast-spreading mid-ocean ridges. Earth Planet. Sci. Lett. 104: 513–534.
- Hyatt TL & Naiman RJ 2001. The residence time of large woody debris in the Queets River, Washington, USA. Ecol. Appl. 11: 191–202.
- Johnson SB, Warén A, Lee RW, Kano Y, Kaim A, Davis A, Strong EE, & Vrijenhoek RC 2010. Rubyspira, new genus and two new species of bone-eating deep-sea snails with ancient habits. Biol. Bull. 219: 166–177.
- Kaim A, Kobayashi Y, Echizenya H, & Jenkins RG 2008. Chemosynthesis based associations on Cretaceous plesiosaurid carcasses Mollusks associated to plesiosaur skeletons. Acta Palaeontol. Pol. 53: 97–104.
- Kiel S 2010. The Vent and Seep Biota Kiel S, ed. Springer Netherlands, Dordrecht. 255-277 pp.
- Kiel S, Kahl W-A, & Goedert JL 2011. Osedax borings in fossil marine bird bones. Naturwissenschaften 98: 51–55.
- Maser C & Sedell JR 1994. From the forest to the sea. The ecology of wood in streams, rivers, estuaries, and oceans. St. Lucie Press, Delray Beach, FL(USA).
- Montgomery DR & Piégay H 2003. Wood in rivers: interactions with channel morphology and processes. Geomorphology 51: 1–5.
- Ralph SC, Poole GC, Conquest LL, & Naiman RJ 1994. Stream Channel Morphology and Woody Debris in Logged and Unlogged Basins of Western Washington. Can. J. Fish. Aquat. Sci. 51: 37–51.
- Rouse GW, Goffredi SK, Johnson SB, & Vrijenhoek RC 2011. Not whale-fall specialists, Osedax worms also consume fishbones. Biol. Lett. 7: 736–739.
- Samadi S, Corbari L, Lorion J, Hourdez S, Haga T, Dupont J, Boisselier MC, & Forges BR De 2010. Biodiversity of deep-sea organisms associated with sunken-wood or other organic remains sampled in the tropical Indo-Pacific. Cah. Biol. Mar. 51: 459–466.
- Savrda CE 1991. Teredolites, wood substrates, and sea-level dynamics. Geology 19: 905–908.
- Sibuet M & Olu K 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. Deep. Res. Part II Top. Stud. Oceanogr. 45: 517–567.

Smith CR & Baco AR 2003. Ecology of whale falls at the deep-sea floor. Oceanogr. Mar. Biol. Vol 41 41: 311–354.

- Somero GN & Hochachka P 1984. Biochemical Adaptation. Princeton University Press, Princeton, NJ. 537 pp.
- Stewart FJ, Newton ILG, & Cavanaugh CM 2005. Chemosynthetic endosymbioses: Adaptations to oxic-anoxic interfaces. Trends Microbiol. 13: 439–448.
- Stockton WL & DeLaca TE 1982. Food falls in the deep sea: occurrence, quality, and significance. Deep Sea Res. Part A. Oceanogr. Res. Pap. 29: 157–169.
- Thiel M & Gutow L 2005. The ecology of rafting in the marine environment. I. The floating substrata. Oceanogr. Mar. Biol. An Annu. Rev. 42: 181–264.
- Tunnicliffe V, McArthur AG, & McHugh D 1998. A Biogeographical Perspective of the Deep-Sea Hydrothermal Vent Fauna. 353-442 pp.
- Turner RD 2002. On the Subfamily Xylophagainae (Family Pholadidae, Bivalvia, Mollusca). Bull. Mus. Comp. Zool. 157: 223–307.
- Voight JR 2005. First report of the enigmatic echinoderm Xyloplax from the North Pacific. Biol. Bull. 208: 77–80.
- Wolff T 1979. Macrofaunal utilization of plant remains in the deep-sa. Sarsia.
- Zbinden M, Pailleret MZ, Ravaux J, Gaudron SM, Hoyoux C, Lambourdière J, Warén A, Lorion J, Halary S, & Duperron S 2010. Bacterial communities associated with the wood-feeding gastropod pectinodonta sp. (Patellogastropoda, Mollusca). FEMS Microbiol. Ecol. 74: 450–463.

Acknowledgements

I am so grateful for every opportunity along my path exploring the marine environment, discovering the excitement of deep-sea ecosystems, and being encouraged to pursue my interests and develop them into the dissertation presented here. None of this would be possible without all the individuals, museums, and organizations that have supported me intellectually, emotionally, and financially.

My interest in the deep sea started in high school, where Ms. Zezula, my AP Biology teacher, told us about the wonders of hydrothermal vents, ecosystems fueled by a novel form of primary production: chemosynthesis. Thus the seed was planted for me to major in Aquatic Biology at UC Santa Barbara, where I took classes with Rachel Haymon and Jim Childress, two pioneers in deep-sea science. My interests in marine biology were further galvanized when I studied abroad at the University of Queensland, where a diverse range of experiences in the field fueled me to apply to graduate school in my final year at UCSB. Before I had fully decided where to apply, I had an opportunity to work at MBL in Woods Hole as a course assistant and although the focus of the course was embryology rather than marine biology, I learned a lot and most importantly became acquainted with Nipam Patel, a UC Berkeley professor. I had not considered applying to UC Berkeley for graduate school as it does not have a marine biology or oceanography program, but Nipam convinced me I should talk to David Lindberg and apply to be in his lab at Berkeley. Nipam could not have been more right about the intellectual connection I would make and further develop with Dave, and I don't think I could have found a better fit or a more supportive advisor. I am so grateful that my path led me to Dave's lab, where I have benefitted from his big picture perspective, encouragement, and connections to a broad network of colleagues. I would also like to thank my qualifying exam committee members Charles Marshall, Rosie Gillespie, Jonathon Stillman, and Jere Lipps who helped develop my framework for tackling scientific problems and making my skin a little thicker.

Throughout my graduate career, I pursued many opportunities that led me to learn from and collaborate with researchers across the globe, and I am eternally grateful for their confidence and willingness to help develop my scientific mind. In order of encounter, I'd like to thank Bob Vrijenhoek (MBARI, also a committee member), Takenori Sasaki (University of Tokyo), Yasunori Kano (University of Tokyo), Anders Warén (Natural History Museum Stockholm), Gerhard Haszprunar (ZSM, Munich), Janet Voight (FMNH, Chicago), Craig McClain (Nescent) and Jim Barry (MBARI, CA). I would also like to thank the museums and individuals that loaned me specimens and trained me in techniques during my exploration of limpet diversity and anatomy: Philippe Bouchet (NMNH, Paris), Bruce Marshall (Te Papa, NZ), Lisa Levin (Scripps), Reena Zalpuri (EM lab, UC Berkeley), Eva Lodde (ZSM, Munich), and Shannon Johnson (MBARI).

I give my thanks to the following organizations and grants that enabled me to follow through with my ideas and travel to many museums and conferences: UCMP, Integrative Biology, UCB Graduate Division, AMNH Lerner Gray, Conchologists of America, American Malacological Society, Sigma Xi (Berkeley Chapter), NSF/JSPS EAPSI, DAAD (Germany), and Unitas Malacologica.

I treasure the years of conversations, seminars, feedback, and camaraderie I have found in the Museum of Paleontology and dept. of Integrative Biology, especially via Fossil Coffee, Fossil Fuel, Marine Tea (even though nobody else calls it that), and Eco Coffee. Although I prize the wisdom shared by those in higher ranks, it has been my fellow graduate students that have always been there to help me figure out a new method and get through the inevitable roadblocks that cropped up during one pursuit or another. In particular I'd like to thank Erin Meyer-Beetham, Rosemary Romero, and Joey Pakes, for many marine biology chats, Camilla Souto for her Echinodermata enthusiasm, and Juan Guevara and Meagan Oldfather for passing on their R knowledge and skills. I would also like to thank my undergraduate research assistants, without whom this dissertation would not exist because I would have given up on counting and sorting the thousands of woodfall invertebrates long ago: Priscilla Chen, Chris Castaneda, Allegra Nottoli, Connie Martin, Alyssa Kehlenbach, Jennifer Yu, and Jessie Kendall-Bar.

Perhaps most importantly, I would like to extend my gratitude to my friends and family that have helped me to keep things in perspective. My father has always supported me in whatever I chose to pursue, and I am grateful that his love of the outdoors rubbed off on me at a young age. I am grateful to my friends who understand the need for a "life" in work/life balance, especially Tesla. Jeremy Chase and Liz started as supportive comrades in the foreign land of Japan, and the bond has remained strong even across state lines. Khaki and Juniper have been with me since my first year here, and even though I have left them for months at a time, they never fail to provide me warm purring comfort. I cannot express how fortunate I feel to have found such a supportive companion in Andy, who inspires me every day to only put energy into the good in my life, of which there is plenty.

Chapter 1

Evolutionary patterns of specialization in deep-sea gastropods: a supertree perspective

Jenna Judge & David Lindberg

Abstract

Gastropods are one of the most diverse groups to occupy and specialize to deep-sea reducing habitats including hydrothermal vents, cold seeps, whale falls and wood falls. However, the phylogenetic occurrence of this habitat across the gastropods has not been previously investigated. Here we construct a supertree for the Gastropoda, composed of 462 genera. We used the supertree to display and reconstruct patterns of specialization in reducing habitats with an unordered parsimony ancestral state reconstruction. Although all major gastropod lineages (ordinal rank) have invaded these deep-sea habitats, they consist primarily of independent invasions that have rarely resulted in major diversifications with the exception of the Neomphalina, several vetigastropod lineages, and Abyssochrysoidea within the Ceanogastropoda. In addition, rather than suggesting a common trajectory from generalist to specialist habitat occupancy, there is a wide range of patterns across the tree, and our data further suggests that lineages can more easily expand into multiple reducing habitat types after first invading a single reducing habitat.

Introduction

Most of the deep-sea is characterized by low biomass and low metabolic rates, except at chemosynthesis-based ecosystems, most famously hydrothermal vents (Corliss et al. 1979, Somero & Hochachka 1984, Tunnicliffe 1992). Unlike most ecosystems, which are photosythentically driven, these deep-sea ecosystems are fueled by chemosynthesis, the process by which microbes utilize energy derived from the oxidation of reduced inorganic molecules, such as sulfide and methane, to fuel the conversion of inorganic carbon and nutrients to organic matter (Van Dover et al. 2002, Levin & Mendoza 2007, Dubilier et al. 2008, Bernardino et al. 2012). The best studied deep-sea habitats fueled by chemosynthesis are hydrothermal vents, hydrocarbon seeps, decomposing wood, and sunken carcasses, and each has a unique distribution (Tunnicliffe et al. 1998, Van Dover et al. 2002, Smith & Baco 2003, Ramirez-Llodra et al. 2010, Fornari et al. 2012).

Hydrothermal vents occur at mid-ocean ridges, back-arc basins, and volcanic hot spots where seawater is heated by magma below. This heat causes a pressure increase in the water flowing through lithospheric cracks, which is then forcefully expelled into the ambient seawater. Low levels of hydrogen sulfide remain in the hydrothermal plume of water and this reduced molecule fuels chemoautotrophic microbes at vents (Haymon et al. 1991, Fornari et al. 2012). Like hydrothermal vents, hydrocarbon seeps also release compounds that act as fuel for chemosynthesis through the action of geologic processes.

Seeps typically occur along active tectonic margins such as subduction zones and back-arc basins. These active tectonic zones cause petroleum reserves to leak methane, carbon dioxide, and traces of hydrogen sulfide, which fuel chemosynthesis (Barry et al. 1996, Sibuet & Olu 1998). However, not all chemosynthesis is fueled by geology (Boetius et al. 2000). Sunken

whale carcasses (Smith et al. 1989, Smith & Baco 2003, Kiel & Goedert 2006, Lundsten et al. 2010) and sunken wood (Turner 1973; Deming et al. 1997, 2013; Kiel et al. 2012; Laurent et al. 2013) provide a range of resources supporting both heterotrophs and chemoautotrophs.

In the case of large vertebrate falls, like whales, there are several successional stages of the community. First, scavengers such as hagfish, sleeper sharks, and lysianassid amphipods, will arrive whether it's a piece of mackerel on a baited trap or a whale carcass (Smith & Baco 2003, Kemp et al. 2006, Higgs et al. 2014). Carrion is the most ephemeral part of a sunken carcass, so most animals that are specialized as scavengers are highly mobile, and constantly seek new sources of fallen food. However, the hard skeleton has a longer residence time before it will fully decompose due to the work of microbes and certain specialized animals that can actually consume bone, and the fatty lipids within (Smith & Baco 2003, Fujiwara et al. 2007, Lundsten et al. 2010). These animal specialists include bone-eating worms of the genus Osedax and gastropods of the genus Rubyspira (Vrijenhoek et al. 2008, Johnson et al. 2010, Rouse et al. 2011). Both of these genera are members of clades that have diversified to live not only on bones, but also in the unique geological settings of hydrothermal vents and methane seeps (Halanych 2005, Johnson et al. 2010, Hilário et al. 2011). However, it should be noted that when proper studies of the background fauna have been made, many animals that had been considered specialists turn out to be general background opportunists (Lundsten et al. 2010). Compared to whale falls, sunken wood has analogous patterns of succession with a range of exploiters from scavengers, opportunistic grazers, predators and wood-consuming specialists.

Sunken wood is colonized by specialized wood-boring bivalves of the family Xylophagaidae that digest wood with symbiotic microbes in their gills (Distel 2003), enrich the surrounding sediment with wood chips and fecal pellets, and alter the wood surface, facilitating colonization by other animals (Bienhold et al. 2013, Fagervold et al. 2014, Voight 2015). The action of these bivalves along with specialized lignin- and cellulose-digesting microbes in the sediment increases the overall oxygen uptake, enabling sulfate-reducing microbes to produce sulfide. Sulfide then fuels chemosynthetic metabolisms of free living microbes and symbionts of chemosymbiotic animals such as the mussel, *Idas* (Bienhold et al. 2013, Fagervold et al. 2014). In addition to these wood ingesting and chemosymbiotic specialists, other fauna are drawn to the wood and utilize it as a hard substrate, grazing surface, or predation opportunity (Wolff 1979, Samadi et al. 2010), Gaudron et al. 2010), resulting in a similar ecosystem structure to whale falls.

In a review of biogeographic patterns at vents by Tunnicliffe et al. (1998), the authors asserted that after 20 years since the discovery of hydrothermal vents, there were high levels of endemicity at the species level to particular sulfidic habitats, but that this diminished at higher taxonomic levels and that this pattern was likely partially due to sampling bias. Tunnicliffe et al. (1998) also put forth the challenge to utilize a phylogenetic approach in comparing taxonomic distributions. At the time, they were severely limited by a lack of systematic knowledge of the relationships of taxa inhabiting reducing habitats, and while this is still a limitation today with the continual discovery of new deep-sea taxa, we have come a long way since 1998. One of the major debates in this field has surrounded the timing of diversification in chemosynthetic ecosystems, and it was hypothesized that most of the fauna was composed of 'living fossils' that had survived extinctions in chemosynthetic refugia (McArthur & Tunnicliffe 1998). However,

many lines of evidence since then indicate that this is likely not the case for most of the modern chemosynthetic fauna, as reviewed in Vrijenhoek (2013).

Exploratory sampling expeditions and experimental deployments of organic and inorganic substrates have returned both new taxa and new occurrences of known taxa expanding their ranges and known habitat associations (Waren & Bouchet 1993, Tunnicliffe et al. 1998, Baco & Smith 2003, Voight 2005, 2007, Vrijenhoek 2009, Lundsten et al. 2010). When Smith et al. (1989) discovered chemosynthetic communities on a whale fall, they recognized the chemical and faunal similarities between the whale fall environment and vents and seeps and even drew a connection to sunken wood. They proposed the stepping stone hypothesis that predicts whale falls may be important "stepping stones" to facilitate dispersal of chemosynthetic organisms to vents and seeps (Smith et al. 1989). This idea ignited research on the connections between reducing habitats and has been investigated through comparing faunal lists (Waren & Bouchet 1993, Tunnicliffe et al. 1998), paleontology (Kiel & Goedert 2006), phylogenetics of certain taxa (e.g., bathymodiolin mussels (Distel et al. 2000; Lorion et al. 2013)), and was most recently tested with an ecological experiment deploying small pieces of different substrate types (Cunha et al. 2013).

Recently, several studies have attempted to test the hypothesis that organic falls provide stepping stones to vents and seeps on both ecological and evolutionary timescales. Bernardino et al. (2012) made a comparison of macroinfauna in chemosynthetic localities and they noted that there was lower endemicity at seeps and vents for heterotrophs compared to chemosymbiotic taxa. They postulated that the density and diversity in chemosynthetic sediments are regulated by geochemistry and that higher sulfide levels support higher densities, but that there is a diversity tradeoff since many background fauna are intolerant of sulfide in high concentrations. It follows then that vents have the lowest diversity but can support high densities of chemosymbiotic specialists (Bernardino et al. 2012). Cunha et al. (2013) tested the potential of organic falls to be reliable stepping stones on ecological timescales and found that it is highly likely they can support organisms for the duration of a reproductive cycle and must be important recurring evolutionary connections to the deep sea. The most recent review of whale falls by Smith et al. (2015) summarized the evidence for ecological and evolutionary connections between sunken wood, whale falls, seeps, and vents and they acknowledge that different lineages show different patterns and that each scenario is context specific. By incorporating new data, Smith et al. (2015) have reconfigured the original stepping stone hypothesis (Smith et al. 1989) to include three stepping stone scenarios implicating whale falls; (1) as "biodiversity generators" facilitating the evolutionary transition from organic falls to vents and seeps [e.g., repeated invasion of bathymodiolin mussels to vents and seeps from whale falls/sunken wood (Thubaut et al. 2013)]; (2) as "ecological stepping stones" enabling diversification (e.g., the vesicomyid clams likely originated at seeps, but the timing of their diversification is parallel to that of whales, so potentially whale falls could have enabled dispersal to other chemosynthetic sites); and (3) as "hot spots of adaptive radiation" in the case of the bone-eating siboglinid genus, Osedax (Vrijenhoek et al. 2009, Rouse et al. 2011). The growing evidence from these ecological, phylogenetic, and metadata analyses indicate that there are many routes to deep-sea chemosynthetic environments and many roles organisms have as community members there, from opportunistic scavengers to obligate chemosymbiotic specialists.

Many ecological and evolutionary questions require a finely sampled phylogeny, especially if the morphological and/or ecological characters of interest are highly variable within and among clades. However, if one wants to investigate evolutionary patterns across a large group, such as the living gastropods, it is a daunting task to collect enough shared sequences or common morphological characters with which to construct such massive trees. Gastropods are one of the most diverse taxonomic groups in reducing deep-sea habitats and also exhibit a wide range of roles from grazers, to predators, to chemosymbiotic specialists (Sasaki et al. 2010). Here we produce a supertree of the Gastropoda and use it to investigate patterns of gastropod invasion and specialization in deep-sea environments. The implementation of supertree algorithms offers one solution by combining phylogenies produced from disparate datasets and tree building methods, and the resulting tree(s) can be used to test a variety of questions beyond what any single source tree can provide.

We focused on patterns of gastropod evolution in chemosynthetic habitats at the generic level, using a supertree approach. Rather than assigning genera to vents, seeps, whale falls, or sunken wood, we have acknowledged that sampling bias towards certain habitat types and undersampling of the background fauna may erroneously reflect higher endemism than exists. Additionally, others have recognized that although there are patterns of endemism at the species level when comparing faunal lists, it declines at higher taxonomic levels (Tunnicliffe et al. 1998, Smith et al. 2015). Thus, in addition to applying a phylogenetic framework, we have taken a different approach in categorizing gastropod genera and assigned them to 1 of 4 states relating to their occurrence in vent, seep, whale fall, and/or sunken wood habitats (hereafter "reducing habitat"): (1) no known occurrence at reducing habitats, (2) known occurrence at both reducing and non-reducing habitats, (3) known from multiple reducing habitats and no non-reducing habitats, (4) only known from one of the four reducing habitat types. We chose this scheme to emphasize patterns of specialization to reducing habitats rather than transitions between them. With this approach we tested whether certain clades were more likely to invade reducing deepsea habitats and of those that did, whether the tendency to specialize was conserved phylogenetically. We hypothesized that the major gastropod clades would either exhibit tendencies toward increasing specialization to certain reducing habitats, or conversely would remain as generalists able to exploit both reducing and non-reducing habitats.

Methods

Supertrees

To examine the generic distribution of chemosynthetic gastropod taxa we required a hypothesis of gastropod phylogenetic relationships. Unfortunately, no such analysis exists with the required coverage across the gastropods. However, there are analyses of smaller groups within these clades that were available for amalgamation using supertree approaches (Bininda-Emonds 2004). Due to the patchiness or phylogenetic coverage across gastropod groups, and limited overlap between published trees, we incorporated several methods to produce a tree with adequate coverage at the generic rank to allow mapping of the chemosynthetic states. For instance, most available phylogenies focused on relatively limited regions of Gastropoda, and few addressed the relationships between the major clades. Thus, we first made a backbone phylogeny using source trees that did sample across all the major clades, then made supertree for each clade and attached those to the backbone supertree. Although, not ideal, the pool of available trees forced us to apply this solution. Based on the available trees, we applied analyses to the following clades:

Patellogastropoda, Vetigastropoda, Neomphalina, Neritimorpha, Heterogastropoda, and Caenogastropoda.

We began by estimating a backbone phylogeny of the major gastropod clades (at the ordinal rank) using both liberal (CLANN 3.0.0, sfit) (Creevev & McInerney 2005, 2009), and conservative (PhySIC IST) (Scornavacca et al. 2008) supertree algorithms. Subclade supertrees were then generated for each of the gastropod orders, with the exception of the Patellogastropoda and Heterobranchia where recent single phylogenies were placed directly on the backbone (Table 1). As pointed out by Sigwart and Lindberg (2014), most molluscan phylogenetic studies focus on small taxonomic groups with the intent to determine the placement of one taxon of interest, rather than the structure of the total group. This was particularly problematic in the caenogastropods where there was insufficient taxon overlap amongst the source trees to produce an integrated result at the generic level. We therefore elevated the level of the caenogastropod analysis to family rank using the authors' familial assignments. This provided greater overlap of taxa as well as enabled us to use additional source trees that had originally been done at the familial level. The result of the caenogastropod familial supertree analysis was then placed on the gastropod backbone and the family tips replaced by the generic level results from the individual source tree analyses using the decomposition method of Mishler et al. (1994). For source trees that were resolved only to the family level, we replaced the family name with the type genus of the family.

For CLANN analyses, a heuristic search using the maximum split fit algorithm (sfit) with subtree pruning and re-grafting (SPR), 100 search repetitions (1000 for backbone determination) and split weighting (every tree had an equal weight) was used. SPR allows for more thorough searching of tree space compared to the other heuristic search algorithm in CLANN, nearest neighbor interchange (NNI). The sfit algorithm returns a score reflecting the fit between the source trees and the resulting supertree by remoing the effect of missing data and allowing the option to equally weight trees of different sizes, so that each taxon split represented in a source tree has an equal vote for the split determined in the supertree between those taxa (Creevey 2005). For the PhySIC_IST analyses we used the online portal (http://www.atgcmontpellier.fr/physic_ist/). Default values were used for bootstrap threshold (0) and the correction threshold for source trees (0.9). CLANN was also used to calculate majority-rule and strict consensus trees when multiple supertrees were identified. All ambiguous regions of the consensus trees that included taxa that occurred in reducing habitats were found to have strong support prior to being placed in the final supertree.

We regard this as the most conservative approach. We analyzed 462 genera from a total of 31 source tree topologies to produce a gastropod supertree. The number of source trees and the total number of taxa in each subclade analysis are presented in Table 1. See Appendix A.1 for a list of source trees and their references.

Character State Reconstruction

Records of gastropod genera found at hydrothermal vents, cold seeps, whale falls, and wood falls were compiled from the literature and the Chessbase database. Genera within the supertree were then categorized based on their occurrence: (1) only in non-reducing habitats (including general deep-sea environments), (2) in both reducing and non-reducing habitats, (3) in multiple reducing

habitats, or (4) only known from one of these reducing habitats. Each of these categories was treated as a character state and mapped onto the supertree using an unordered parsimony ancestral reconstruction method in Mesquite (Maddison & Maddison 2001). We were limited to the parsimony analysis because the supertree does not have branch lengths and maximum likelihood ancestral reconstruction in Mesquite requires branch lengths. Transition rates between each state were calculated. It should be noted that the parsimony reconstruction does not allow ambivalent states, and this restriction may have biased the pattern for some lineages. See Appendix A.2 for data sources and references that provided taxon occurrence information.

Results

Supertrees

Results of the conservative PhySIC_IST analyses are given in Table 1. The number of taxa rejected by the analyses, due to insufficient overlap of those taxa in source trees, ranged between 23% and 46% of the taxa. In the gastropod backbone analysis, the taxa not inserted into the analysis were the Cocculinoidea, Neomphalina, and the Vetigastropoda – three taxa that include many prominent chemosynthetic taxa. The inability to include these taxa is related to their variable placement in the source trees for the gastropod backbone. Because of these results, we did not include the PhySIC_IST trees in our analysis of chemosynthetic taxa and proceeded with the more liberal CLANN trees.

The CLANN analyses produced multiple supertrees for each of the subclades (Table 1). The best fit of the supertrees with the source trees (sfit) was found in the Neritimorpha (0.0468) and the gastropod backbone tree (1.1810); the worst fit in the caenogastropods (9.0986). Each sfit score was interpreted in the context of the each supertree compared to its respective source trees and should not be compared between analyses. A score of zero indicates that all of the source trees are identical to the pruned supertree (Creevey & McInerney 2009). Higher scores indicate more variability between relationships in source trees, therefore the resulting supertree will not have a perfect fit with all source trees, returning an sfit score larger than zero. The sfit score provides an optimality criterion for selecting the supertree that best agrees with splits represented in source trees (Creevey 2005).

A majority rule consensus tree was determined for the backbone and each subclade (CLANN, consensus). Each subclade tree was rooted based on the original analyses, and placed on the backbone phylogeny. For each subclade, majority rule consensus trees were compared to strict consensus trees. Although some regions of the tree are not well resolved (indicated by lower support values on majority rule trees and polytomies on strict consensus trees), these regions with weaker support did not influence patterns of specialization to reducing habitats.

Character State Reconstruction

Across all 462 gastropod genera represented in the supertree, 62 are known from reducing habitats (Fig. 1). There are 25 lineages in a mix of reducing and non-reducing habitats, 14 in multiple reducing habitats, and 23 in a single reducing habitat type (Table 2). The state change matrix (Table 3) summarizes the transitions between states along branches of the supertree. The main pattern observed from the transition matrix is that marginally more lineages starting from non-reducing habitats expanded their habitat range to include reducing habitats, but several lineages went directly to the multiple or single reducing habitat state. Additionally, once lineages

were only in a single reducing habitat, further derived branches from that lineage primarily remained in one reducing habitat or expanded to include multiple reducing habitats (Table 3, Fig. 2).

Clade comparisons

Starting at the base of the tree, Patellogastropoda is a relatively small clade (16 of 462 genera represented), but has a moderate percentage of represented taxa occupying reducing habitats (18.75%) (Table 2, Fig. 3). Vetigastropoda had the most lineages in these habitats compared to any other clade (23) and a moderate percentage (23%) (Table 2, Fig. 4). Neomphalina showed by far the highest percentage of taxa occupying reducing habitats (100%) and also had the highest degree of specialization, but is only represented by a total of 13 genera (Table 2, Fig. 5). Nerites had four lineages coded, with three of those limited to one or multiple reducing habitats (Fig. 6). Heterobranchs had only three lineages represented in a mix of reducing and non-reducing habitats (Fig. 7). Caenogastropods, the most diverse clade in the tree with 226 genera, had only a 7.08% occupancy rate in reducing habitats, primarily in a mix of reducing and non-reducing habitats (Table 2, Fig. 8). However, the Abyssochrysoidea are one clade within the caenogastropods that has seen increased specialization.

Discussion

Gastropod relationships

The supertree presented here represents one estimate of gastropod relationships (Fig. 1). Although many parts of the tree were resolved with high confidence, there are clades for which there was low overlap between source trees, or poor agreement on relationships between source trees that resulted in poor resolution. These regions of low overlap or disagreement in relationships indicate clades that require further systematic work to resolve relationships (see comparisons between majority rule and strict consensus trees in Figs. 4, 6, 8, and Appendix B). One recurring issue that contributed to poor placement of lineages was inadequate outgroup choice in phylogenies focused on specific clades. Often authors chose outgroups that were either too distant or too close for our purposes, making it difficult for the supertree algorithm to place a smaller clade within a larger one or to relate larger clades amongst one another. The supertree programs responded to this issue by placing taxa at the base of the clade, clearly not where many of them belonged. These issues led to our solution of producing the final tree in several steps, first with the backbone, and then constructing subtrees of each major subclade. Each clade presented its own quirks as far as what kind of trees and how many were available. Patellogastropods and heterobranchs each only had one tree that spanned enough diversity to be informative and adequate for our analysis. The caenogastropods were particularly problematic, due again to few trees covering the tremendous diversity of caenogastropods, with most published phylogenies focused either on very restricted taxa, particular regions, or broad course sampling that did not provide much overlap between trees. Despite these challenges, we were able to reconstruct a supertree for the Gastropoda that provides the largest summary of known relationships in this group, is based on the latest available phylogenies and is reproducible.

Specialization to reducing habitats in the deep sea

Despite significant undersampling in the deep sea at global scales, and incomplete inclusion of known taxa from these habitats in published phylogenies, clear patterns emerged showing different levels of specialization amongst gastropod clades. Although there are poorly supported

topologies in the supertree, the lineages occupying reducing habitats are widely dispersed across the tree. The overall patterns of specialization to deep-sea habitats reconstructed for each major subclade are robust given the current state of knowledge for gastropod phylogeny.

The main pattern for gastropods is that most genera do not occupy any reducing habitats, and the lineages that do represent multiple independent invasions of these habitats, either expanded their range to include reducing and non-reducing habitats, or shifted to a range restricted to one or more reducing habitat types. Rather than observing a common trajectory of lineages moving towards further specialization, there are many routes that result primarily in lineages either (1) retaining flexibility to be opportunistic members of chemosynthetically-based ecosystems, or (2) being limited to one reducing habitat type, with fewer lineages occupying multiple habitat types. When examining the directionality of these habitat expansions and restrictions, there is a stronger pattern of lineages moving directly to a single reducing habitat type before expanding to multiple reducing habitat types (Fig. 2). This overall pattern in the tree is corroborated by knowledge that there are very few species that occupy more than one reducing habitat type (Tunnicliffe et al. 1998), so these transitions of genera from one to multiple habitat types likely represent diversification events. This pattern is in contrast to the alternative hypothesis of lineages first being widespread and across several habitat types that are higher in sulfide or other reduced compounds, and then subsequently specializing to a particular habitat type, losing their range across varied reducing habitats.

In general, diversifications into reducing habitats are rare across the gastropod supertree. The clear exception to this pattern is the Neomphalina, a clade that has 100% of its branches coded as specialists in one or multiple reducing habitat types. Although most neomphalids are vents specialists, some lineages coded as single habitat occupants are not at vents. For instance, *Bathysciadium* lives on sunken squid beaks and *Cocculina* lives on a mix of organic substrates such as wood and bone (Haszprunar 1988a, b, McLean 1992, Sasaki et al. 2005, Hess et al. 2008). Additionally, the Vetigastropoda have the highest number of lineages in reducing habitats with several independent specialization events. Most of the specialized vetigastropod lineages have not been subject to ecological or physiological studies, but a summary of research and taxonomic knowledge can be found in Sasaki et al. (2010). Although the patellogastropods only have three specialist lineages, they have no non-specialist taxa in reducing habitats, and include one of the only gastropod genera that is confirmed as a direct wood consumer, *Pectinodonta* (Lindberg & Hedegaard 1996, Zbinden et al. 2010). Thus, specialists in reducing habitats tend to be concentrated in the three earliest branching clades of the gastropods; the Patellogastropoda, Vetigastropoda, and Neomphalina.

The Neritimorpha, Heterobranchia, and Caenogastropoda have a pattern of fewer genera occupying reducing habitats and of those that do, most live in a mix of reducing and non-reducing habitats and are typically opportunistic members of chemosynthetic ecosystems. The exception to this pattern is found in the radiation of the Abyssochrysoidea, an early diverging lineage within Caenogastropoda that includes chemosymbiotic vent specialists, like *Alvinoconcha* and *Ifremaria*, and the only known gastropod to consume bone directly, *Rubyspira* (Johnson et al. 2010, Sasaki et al. 2010).

Although this analysis provides an additional perspective towards understanding large scale patterns of specialization to deep-sea habitats, it cannot directly test hypotheses regarding the timing of major diversifications of chemosynthetic faunas. The classical "antiquity hypothesis" viewed chemosynthetic habitats, and especially hydrothermal vents, as stable refuges where relict Paleozoic taxa have remained undisturbed through evolutionary time (McArthur & Tunnicliffe 1998). However, the dynamic nature of reducing habitats on short and long timescales, evidence from the fossil record and molecular clock estimates contest the antiquity hypothesis and suggest that most lineages radiated or re-radiated in the Cenozoic (Vrijenhoek 2013). Although the ancestral reconstructions presented here cannot address the timing of radiations directly, there is a general pattern that most lineages occupying reducing habitats are relatively derived, with few long branches reaching back to more basal splits. However, caution should be used when interpreting the ancestral reconstruction of long branches due the possibility that extinction of taxa in non-reducing habitats is artificially amplifying a chemosynthetic signal. For instance, the placement of the Neomphalina within the Gastropod backbone did not have high support, and although McArthur and Tunnicliffe (1998) found support for a mid-Mesozoic radiation, this clade merits further systematic investigation.

In general, the data presented here do not support a singular pattern of specialization in either topology or relative timing of invasion in chemosynthesis-based ecosystems. The variation in patterns amongst clades is likely due to fluctuations in the environment that have affected the general timing of origination for deep water taxa (Jacobs & Lindberg 1998), variation in the ability of clades to readily take advantage of reducing habitats when exposed to them, and length of time during which lineages might have been exposed and able to further specialize to reducing habitats. This supertree investigation should inform both future systematic research to further resolve gastropod relationships and direct more detailed investigations of the ecology, anatomy, and possible symbiotic relationships of the lineages examined here, most of which have sparse records and limited data in their descriptions.

Acknowledgements

We thank Lucy Chang for participating in many discussions of supertree methods and look forward to doing this again for the bivalves. We would also like to thank Anders Warén for early feedback on gastropod habitat occurrences.

References

- Baco AR & Smith CR 2003. High species richness in deep-sea chemoautotrophic whale skeleton communities. Mar. Ecol. Prog. Ser. 260: 109–114.
- Barry J, Garygreene H, Orange D, Baxter C, Robison B, Kochevar R, Nybakken J, R D, & Mchugh C 1996. Biologic and geologic characteristics of cold seeps in Monterey Bay, California. Deep Sea Res. Part I Oceanogr. Res. Pap. 43: 1739–1755.
- Bernardino AF, Levin LA, Thurber AR, & Smith CR 2012. Comparative composition, diversity and trophic ecology of sediment macrofauna at vents, seeps and organic falls. PLoS One 7.
- Bienhold C, Ristova P, & Wenzhöfer F 2013. How deep-sea wood falls sustain chemosynthetic life. PLoS One 8: 10–15.
- Bininda-Emonds ORP 2004. Phylogenetic Supertrees: Combining Information to Reveal the Tree of Life. Springer Science & Business Media. 550 pp.

- Boetius A, Ravenschlag K, Schubert CJ, Rickert D, Widdel F, Gieseke A, Amann R, Jørgensen BB, Witte U, & Pfannkuche O 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407: 623–626.
- Corliss JB, Dymond J, Gordon LI, Edmond JM, Herzen RP von, Ballard RD, Green K, Williams D, Bainbridge A, Crane K, & Andel TH van 1979. Submarine thermal springs on the galapagos rift. Science 203: 1073–1083.
- Creevey CC 2005. Clann : Construction of Supertrees and exploration of phylogenomic information from partially overlapping datasets. Clann Man. version 3.0.0: 1–86.
- Creevey CJ & McInerney JO 2005. Clann: Investigating phylogenetic information through supertree analyses. Bioinformatics 21: 390–392.
- Creevey CJ & McInerney JO 2009. Trees from Trees: Construction of Phylogenetic Supertrees Using Clann. Methods Mol Biol 537: 1–13.
- Cunha MR, Matos FL, Génio L, Hilário A, Moura CJ, Ravara A, & Rodrigues CF 2013. Are Organic Falls Bridging Reduced Environments in the Deep Sea? - Results from Colonization Experiments in the Gulf of Cádiz. PLoS One 8: 1–17.
- Deming JW, Reysenbach AL, Macko S a., & Smith CR 1997. Evidence for the microbial basis of a chemoautotrophic invertebrate community at a whale fall on the deep seafloor: Bone-colonizing bacteria and invertebrate endosymbionts. Microsc. Res. Tech. 37: 162–170.
- Distel DL 2003. The Biology of Marine Wood Boring Bivalves and Their Bacterial Endosymbionts. In:Wood Deterior. Preserv., pp. 253–271.
- Distel DL, Baco A, Chuang E, Morrill W, Cavanaugh C, & Smith CR 2000. Do mussels take wooden steps to deep-sea vents? Nature 403: 725–726.
- Dover CL Van, German CR, Speer KG, Parson LM, & Vrijenhoek RC 2002. Evolution and biogeography of deep-sea vent and seep invertebrates. Science 295: 1253–1257.
- Dubilier N, Bergin C, & Lott C 2008. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. Nat. Rev. Microbiol. 6: 725–740.
- Fagervold SK, Romano C, Kalenitchenko D, Borowski C, Nunes-Jorge A, Martin D, & Galand PE 2014. Microbial communities in sunken wood are structured by wood-boring bivalves and location in a submarine canyon. PLoS One 9.
- Fornari DJ, Damm KL Von, Bryce JG, Cowen JP, Ferrini V, Fundis a, Lilley MD, Luther GW, Mullineaux LS, Perfit MR, Meana-Prado MF, Rubin KH, Seyfried WE, Shank TM, Soule S a, Tolstoy M, & White SM 2012. The East Pacific Rise Between 9 degrees N and 10 degrees N: Twenty-Five Years of Integrated, Multidisciplinary Oceanic Spreading Center Studies. Oceanography 25: 18–43.
- Fujiwara Y, Kawato M, Yamamoto T, Yamanaka T, Sato-Okoshi W, Noda C, Tsuchida S, Komai T, Cubelio SS, Sasaki T, Jacobsen K, Kubokawa K, Fujikura K, Maruyama T, Furushima Y, Okoshi K, Miyake H, Miyazaki M, Nogi Y, Yatabe A, & Okutani T 2007. Three-year investigations into sperm whale-fall ecosystems in Japan. Mar. Ecol. 28: 219– 232.
- Gaudron SM, Pradillon F, Pailleret M, Duperron S, Bris N Le, & Gaill F 2010. Colonization of organic substrates deployed in deep-sea reducing habitats by symbiotic species and associated fauna. Mar. Environ. Res. 70: 1–12.
- Halanych KM 2005. Molecular phylogeny of siboglinid annelids (a.k.a. pogonophorans): A review. Hydrobiologia 535: 297–307.
- Haszprunar G 1988a. Comparative Anatomy of Cocculiniform Gastropods and its Bearing on Archaeogastropod Systematics. Malacol. Rev. 4: 64–84.

- Haszprunar G 1988b. Anatomy and relationships of the bone-feeding limpets, Cocculinella minutissima (smith) and Osteopelta mirabilis marshall (archaeogastropoda). J. Molluscan Stud. 54: 1–20.
- Haymon RM, Fornari DJ, Edwards MH, Carbotte S, Wright D, & Macdonald KC 1991. Hydrothermal vent distribution along the East Pacific Rise crest (9°09'–54'N) and its relationship to magmatic and tectonic processes on fast-spreading mid-ocean ridges. Earth Planet. Sci. Lett. 104: 513–534.
- Hess M, Beck F, Gensler H, Kano Y, Kiel S, & Haszprunar G 2008. Microanatomy, shell structre and molecular phylogeny of Leptogyra, Xyleptogyra and Leptogyropsis (Gastropoda: Neomphalida: Melanodrymiidae) from sunken wood. J. Molluscan Stud. 74: 383–401.
- Higgs ND, Gates AR, & Jones DOB 2014. Fish food in the deep sea: revisiting the role of large food-falls. PLoS One 9: e96016.
- Hilário A, Capa M, Dahlgren TG, Halanych KM, Little CTS, Thornhill DJ, Verna C, & Glover AG 2011. New perspectives on the ecology and evolution of siboglinid tubeworms. PLoS One 6.
- Jacobs DK & Lindberg DR 1998. Oxygen and evolutionary patterns in the sea: onshore/offshore trends and recent recruitment of deep-sea faunas. Proc. Natl. Acad. Sci. U. S. A. 95: 9396–9401.
- Johnson SB, Warén A, Lee RW, Kano Y, Kaim A, Davis A, Strong EE, & Vrijenhoek RC 2010. Rubyspira, new genus and two new species of bone-eating deep-sea snails with ancient habits. Biol. Bull. 219: 166–177.
- Kemp K, Jamieson A, Bagley P, McGrath H, Bailey D, Collins M, & Priede I 2006. Consumption of large bathyal food fall, a six month study in the NE Atlantic. Mar. Ecol. Prog. Ser. 310: 65–76.
- Kiel S & Goedert JL 2006. Deep-sea food bonanzas: early Cenozoic whale-fall communities resemble wood-fall rather than seep communities. Proc. Biol. Sci. 273: 2625–2631.
- Kiel S, Gotz S, Pascual-Cebrian E, & Hennhofer DK 2012. Fossilized digestive systems in 23 million-year-old wood-boring bivalves. J. Molluscan Stud. 78: 349–356.
- Laurent MCZ, Bris N Le, Gaill F, & Gros O 2013. Dynamics of wood fall colonization in relation to sulfide concentration in a mangrove swamp. Mar. Environ. Res. 87-88: 85–95.
- Levin LA & Mendoza GF 2007. Community structure and nutrition of deep methane-seep macrobenthos from the North Pacific (Aleutian) Margin and the Gulf of Mexico (Florida Escarpment). Mar. Ecol. 28: 131–151.
- Lindberg DR & Hedegaard C 1996. A deep water patellogastropod from Oligocene water-logged wood of Washington state, USA (Acmaeoidea: Pectinodonta). J. Molluscan Stud. 62: 299–314.
- Lorion J, Kiel S, Faure B, Kawato M, Ho SYW, Marshall B, Tsuchida S, Miyazaki JI, & Fujiwara Y 2013. Adaptive radiation of chemosymbiotic deep-sea mussels. Proc. R. Soc. B Biol. Sci. 280: 20131243.
- Lundsten L, Schlining KL, Frasier K, Johnson SB, Kuhnz L a., Harvey JBJ, Clague G, & Vrijenhoek RC 2010. Time-series analysis of six whale-fall communities in Monterey Canyon, California, USA. Deep. Res. Part I Oceanogr. Res. Pap. 57: 1573–1584.
- Maddison W & Maddison DR 2001. Mesquite: a modular system for evolutionary analysis.
- McArthur a. G & Tunnicliffe V 1998. Relics and antiquity revisited in the modern vent fauna. Geol. Soc. London, Spec. Publ. 148: 271–291.

- McLean JH 1992. Cocculiniform Limpets (Cocculinidae and Pyropeltidae) Living on Whale Bone in the Deep Sea Off California. J. Molluscan Stud. 58: 401–414.
- Mishler BD, Lewis LA, Buchheim MA, Renzaglia KS, Garbary DJ, Delwiche CF, Zechman FW, Kantz TS, & Chapman RL 1994. Phylogenetic Relationships of the "Green Algae" and "Bryophytes." 451-483 pp.
- Ramirez-Llodra E, Brandt A, Danovaro R, Mol B De, Escobar E, German CR, Levin LA, Martinez Arbizu P, Menot L, Buhl-Mortensen P, Narayanaswamy BE, Smith CR, Tittensor DP, Tyler P a., Vanreusel A, & Vecchione M 2010. Deep, diverse and definitely different: Unique attributes of the world's largest ecosystem. Biogeosciences 7: 2851–2899.
- Rouse GW, Goffredi SK, Johnson SB, & Vrijenhoek RC 2011. Not whale-fall specialists, Osedax worms also consume fishbones. Biol. Lett. 7: 736–739.
- Samadi S, Corbari L, Lorion J, Hourdez S, Haga T, Dupont J, Boisselier MC, & Forges BR De 2010. Biodiversity of deep-sea organisms associated with sunken-wood or other organic remains sampled in the tropical Indo-Pacific. Cah. Biol. Mar. 51: 459–466.
- Sasaki T, Okutani T, & Fujikura K 2005. Molluscs from Hydrothermal Vents and Cold Seeps in Japan : A Review of Taxa Recorded in Twenty Recent Years (1984-2004). Venus J. Malacol. Soc. Japan 64: 87–133.
- Sasaki T, Warén A, Kano Y, Okutani T, & Fujikura K 2010. Gastropods from Recent Hot Vents and Cold Seeps: Systematics, Diversity and Life Strategies. In:Vent Seep Biota, pp. 333–378.
- Scornavacca C, Berry V, Lefort V, Douzery EJP, & Ranwez V 2008. PhySIC_IST: cleaning source trees to infer more informative supertrees. BMC Bioinformatics 9: 413.
- Sibuet M & Olu K 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. Deep. Res. Part II Top. Stud. Oceanogr. 45: 517–567.
- Sigwart JD & Lindberg DR 2014. Consensus and confusion in molluscan trees: evaluating morphological and molecular phylogenies. Syst. Biol. In press.
- Smith CR & Baco AR 2003. Ecology of whale falls at the deep-sea floor. Oceanogr. Mar. Biol. Vol 41 41: 311–354.
- Smith CR, Glover AG, Treude T, Higgs ND, & Amon DJ 2015. Whale-Fall Ecosystems: Recent Insights into Ecology, Paleoecology, and Evolution. Ann. Rev. Mar. Sci. 7: 571–596.
- Smith CR, Kukert H, Wheatcroft RA, Jumars PA, & Deming JW 1989. Vent fauna on whale remains. Nat. Commun.
- Somero GN & Hochachka P 1984. Biochemical Adaptation. Princeton University Press, Princeton, NJ. 537 pp.
- Thubaut J, Puillandre N, Faure B, Cruaud C, & Samadi S 2013. The contrasted evolutionary fates of deep-sea chemosynthetic mussels (Bivalvia, Bathymodiolinae). Ecol. Evol. 3: 4748–4766.
- Tunnicliffe V 1992. The Nature and Origin of the Modern Hydrothermal Vent Fauna. Palaios 7: 338–350.
- Tunnicliffe V, McArthur AG, & McHugh D 1998. A Biogeographical Perspective of the Deep-Sea Hydrothermal Vent Fauna. 353-442 pp.
- Turner RD 1973. Wood-Boring Bivalves, Opportunistic Species in the Deep Sea. Science (80-.). 180: 1377–1379.
- Voight JR 2005. First report of the enigmatic echinoderm Xyloplax from the North Pacific. Biol. Bull. 208: 77–80.

- Voight JR 2007. Experimental deep-sea deployments reveal diverse Northeast Pacific woodboring bivalves of Xylophagainae (Myoida: Pholadidae). J. Molluscan Stud. 73: 377–391.
- Voight JR 2015. Xylotrophic bivalves: aspects of their biology and the impacts of humans. J. Molluscan Stud.: 1–12.
- Vrijenhoek RC 2009. Cryptic species, phenotypic plasticity, and complex life histories: Assessing deep-sea faunal diversity with molecular markers. Deep Sea Res. Part II Top. Stud. Oceanogr. 56: 1713–1723.
- Vrijenhoek RC 2013. On the instability and evolutionary age of deep-sea chemosynthetic communities. Deep. Res. Part II Top. Stud. Oceanogr. 92: 189–200.
- Vrijenhoek RC, Johnson SB, & Rouse GW 2008. Bone-eating Osedax females and their "harems" of dwarf males are recruited from a common larval pool. Mol. Ecol. 17: 4535– 4544.
- Vrijenhoek RC, Johnson SB, & Rouse GW 2009. A remarkable diversity of bone-eating worms (Osedax; Siboglinidae; Annelida). BMC Biol. 7: 74.
- Waren A & Bouchet P 1993. New records, species, genera, and a new family of gastropods from hydrothermal vents and hydrocarbon seeps*. Zool. Scr. 22: 1–90.
- Wolff T 1979. Macrofaunal utilization of plant remains in the deep-sa. Sarsia.
- Zbinden M, Pailleret MZ, Ravaux J, Gaudron SM, Hoyoux C, Lambourdière J, Warén A, Lorion J, Halary S, & Duperron S 2010. Bacterial communities associated with the wood-feeding gastropod pectinodonta sp. (Patellogastropoda, Mollusca). FEMS Microbiol. Ecol. 74: 450–463.

, -	# of	# of	CLANN (sfit)		PhySIC_IST		
Taxon	source	unique	# of super		# taxa	% taxa	
	trees	taxa	trees	Score	rejected	rejected	CIC
Backbone	11	10	3	1.1810	3	33%	0.4737
Vetigastropoda	4	102	838	2.8992	42	41%	0.5039
Neomphalina +	4	13	3	0.6500	0	-	0.8348
Cocculinoidea							
Neritimorpha	3	31	19	0.2105	7	23%	0.6883
Caenogastropoda	6	90 (225)	39	9.0986	41	46%	0.4443
Patellogastropoda	1	16	-	_	-	-	-
Heterobranchia	1	74	_	_	_	_	_

Table 1. Gastropod supertree construction parameters. For Caenogastropoda the number of families (and genera) is presented. CIC = cladistics information content

Таха	total n	n coded	% coded	mixed	multiple	single
Patellogastropods	16	3	18.75%	0	2	1
Vetigastropods	100	23	23.00%	10	6	7
Neomphalines	13	13	100.00%	0	3	10
Nerites	33	4	12.12%	1	1	2
Heterobranchs	74	3	4.05%	3	0	0
Caenogastropods	226	16	7.08%	11	2	3
Totals	462	62	13.42%	25	14	23

Table 2: Summary of lineages with each character state relating to their occurrence in reducing habitats.

Table 3: State change matrix summarizing how many times each state changed to another state throughout the supertree. State names refer to how many reducing habitats the lineage occupied or if it was in a mix of reducing and non-reducing ones.

	Starting State					
		none	mixed	multiple	single	
Derived	none	0	2	0	0	
State	mixed	21	1	0	2	
	multiple	8	0	1	5	
	single	10	1	1	11	

Figure Legends:

Figure 1: Gastropod supertree with unordered parsimony ancestral reconstruction of specialization levels in reducing habitats. Green indicates a mix of reducing and non-reducing habitats, orange is multiple reducing habitats, and purple is a singular habitat.

Figure 2: Schematic illustrating transitions between states in Table 3. Line thicknesses approximate relative numbers of lineages making transitions, but are not drawn to scale. Blue arrows indicate right to left transitions in the schematic and pink indicate the opposite direction and those remaining in single reducing habitat types across lineage splits.

Figure 3: Patellogastropoda subtree with unordered parsimony ancestral reconstruction of reducing habitat occupancy. Orange indicates multiple reducing habitats, and purple indicates a single reducing habitat

Figure 4: Vetigastropoda supertrees. **A.** Majority rule consensus tree with confidence indicated on nodes and an unordered parsimony reconstruction of occupancy in reducing habitats indicated by color. Green indicates the lineage occupies a combination of reducing and non-reducing habitats, Orange indicates multiple reducing habitats, and Purple indicates only known occurrences from one reducing habitat type. **B.** Strict consensus supertree, polytomies indicate areas with little agreement or resolution in source trees.

Figure 5: Neomphalina strict consensus supertree with unordered parsimony reconstruction of occupancy in reducing habitats indicated by color. Orange indicates multiple reducing habitats and no non-reducing habitats, and purple indicates only known occurrences from one reducing habitat type.

Figure 6: Neritimorpha supertrees. **A:** Majority rule consensus tree with node confidence indicated and unordered parsimony reconstruction of occupancy in reducing habitats indicated by color. Green indicates the lineage occupies a combination of reducing and non-reducing habitats, orange indicates multiple reducing habitats, and purple indicates only known occurrences from one reducing habitat type. **B:** Strict consensus supertree, polytomies indicate areas with little agreement or resolution in source trees.

Figure 7: Heterogastropoda subtree with unordered parsimony ancestral reconstruction of reducing habitat occupancy. Green lineages occupy a mix of reducing and non-reducing habitats.

Figure 8: Caenogastropoda supertree produced by attaching generic level topologies from source trees to a majority rule consensus supertree at the family level (see Appendix B for comparison of family level majority rule and strict consensus supertrees). Green indicates the lineage occupies a combination of reducing and non-reducing habitats, orange indicates multiple reducing habitats, and purple indicates only known occurrences from one reducing habitat type.









Figure 3:











Figure 7:






Appendix A.1: Source Trees for Supertree construction

Smith et al. (2011)	((Patellogastropoda, Vetigastropoda), (Caenogastropoda, Heterobranchia));
Aktipis & Giribet (2011)	(Neritimorpha, (((Patellogastropoda, Vetigastropoda), (Cocculinoidea, Vent)),
	((Caenogastropoda, Cyclophoroidea, Ampullariodea), Heterobranchia)));
Aktipis & Giribet (2008),	(Patellogastropoda, ((Cocculinoidea, (Neritimorpha, ((Caenogastropoda,
Fig. 9.3	(Vivipariodea, Cyclophoroidea)), Heterobranchia))), (Vetigastropoda, Vent)));
Aktipis & Giribet (2008),	(((Patellogastropoda, Vetigastropoda), (Cocculinoidea, Vent)), (Neritimorpha,
Fig. 9.7	((Caenogastropoda, (Cyclophoroidea, Vivipariodea)), Heterobranchia)))
Colgan et al. (2003)	(Patellogastropoda, (Vetigastropoda, (Neritimorpha, ((Cocculinoidea,
	Heterobranchia), (Caenogastropoda, (Cyclophoroidea, Vivipariodea))))))
Kocot et al. (2011)	(Patellogastropoda, (Vetigastropoda, (Neritimorpha, (Caenogastropoda,
	Heterobranchia))));
Lindberg & Guralnick	(Patellogastropoda, (Vetigastropoda, (Neritimorpha, (Caenogastropoda,
(2003)	(Ampullariodea, Heterobranchia)))));
McArthur & Harasewych	(Patellogastropoda, (((Vent, Vetigastropoda), (((Caenogastropoda, (Vivipariodea,
(2003), Fig. 6.2	Cyclophoroidea)), Heterobranchia), Neritimorpha)), Cocculinoidea));
McArthur & Harasewych	(Neritimorpha, ((((Patellogastropoda, Cocculinoidea), Vetigastropoda), Vent),
(2003), Fig. 6.3	(Heterobranchia, (Caenogastropoda, (Cyclophoroidea, Vivipariodea)))));
McArthur & Harasewych	(Patellogastropoda, (Vetigastropoda, ((Neritimorpha, (Heterobranchia,
(2003), Fig. 6.4	(Caenogastropoda, Cyclophoroidea, Vivipariodea))), (Cocculinoidea, Vent))));
Ponder & Lindberg 1997	(Patellogastropoda, ((Neritimorpha, Cocculinoidea), ((Vetigastropoda, Vent),
	(Heterobranchia, (Caenogastropoda, (Cyclophoroidea, Ampullariodea))))));

Gastropod Backbone Input Trees

Patellogastropoda Input Tree

Nakano & Ozawa (2007)	(((Patella, (Scutellastra, (Cymbula, Helcion))), (((Cellana, Nacella), (Lepeta,
	(Bathyacmaea, Pectinodonta))), (Erginus, (Acmaea, (Neolepetopsis, (Asteracmea,
	(Tectura, Lottia)))))), Eoacmaea)

Vetigastopoda Input Trees

Aktipis & Giribet (2012)	((((((Phasianella, Tricolia), Pseudostomatella), ((Collonista, Homalopoma), (Collonia,							
	Cantrainea))), (Angaria, (Marevalvata, Arene))), (((Munditiella, ((Scissurella,							
	Sinezona), (Bathyxylophila, (Clypeosectus, (Gorgoleptis, Lepetodrilus))))), ((Haliotis,							
	(Cataegis, ((Granata, (Calliotropis, Lischkeia)), (Ventsia, (Bathymargarites,							
	Fluxinella))))), ((Cittarium, (Margarites, Liotina)), (((Lithopoma, Turbo), (Tegula,							
	(Protolira, Dillwynella))), ((Calliostoma, (Gibbula, ((Clanculus, Monodonta),							
	Umbonium))), Microgaza))))), ((Hemitoma, (Puncturella, Cranopsis)), ((Tugali,							
	Emarginula), (Fissurella, (Lucapina, Diodora)))))), (Bayerotrochus,							
	Entemnotrochus));							
Kano (2008) Fig.2	((((((Anatoma, Bathyxylophila), ((Homalopoma, (Rimula, ((Diodora, Macroshisma),							
	(Scutus, (Emarginula, Montfortula))))), (Neocollonia, (Notocrater,							
	Pseudococculina)))), (((Bruciella, (Dilwynella, Cirsonella)), (Tegula, Lodderena)),							
	(Astraea, Turbo))), (Sinezona, Scissurella)), Haliotis), (((Calliostoma, Liotina),							
	(((Minolia, Margarites), (Umbonium, Tricolia)), ((Conradia, ((((Turcica, Cataegis),							
	(Ginebis, Calliotropis)), (Agathodonta, Granata)), ((Fluxinella, (Hadroconus,							

	Seguenzia)), (Adeuompalus, (Ventsia, Xyloskenea))))), (Trochus, Munditiella)))),								
	(Pseudorimula, Lepetodrilus))), (Entemnotrochus, Perotrochus));								
Williams et al. (2010)	(Entemnotrochus,((Angaria,Arene),((Munditiella,(((Microgaza,(((Cittarium,Tectus),(
	Tegula,(Chlorostoma,Omphalius))),((Margarites,Dillwynella),(Prisogaster,((Cookia,L								
	unella),((Lithopoma,(Megastraea,Pomaulax)),((Astraea,(Bolma,(Bellastraea,Guildfor								
	dia))),(Astralium,Turbo))))))),((Monodonta,(((Broderipia,Synaptocochlea),((Stomate								
	lla,(Gibbula,((Phasianotrochus,Prothalotia),(Jujubinus,Cantharidus)))),(Monilea,(Etha								
	lia,(Umbonium,(Ethaliella,Isanda)))))),((Eurytrochus,Notogibbula),Trochus))),(Liotin								
	a,Calliostoma))),(Haliotis,(((Emarginula,(Macroschisma,Diodora)),((Scissurella,Sinez								
	ona),Lepetodrilus)),(Granata,(Bathymargarites,(Lischkeia,Ginebis))))))),((Gabrielona,								
	(Tricolia, Phasianella)),((Bothropoma, Homalopoma), Collonista)))));								
Geiger & Thacker (2005)	(Perotrochus, (((Homalopoma, (Clypeosectus, (Anatoma, (Diodora, (Fissurella,								
	(Emarginula, (Montfortula, Scutus)))))), ((Trochus, Tegula), (Turbo, (Astraea,								
	Batillus)))), ((Haliotis, (Lepetodrilus, (Scissurella, Sukashitrochus))), (Stomatella,								
	Gibbula))));								

Neomphalina Input Trees

Heß et al. (2008), Fig. 9	(Cocculina,((Melanodrymia,(Leptogyra,Leptogyropsis)),((Cyathermia,Lacunoides),((
	Peltospira,Rhynchopelta),Mediapex),(Depressigyra,Pachydermia))));
Kano (2010)	((Leptogyra, (Leptogyropsis, (Depressigyra, (Cyathermia, (Peltospira,
	Rhynchopelta))))), (Coccopigya, Cocculina));
Aktipis & Giribet (2012)	((Cocculina, Bathysciadium), ((Melanodrymia, Leptogyropsis), ((Depressigyra,
	Cyathermia), Peltospira)));
Kano (2008)	((Leptogyra, (Leptogyropsis, (Depressigyra, (Cyathermia, (Peltospira,
	Rhynchopelta))))), (Coccopigya, Cocculina))
Geiger & Thacker (2005)	(Cyathermia,((Depressigyra,(Peltospira, Rhynchopelta)), Cocculina));

Neritimorpha Input Trees

Hedegaard (1996)	(Neritopsis,(Titiscania,((((Alcadia,Helicina,(Hendersonia,Oligyra)),(Emod							
	a,Eutrochatella),(Stoastoma,Viana)),(Georissa,Hydrocena)),((Bathynerita,(
	Neritilia,(((Puperita,(Nerita,Smaragdia)),(Septaria,Neritina)),(Neritodryas							
	Theodoxus)))),(Phenacolepas,(Olgasolaris,Shinkailepas))))));							
Kano et al. (2002)	((Neritopsis, Titiscania), (Georissa, ((Pleuropoma, (Neritilia, Pisulina)),							
	(Cinnalepeta, Nerita))));							
Holthuis (1995)	(Neritopsis,(Neritilia,((Bathynerita,Phenacolepas),(Nerita,(Theodoxus,(Pu							
	perita,((Fluvinerita,Nentodryas),(Clithon,Smaragdra,(Vitta,(Neritina,(Neri							
	pteron,Septaria))))))))))));							

Heterogastropoda Input Tree

Jörger et al. 2010	(Valvata,(Cima,((Rissoella,(Pupa,(Rictaxis,Hydatina))),(((((Diaphana,(((T
	oledonia,Cylichna),(Philine,(Bulla,Haminoea))),(Philinoglossa,Scaphander
))),(Runcina,(((Cavolinia,Hyalocylis),(Pneumoderma,Spongiobranchaea)),
	(Aplysia, Akera)))), (Tylodina, Umbraculum)), ((Glacidorbis, ((Salinator, (Am
	phibola,Phallomedusa)),((Eulimella,Turbonilla),(Boonea,Odostomia)))),(((
	Lymnaea,(Acroloxus,(Physella,Ancylus))),(Chilina,Latia)),(((Rumina,(((E

na,Cerion),Arianta),Arion)),(((Semperula,Laevicaulis),((Onchidium,Atopo
s),Onchidella)),((Carychium,(Ophicardelus,Myosotella)),(Trimusculus,(S
meagol,Otina))))),(((Pontohedyle,(Paraganitus,Microhedyle)),Asperspina),
((Aitengidae,(Pseudunela,(Strubellia,Acochlidium))),Hedylopsis))))),(Siph
onaria,((Gascoignella,(Cyerce,(Plakobranchus,(Thuridilla,Elysia)))),(Cylin
drobulla,Volvatella)))),((Pleurobranchus,Tomthompsonia),Bathydoris))),O
rbitestella));

Caenogastropoda Input Trees

Criscione & Ponder (2013), Fig. 2	(Pomacea,((((Alaba,(Epitonium,Serpulorbis)),(Mesophora,Strombus),(Aus trolittorina,Lacuna)),(((Scrupus,(Badepigrus,(Anabathron,Pisinna))),(((Cae cum,(Calopia,Clenchiellidae)),(Nozeba,(Circulus,Pseudoliotia))),(Iravadia, (Fluviocingula,Fairbankia))),(Hydrococcus,(Tatea,(Nodulus,(Hydrobia,Ve ntrosia))),(Stenothyra,(Truncatella,(Coxiella,Falsicingula),(Suterilla,Assim ineaii))))),((Hebeulima,(Rissoina,(Barleeia,Fictonoba))),((Subestea,(Alvan ia,Rissoa)),(Emblanda,Lironoba))))),(Rastodens,(Eatoniopsis,(Crassitoniel la,Eatoniella),(Eatonina,Tubbreva)))));
Colgan et al. (2007), Fig. 6	(Gibbula,Haliotis,((Cyclophorus,(Campanile,(Cacozeliana,((Planaxis,(((Se misulcospira,(Pomacea,Pleurocera)),(Melanopsis,Modulus,(Syrnolopsis,((Telescopium,Cerithidea),(Scaliola,Finella))))),((Batillaria,(Brotia,Pyrazus)),(Protomella,Maoricolpus)))),(Alaba,(Clypeomorus.,Cerithium)))))),((Thi ara,Melanoides),(Viviparus,(((Setia,Rissoa),(Cheilea,(Sabia,Leptonetis))),(((Erronea,(Cypraeovula,(Cribrarula,Umbilia))),(Simnia,(Prosimnia,Ovula))),((((Alvinoconcha,Austrolittorina),((Marstoniopsis,(Potamopyrgus,(Hydr obia,Pyrgula)),(Lithoglyphus,(Bithynia,Bythinella))),(Neotricula,Oncomel ania))),(Strombus,Xenophora)),((Trichotropis,(Hyalorisia,Capulus)),((Siga patella,(Crucibulum,(Trochita,Crepidula))),(Semicassis,(Conus,(((Terebra, (Trivirostra,Lamellaria)),(Fasciolaria,Melongena)),(Neptunea,(Ocinebrellu s,(Busycon,Buccinum))))))))))))))
Ponder et al. (2008), Fig.13.16	(((((Natica, ((Pterotrachea, (Eulimnia, (Janthina, Epitonium))), (Ficus, Triphora, ((Cypraea, (Erato, Velutina)), ((Cabestana, Tonna), (Buccinum, (Nassarius, (Mitra, (Murex, (Vexillum, Marginella, Cancellaria, Conus), (Olivia, Voluta)))))))), (Capulus, (((Bithynia, (Hydrobia, (Pisinna, Rissoa))), (Hipponix, Crepidula)), ((Struthiolaria, Strombus), (Vermetidae, Xenophora))))), ((Provanna, Littorinia), (Eatoniella, Eatonina))), ((Campanile, Plesiotrochus), (Turritella, (Batillaria, Cerithium)))), (Viviparus, (Cyclophora, Ampullaria))), ((Valvata, Architectonica), (Amphibola, (Micromelo, Aplysia))));
Johnson et al. (2010), Fig. 1	(Littorina,(Neptunea,(Provanna,((Debruyeresia,(Rubyspira,Abyssochrysos)),(Alviniconchia,Ifremeria)))));
Xu (2015)	(Abyssocchrysos,(((Alviniconchia, Ifremeria),Provana),(Desbruyeresia, Rubyspira)));
Puillandre et al. (2011)	(Strombus, Xenophora, (Belomitra, (Turrilatirus, (((((Benthofascis, ((Genota, ((Bathytoma, Zemacies), (Borsonia, Tomopleura)))), (Microdrillia, (Borsoniidae_gen_1, Typhlomangelia)))), (Clathurella, (Etrema, Nannodiella)), (Profundiconus, (Californiconus, (Conasprella, (Conus, Taranteconus))))), (((Benthomangelia, (((Eucithara, (Anticlinura, Mangeliidae_gen_1)), (Heterocithara, Mangeliidae_gen_2)), (Oenopota, Toxicochlespira))), (((Glyphostomoides, Tritonoturris, ((Pleurotomella, (Taranis, (Daphnella, (Teretiopsis, (Gymnobela, Thatcheria))))), (Eucyclotoma, Kermia))), (Rimosodaphnella, Veprecula)), (Hemilienardia, Raphitoma))), (Lovellona, Mitromorpha))), ((((Gemmuloborsonia,

((Turricula, (Pusionella, (Clavatula, Perrona))), (Paradrillia, (((Anacithara,
Horaiclavus), (Anguloclavus, Horaiclavidae_gen_1)), (Carinapex,
Ceritoturris))))), (Lucerapex, ((Euterebra, ((Hastula, (Cinguloterebra,
Oxymeris)), (Myurella, (Clathroterebra, (Hastulopsis, Terenolla)),
(Strioterebrum, Terebra)))), (Polystira, (Ptychosyrinx, (Turridrupa,
((Gemmula, Lophiotoma), (Turris, (Iotyrris, Xenuroturris))))))))),
(((((Agladrillia, (Calliclava, Imaclava)), (Clathrodrillia, Drillia)),
(Conopleura, (Splendrillia, (Iredalea, (Cerodrillia, Clavus))))),
(Cruziturricula, Fusiturricula)), (Leucosyrinx, (((Otitoma, (Carinodrillia,
(Hindsiclava, ((Cheungbeia, Funa), (Inquisitor, (Crassispira,
Ptychobela)))), (Pyrgospira, (Pilsbryspira, Zonulispira)))),
(Pseudomelatoma, Tiariturris)), (Comitas, Knefastia))))), (Cochlespira,
Sibogasyrinx))), (Harpa, Vexillum)))));

Appendix A.2 – Sources of habitat occupancy of gastropod genera

Baker, M.C., Ramirez-Llodra, E., Perry, D. 2010. ChEssBase: an online information system on

species distribution from deep-sea chemosynthetic ecosystems. Version 3.

Bouchet, P., H. Le Guyader, and O. Pascal. 2009. The SANTO 2006 Global Biodiversity

Survey. Zoosystema 31:401-406.

Kiel, S. 2010. The vent and seep biota. Springer, New York.

Warén, A. and Bouchet, P. 1993. New records, species, genera, and a new family of gastropods from hydrothermal vents and hydrocarbon seeps*. Zoologica Scripta 22:1 1-90.



Appendix B: Familial level Caenogastopoda majority rule (A) and strict consensus (B) trees.

Chapter 2

The anatomy of *Lepetella sierrai* (Vetigastropoda, Lepetelloidea): implications for reproduction, feeding, and symbiosis in lepetellid limpets

Originally published as Judge, J. and Haszprunar, G. 2014. The anatomy of *Lepetella sierrai* (Vetigastropoda, Lepetelloidea): implications for reproduction, feeding, and symbiosis in lepetellid limpets. Invertebrate Biology 133: 324–339.

Abstract

The Lepetelloidea, a clade of small limpet-shaped gastropods, can be used as a case study in continental margin and deep-sea diversification. Lineages in this clade have been found associated with a combination of different substrates, including hydrothermal vents, seeps, wood, whale carcasses, polychaete tubes, chondrichthyan egg cases, seagrass rhizomes, algal holdfasts, crab carapaces, and sponges. Members of one lepetelloidean family, Lepetellidae, live on or inside empty tubes of members of the polychaete genus Hyalinoecia. The detailed morphology of a Mediterranean species, Lepetella sierrai DANTART & LUQUE 1994, was reconstructed in three dimensions from serial semithin sections and compared to that of eleven other members of Lepetellidae. The hermaphroditic lepetellid limpets possessed a ciliated seminal groove, distinct testis and ovary with a common distal gonoduct, and a seminal receptacle containing mature sperm. A unique alimentary tract, with huge oesophageal pouches, no true stomach, an extensive multi-lobed midgut, and short intestine was present. Additionally, a bacteriocyte system throughout the entire mantle rim was revealed via light and transmission electron microscopy. This is the first recognized evidence for intracellular microbial symbiosis in lepetelloidean limpets. Semithin sections showed evidence of a parasite, potentially a chitonophilid copepod, penetrating the body wall of the limpet. Hypotheses about reproductive biology, feeding, and symbiosis are presented based on anatomical features and knowledge of the habitat described herein.

Key words: Gastropoda, Lepetellidae, parasitism, Hyalinoecia, Amira®

Introduction

Many metazoan taxa include lineages that have become specialized for life in chemosynthetic and biogenic communities. Molluscs, in particular, have repeatedly invaded these habitats. However, there have been few major diversifications (Kiel 2010). The Lepetelloidea is a clade of small, limpet-shaped vetigastropods that inhabit a high diversity of chemosynthetic or biogenic substrates in the deep sea. Members of Lepetelloidea have been found associated with different substrates, including hydrothermal vents or cold seeps, sunken wood, whale carcasses, fish bone, polychaete tubes, empty chondrichthyan egg cases, seagrass rhizomes, algal holdfasts, crab carapaces, sponges, or some combination of these (Haszprunar 1988a and references therein). Previous researchers have identified nine families within the Lepetelloidea based on unifying morphological characters (Moskalev 1978; Hickman 1983; Marshall 1983, 1987; McLean 1985; McLean & Haszprunar 1987; Haszprunar 1987, 1988a-c, 1989; Haszprunar & McLean 1996; Lesicki 1998). Kano et al (2013) clearly showed that it is difficult and often misleading to reconstruct phylogeny from anatomy in lepetelloidean limpets. Recent molecular data have supported few of the previously described clades, and identified others that will require formal description following the acquisition of additional molecular and morphological data (Kano et al. 2013). With comprehensive morphological data in the context of a well-resolved phylogeny, an integrative and comparative approach can be used to better understand the diversification and ecological differentiation of the group as a whole. Many lineages within Lepetelloidea have had their morphology examined in detail (Haszprunar 1987, 1988a-c, 1998; Haszprunar & McLean 1996), but several gaps remain, including tube-dwelling lepetellid limpets.

Lepetellidae was initially erected based on external and radular morphology and is now also supported by molecular data (Kano et al. 2013). Prior to the present study, information on internal morphology was largely lacking, except for the radula, which is unusual in form. De Rayneval et al. (1854) described the first lepetellid limpet as *Patella laterocompressa* based on fossil material from the Pleistocene of Italy. Verrill (1880, 1881) and Dall (1882) added details on external morphology and the radula. Verrill introduced the genus *Lepetella* (type species: *Lepetella tubicola* VERRILL & SMITH in Verrill 1880), and Dall (1882) introduced the family Lepetellidae. Both had previously reported on the highly specialized habit of lepetellid limpets, which feed exclusively on the tubes of the polychaete genus *Hyalinoecia* MALMGREN 1867, which consist largely of sugar-phosphate polymers (e.g. Graham et al. 1965). However, more than twenty years passed until Thiele (1908) presented the first notes on the anatomy of this family. Thiele placed the Lepetellidae near the Cocculinidae, Bathysciadiidae, and Addisoniidae. This opinion was repeated in Thiele's (1909) overview on the "Cocculinoidea" and also later in his "Handbuch" (Thiele 1929-31). Subsequently, the systematic position of the Lepetellidae remained unstudied and unquestioned for several decades.

Finlay (1927) introduced the genus *Tectisumen* (type species: *Cocculina clypidellaeformis* SUTER 1908) on the basis of differences in shell morphology. Dell (1956) also used shell morphology in recognizing *Tecticrater* (type species: *Cocculina compressa* SUTER 1908). However, neither genus was accepted by Warén (1972) or Moskalev (1978), because they considered shell shape to be highly variable, and the radulae of members of both genera were identical. Warén (1972) reported brood protection in *Lepetella* cf. *laterocompressa*; however, the "brood" later turned out to be parasitic copepods (Huys et al. 2002). Moskalev (1978) studied a large number of specimens (more than 180 of *L. tubicola* and 14 of *T. clypidellaeformis*) and even did some histological sectioning, but did not report on lepetellid anatomy in detail. Histological work by Zharkova (1978) confirmed earlier observations of Verrill (1880) on the presence of eyes in *L. tubicola*.

Although Moskalev's radular drawings were based on scanning electron microscopy (SEM), Hickman (1983) first published scanning electron micrographs of lepetellid radulae, and she described the unique characters of the lepetellid radula. Warén (1991) published a scanning electron micrograph of a lepetellid protoconch, and Dantart & Luque (1994) published scanning electron micrographs of shells, radulae and soft bodies of Iberian species. Mifsud (1996) provided the first photograph of living *L. laterocompressa*.

On the basis of a morphological cladistic analysis of major gastropod groups, Ponder & Lindberg (1997) argued that Cocculiniformia (Cocculinoidea & Lepetelloidea; cf. Haszprunar 1988a), is polyphyletic. Lepetelloidea are now included in the Vetigastropoda, and Cocculinoidea are a separate clade. This determination has subsequently been supported by molecular phylogenetic analyses that included members of Cocculinoidea, Lepetelloidea, and other vetigastropod groups, and is now generally accepted (Kano 2008; Aktipis & Giribet 2010, 2011).

Preliminary descriptions of lepetellid anatomy have been presented by Haszprunar (1988a) and Haszprunar & McLean (1996). Here we present a detailed description of the anatomy and histology of the Mediterranean species *Lepetella sierrai* DANTART & LUQUE 1994 as an exemplar of lepetellid anatomy, and visualize it in a 3D interactive reconstruction. We also present evidence for microbial symbiosis in this taxon, discuss anatomical variation among other lepetellids, and provide additional information on lepetellid parasites. The morphological characters, parasitism, and symbiosis are evaluated and interpreted in an ecological context.

Methods

Material studied

Specimens belonging to 12 tube-dwelling lepetellid taxa were accumulated by GH from various museums and individuals (see below) and investigated by means of serial sections. Several specimens were collected on the BALGIM (Benthos Alboran Golfe Ibéro-Marocain) expedition. For more information and details on collection stations see Salas (1996) and Ramil & Vervoort (1992). For species determination and taxonomy see Dantart & Luque (1994).

Tectisumen clypidelliformis **SUTER 1908**. Two juveniles, one early-stage adult, and three late-stage adult specimens from the National Museum of New Zealand (without detailed information on source locality) were serially sectioned (for details see below). The single sample contained 10 large and 48 very small specimens together with somewhat corroded empty tubes of *Hyalinoecia*, which had round depressions formed by the gastropods.

Lepetella espinosae DANTART & LUQUE 1994. One juvenile in poor condition and two adult paratypes in good condition from the type locality were sectioned. According to Dantart & Luque (1994), this is probably the species repeatedly referred to as *Lepetella laterocompressa* (DE RAYNEVAL, VAN DER HECKE & PONZI 1854), a taxon based on Pleistocene subfossils from Italy (Monte Mario). However, because species identity in lepetellids cannot be established on shells alone, and there are four extant species with similar shells in the Mediterranean region (*L. sierrai, L. espinosae, L. barrajoni, and L.* "from Banyuls"), the name *L. laterocompressa* should be restricted to the original sample.

Lepetella sierrai DANTART & LUQUE 1994. A juvenile, an early-stage adult (parasitized), and two late-stage adult (parasitized) paratypes from the type locality (station 168A) in the Bay of Biscay were sectioned. Three additional adults (not parasitized) from station 185A on the Fauna II expedition were also examined (see Dantart & Luque 1994 for details).

Lepetella barrajoni DANTART & LUQUE 1994. Two paratypes were sectioned from the type locality (Bay of Biscay, off Vizcaya, Spain: 4325.29'-4325.09'N, 231.05'E, station 151A: 82-86 m; 22-06-91; MNCN 15.05/5233).

Species A (*Lepetella* cf. *tubicola*). We examined the single original section series from the Humboldt-Museum in Berlin (see Acknowledgments), on which Thiele's (1908) anatomical paper was based.

Species B (*Lepetella* cf. *laterocompressa*). Three specimens of different sizes and varying in their reproductive status (collected by A. Warén from, Calvi, Corsica) were sectioned. Warén (letter of 29 June 1987 described the animals in this collection as follows: "1 tube with 1 on outside, 3 on inside, 4 on inside + 1 very young (0.3 mm), 110 m, just off Calvi, slit with numerous ascidians. 8 branchial lamellae at right side of pallial cavity. 2 halfgrown specimens, 0.9 and 1.1 mm had numerous eggs attached individually by stalks in pallial cavity. No side flaps on snout. Snout very extensile, anterior edge very finely crenulated. Pallial edge minutely and

irregularly fringed. Foot very mobile, sucker-shaped. Tentacles 3-times as long as when retracted, quite cylindrical. No eyes."

Species C. BALGIM CP18 (36°48′N-09°31′W; 1578 m, 30.5.1984), also BALGIM CP99, CP69, CP106, CP65. Two adult specimens were sectioned.

Species D. BALGIM CP 108 (36°48'N-09°31'W; 1578 m, 30.5.1984); the same species was also found at BALGIM CP99 and CP10. Warén (letter of 10 June 1987) described these as having "tentacles always contracted; smooth mantle margin". Two specimens were sectioned.

Species E. BALGIM CP 106 (36°05′N - 08°05′W; 1906 m, 10.6.1984. Warén (letter of 29 June 1987) described these as "very small, similar to [Species C], but different shells". Three specimens from a single sample were sectioned (for details see Warén 1992).

Species F. BALGIM CP 84 (33°45.4'N - 08°31.'9W; 345 m, 6.6.1984). Warén (letter of 29 June 1987) described these as "shell flatter and the gill is conspicuous". Five specimens (two adults and three juveniles of various sizes) were sectioned.

Species G (*Lepetella* cf. *laterocompressa*). These were collected from a depth of 130 m off Ras-il-Pellegrin, Western Malta, Mediterranean. One adult specimen was collected alive (Mifsud 1996, fig. 4, p. 27), being "found attached to pieces of dead algae stalks and decaying roots of *Posidonia* on a muddy substrate" (Mifsud in letter of 19 Oct 1996). It should be noted that *Posidonia* is an unusual substrate for this species, and further investigation is necessary to determine whether this species also has an affinity for plant substrates such as this.

Lepetella "from Banyuls". Live specimens were collected in tubes of *Hyalinoecia* from Banyuls-sur-Mer, France during June 1996 and June 1999. Serial sections were cut of a juvenile, an early-stage adult, and 2 late-stage adults; some individuals were parasitized by copepods. Some tissues were thin-sectioned and examined by transmission electron microscopy.

Serial sectioning

The early-stage adult and late-stage adult specimens of *T. clypidellaeformis* and Species C were serially sectioned by conventional methods. It was possible to separate the intact shells from the bodies. The bodies were dehydrated in an ethanol series, then embedded in Paraplast. Series of transverse sections (5 μ m thickness) were made. Staining was by Haidenhain's Azan-method (Romeis 1989, p. 501). Thiele's (1908) sections of Species A were obviously stained by haemalaun-eosin, and the staining was still in good condition.

The juveniles of *T. clypidellaeformis* and all remaining specimens were treated differently. In these instances the shells were dissolved by using Bouin's fluid; this treatment caused some additional post-fixation. After washing in 70% ethanol (brought to basic pH by adding a drop of ammonia), the animals were stained in Safranin (1% in 80% ethanol) to facilitate handling of the tiny specimens. After embedding in Araldite or Spurr's (1969) resin, semithin sectioning (transverse, 2 μ m thickness) was done with glass (Ralph) knives. Resin blocks were coated on one side with contact cement to facilitate ribbon formation (Ruthensteiner 2008). The plastic was not removed from sections. Staining was by Regaud's (Romeis 1989, p. 365) iron-haematoxylin or (with better results) by methylene blue (Richardson et al. 1960). Slides were embedded in resin to prevent weakening of the stain over at least twenty years.

Transmission electron microscopy

After relaxation in an isotonic solution of magnesium chloride, specimens of *Lepetella* "from Banyuls" were prefixed in 2.5 % glutaraldehyde in 0.1M phosphate buffer (pH 7.3) for about 20 d. After additional rinses in buffer, postfixation was done in 1% osmium tetroxide

solution in buffer for 2 h, again followed by rinsing in buffer. After dehydration in an ethanol series, the specimens were embedded in epoxy resin. Staining of 70-80 nm ultrathin sections was by uranyl-acetate and lead citrate.

Three-dimensional reconstruction

One section series of L. sierrai was selected for 3D reconstruction with Amira® software following the methods outlined by Ruthensteiner (2008). The section series was prepared using a specimen from the empty tubes of Hyalinoecia collected at a depth of 95 m in Banyuls-sur-Mer, France in June 1996. Every second section in the series was photographed, except for the occasional broken or obstructed section; these were skipped. Images were pre-processed for 3D reconstruction in Adobe Photoshop by converting to eight bit grayscale, auto-scaling, unsharp masking, and resampling to 1600x1200 pixels, a resolution sufficient for the reconstruction of most histological details. The images were renamed with sequential numbers and imported as an image stack into Amira®. The body surface and each individual organ were traced and labeled manually by interpolating through subsets of sections that were consistent or changed shape gradually. All labeling was checked by eye, and sections distorted or broken by scratches were corrected using interpolation. The set of contour lines for each organ and the body surface were separately rendered to create a set of 3D surface models of the body surface and all organs. Snapshots of various organ systems and perspectives were taken in 2D, and the 3D model was embedded in a portable document format (pdf) file following Ruthensteiner & Heß (2008) and Ruthensteiner et al. (2010).

Graphic reconstructions

All graphic reconstructions were based on nearly transverse serial sections. In most cases (dorsal views) the structures and organs were projected vertically on to a horizontal plane, and a transverse line represented each section. In the case of Paraplast-embedded specimens, the midlines of the mantle border, nerve cords, and radular cartilages served as reference lines from which measurements were taken. In the case of resin-embedded material, the blocks were symmetrically trimmed (by a Reichert TM-60 trimming machine) to use the edges of the whole sections as reference lines, enabling very exact measuring in all views. After some jagged lines had been smoothed, the graphic reconstructions were directly used for illustrations, except for some shading and semi-schematic patterning that was added. Photographs of sections were taken to show anatomical and histological details.

Results

The primary morphological descriptions presented below are based on observations of *Lepetella sierrai*. Differences in anatomy from *L. sierrai* that were observed in members of other lepetellid species are also described, and summarized in Table 1.

External morphology

All known lepetellids are "symmetrical limpets", i.e., there is no trace of juvenile coiling in the early teleoconch. The protoconch is a coiled over cup with a fused tip and distinct lateral bulges as in other lepetelloid families (Figure 6B of Warén 1991; A. Warén, personal communication). The external body shape of the animals is also bilaterally symmetrical (Fig. 1).

Lepetella sierrai had a large head with two anteriorly situated slightly papillate cephalic tentacles; the ventral, slit-shaped mouth was flanked by medium-sized oral lappets. From the

outer base of the right cephalic tentacle a seminal groove extended backwards along the right side of the neck, reaching the right side of the mantle cavity (Fig. 1). Eyes were lacking.

The pedal sole had two distinct zones: (1) a densely ciliated zone of very elongate cells around (2) the flat epithelium of the central zone, which was non-ciliated. A pedal gland was not present, but there were laterally situated sole glands. *Lepetella sierrai* also had a single epipodial tentacle in a posterior median position (Fig. 2A) (cf. Figure 51 of Dantart & Luque 1994).

Interspecific variation. The central sole of the foot was not (Species B, D, F) or only sparsely (*T. clypidellaeformis*, Species C) ciliated; conditions in Species A could not be clearly determined. The dorsal side of the pedal flaps consisted of distinct mucous cells in Species F. In Species C, lappets of the oesophageal pouches reached into the lateral flaps of the pedal sole.

Mantle margin and cavity

The mantle had small papillae and a dense region of glandular cells along its margin, surrounding the animal in a horseshoe shape. These cells stained a distinctive pink color (toluidine blue), rather than the typical blue staining of the other cell types. A dense network of bacteriocytes was positioned directly below these glandular cells in the same, symmetrical, horseshoe pattern (Fig. 1).

The mantle cavity was asymmetrical and quite deep on the left side. The pericardium was situated in the left anterior roof of the mantle cavity and contained a single auricle anteriorly and a ventricle posteriorly (Figs. 1,2B). The posterior part of the ventricle enclosed the rectum, which curved to the right side and opened via an anal papilla into the mantle cavity. The left kidney was a small organ in the right anterior roof; it lay alongside the pericardium adjacent to where the auricle met the ventricle. The distal part of the rectum was directly posterior to the left kidney and the anal opening was further right. To the right of the anus, the anterior chamber of the right kidney opened into the mantle cavity and a glandular zone continued forward from this opening to the anterior end of the right shell muscle. The external ciliated seminal groove extended up the right cephalic tentacle and connected to the right dorsal anterior part of the right kidney opening (Figs. 1,2C,D). A hypobranchial gland was lacking.

The gill leaflets occupied the space in the mantle cavity reaching from the central anterior-most part of the mantle cavity and back one third the length of the animal into the right side of the mantle cavity. The gill in the reconstructed specimen had 10 leaflets. The gill was equipped with short pockets ("sensory bursicles") at its efferent axis (Figs. 1,2E).

Interspecific variation. Species C was somewhat exceptional, since the gill leaflets were here reduced to short ciliated spots providing water circulation, but still equipped with bursicles (Fig. 3). In *T. clypidellaeformis* the heart was positioned in the central area, whereas the anterior left area was occupied by the "sinus venosus" (Fig. 4A). In Species D the kidney-rectum complex was shifted to the right and somewhat detorted so that the left kidney was situated anteriorly of the rectum, and the urogenital opening was placed more posteriorly.

Muscle systems

The shell muscle was a compact horseshoe shaped structure penetrated by nerves (see Haszprunar 1985, 1988d). Smooth muscle fibers were oriented longitudinally and met mid-ventrally in the foot, with clear inter-crossing of these fibers (Fig. 1).

Two distinct head retractor muscles had their insertion areas at the inner anterior ends of the shell muscle. They followed the cerebral connective nerves and connected in the center,

immediately dorsal to the buccal apparatus (Fig. 1). Cross-striated buccal muscles surrounded and supplied the buccal apparatus.

Interspecific variation. In Species B only the anterior portions of the shell muscles were thick, whereas the posterior portion and the ventral intercrossing area were very weakly developed. Most remaining species had thicker shell muscles that were compact and only penetrated by nerves. Species C, however, showed a shell muscle divided by blood sinuses like Patellogastropoda or Cocculinida (Fig.3).

Heart and excretory system

The heart was monotocardian (one auricle and one ventricle) and rested within a large, flattened pericardium located in the left anterior mantle roof. The auricle was positioned on the left side of the pericardium, while the ventricle was posterior and slightly more to the right. The posterior part of the ventricle surrounded the rectum. The aortic vessel was very short, and opened into the body sinuses.

Lepetella sierrai had two kidneys. The left one was substantially smaller than the right one, was situated in the roof of the mantle cavity in front of the rectum, and was connected with the pericardium via a thin, short, and densely ciliated duct. The right kidney occupied the space between the gonads and extensive midgut. It had a much thinner epithelium than the left kidney and did not connect to the pericardium, but it shared an opening in the mantle cavity with the gonoduct (Fig. 1).

Interspecific variation. In all species except *Tectisumen clypidellaeformis* the main portion of the heart was located in the mantle roof (Fig. 4A). In Species B the blood stream passed the kidneys, then surrounded the anterior end of the right shell muscle, and was oxygenated by passing the mantle sinus, the gill leaflets, or the mantle roof. The blood streams united immediately before entering the auricle anteriorly left. In Species C the blood stream passed the right kidney, then extended between the shell muscle bundles and formed a large mantle sinus, which entered the auricle at the left side. A second stream was filtered by the left kidney, then spread over the mantle roof and collected in numerous small sinuses entering the auricle anteriorly.

Due to the well-developed gill, the heart conditions differed in adults of *T*. *clypidellaeformis* (Fig. 4A). The heart was mainly viscerally situated and showed a somewhat hypertorted orientation in that the auricle-ventricle axis was nearly horizontal to the left kidney. In addition, the pericardium had a large but depressed pouch, which reached far backwards. Early-stage adults had their heart much more pallially situated and lacked the posterior pouch of the pericardium (Fig. 4B). Azan-staining suggested that the blood was oxygenated mainly in the subpallial cavity, in the mantle roof, bypassing the gill-leaflets (if present). The anterior right leaflets released their blood into the anterior portion of the mantle sinus, whereas the blood of the central (truly pallial) leaflets was collected in an efferent sinus proper. Both streams united in a large "sinus venosus" before entering the auricle.

Reproductive system

Adults of *L. sierrai* were simultaneous hermaphrodites. The gonad occupied over half of the visceral mass and possessed distinct testicular and ovarian tissue in separate chambers. The maximum egg diameter observed was 180-230 μ m, all with a large proportion of yolk.

In *L. sierrai* the testis began directly posterior to the buccal apparatus and occupied the left side of the body. All stages of spermatogenesis were observed within the testis (Fig. 5A).

The ovary was situated more posteriorly and occupied the right dorsal side of the body (Fig. 5B). Between the testis and ovary sat a seminal receptacle containing mature spermatozoa (Figs. 1,5C). The oviduct and receptacular duct joined initially and then met the vas deferens to form a common hermaphroditic gonoduct (Fig. 1). The gonoduct itself was a simply ciliated tube and lacked special glands or vesicles. It extended forward to the right and opened into the distal portion of the right kidney forming a common urogenital opening into the mantle cavity. The urogenital pore opened into the right posterior corner of the mantle cavity, and from there a glandular groove extended forward a short distance reaching the anterior end of the right shell muscle. It was continued by a simple ciliated groove, which extended forward along the right neck to reach the outer side of the right cephalic tentacle (Figs. 1,2D). Aside from the ciliary groove, the right cephalic tentacle resembled the left one.

Interspecific variation. In Species B, the smallest of the three examined specimens showed only a testis with ripe sperm but no trace of an ovary, in the intermediate-sized specimen both gonads were present including ripe gametes, and in the largest specimen only traces of a testis were detected, whereas the ovary was enormously developed. In Species A, C, D, and E only adults were studied, which had both gonads well developed, and the same can be stated for Species F and all stages of *T. clypidellaeformis* (Fig. 4), though in the early-stage adult the testis was relatively much larger than in the adults (Fig. 4B). There were no differences between the species with respect to their copulatory organs. In one specimen of Species C, mature sperm were found in the mantle cavity, but we could not determine whether these were auto- or allosperm.

Thiele's (1908, p. 88, his figure 16) description of the genital openings of Species A must be corrected. Thiele's "gonoduct" (labeled gd) is the distal chamber of the right kidney, and Thiele's "receptaculum" (labeled rec) is the genital duct. Nevertheless, a quite large seminal receptacle was indeed present in the right side of the mantle cavity of Species A. It contained a mass of dense sperm, which was not orientated. The seminal receptacle opening was to the right of the urogenital opening.

Alimentary tract

The voluminous buccal cavity was lined by a cuticularized epithelium. Jaws and salivary glands were lacking, and the sublingual pouch was shallow and lacked a subradular sense organ or distinct glands. A ciliated dorsal food channel extended up from the mouth between two large buccal cartilages. Prominent cross-striated muscles, the fibers of which looked hollow in crosssection, supported the buccal cartilages (Fig. 6A). The horizontal muscle inserted ventrally and a thick sphincter muscle surrounded the mouth opening. The anterior end of the radula extended vertically up the dorsal food channel and met the oesophagus near the top of the channel (Fig. 6B). An additional small cartilage was located ventral to the radula, which extended posteriorly and downward, ending where the midgut began. The radula was described from SEM images by Dantart & Luque (1994). The radular sheath was quite short and the radular caecum, which was short but broad and depressed, containing many small odontoblasts, was situated between the posterior ends of the cartilages. The anterior oesophagus had huge lateral pouches, which folded up and extended backward along the top of the buccal cartilages (Fig. 6A,B). After a short distance the enlarged oesophagus was narrowed abruptly and extended backward as a thin tube, approaching the ventral side of the body. The epithelium of the posterior oesophagus consisted of few vacuolated and ciliated cells. The lumen was very narrow. Running backwards at the very ventral side, the oesophagus finally entered the stomach region, which was situated in the center of the animal (Fig. 1).

The stomach could not be clearly delimited, but was combined with a rather complex multi-lobed midgut with extensive folding in the lumen. The stomach region lacked a gastric shield, caecum, sorting areas, and typhlosole, and could be defined only by the entrance of the oesophagus and the emergence of the intestine. The stomach region and midgut epithelium were very flat and not ciliated. There were two distinct anterior lobes of the midgut that were thin-walled compared to the rest of the organ. Several openings into the midgut from the stomach region were present. The extensive midgut occupied the ventral posterior body cavity. Small particles of food were evident in the lumen of the gut, but the material was not very dense (Fig. 5C).

At the anterior left side of the stomach region the intestine emerged as a short, narrow tube with a flat and simply ciliated epithelium; a typhlosole was not present. After a short loop at the ventral side of the body, the intestine extended dorsally near the posterior end of the buccal apparatus (Fig. 1). It emerged anterior to the gonoduct and transitioned into the rectum extending to the right along the posterior edge of the left kidney. After having penetrated the posterior part of the heart ventricle (Fig. 2B), the rectum extended to the right and opened via a papillate anus into the right mantle cavity.

Interspecific variation In Species A, B, D, and F the oesophageal pouches were somewhat smaller than in *T. clypidellaeformis*, where they occupied the anterior third of the animal's body (Fig. 4A). In Species C these pouches were enormously developed and ran laterally backwards, filling free space between the shell muscle bundles and in the lateral pedal flaps (Fig. 3).

Nervous system

The weakly concentrated lepetellid nervous system had a hypoathroid (adjacent pleural and pedal ganglia) cerebropedal nerve ring enclosing the buccal apparatus, and a streptoneurous visceral loop (Figs. 1,7). The cerebral ganglia were laterally situated and interconnected by a long commissure. Anteriorly they sent out thick but simple tentacular nerves into the cephalic tentacles. An optic nerve could not be detected. Ventrally, three main nerves emerged from the cerebral ganglia that supplied the region of the mouth. The posterior-most nerve had a common origin with the nerve supplying the oral lappet region. Immediately beneath the cerebral ganglia, the buccal connectives emerged. They extended downward and then upward between the buccal muscles to meet the buccal ganglia. The latter were laterally situated at the line of the emergence of the oesophagus and were interconnected by a long commissure.

The connectives to the pleural and pedal ganglia were nearly equal in length. The pedal system lacked pedal cords, and the large pedal ganglia were interconnected by two commissures only, a very thick anterior and a very thin posterior one. Furthermore, there were no anterior pedal nerves as is usual in vetigastropods. Aside from the pedal nerves, which supplied the pedal sole, each pedal ganglion sent out a shell muscle nerve immediately below the emergence point of the mantle nerve. The statocysts were situated immediately above the anterior pedal commissure. The pleural ganglia were positioned laterally and somewhat above the pedal ganglia. Thick mantle nerves emerged laterally from the pleural ganglia and penetrated the anterior bundles of the shell muscle to eventually reach the mantle region, where they formed a nerve net surrounding the whole animal.

Whereas the anatomy of the cerebropedal ring and the osphradial ganglion could be studied in all species investigated, the details of the visceral loop could only be resolved in Species F (Fig. 7). In that species, from the left pleural ganglion the visceral loop started to the right side leading under the radular sheath and the right pleural ganglion. The suboesophageal ganglion was small, elongated, and quite indistinct, being situated above the posterior pedal commissure. From there, the visceral loop extended further to the right, then upward along the right dorsoventral shell muscle, finally looping to the left along the posterior end of the mantle cavity. The visceral ganglion was long but dorsoventrally compressed, reaching from the region of the urogenital opening to the point of entrance of the rectum into the heart ventricle. From this latter position, two nerves emerged, supplying the urogenital chamber and the rectum. The visceral loop continued anteriorly along the downward bending intestine and reached the supracesophageal ganglion after a short distance. This ganglion was situated near the center of the animal and was very small and indistinct and was embedded in the gonad. From this position a long and very thin connective was oriented between gonad and viscera to the left. At the anterior end of the left shell muscle this connective entered the mantle roof, then extended anteriorly. After a short distance it formed the osphradial ganglion. From the supraoesophageal ganglion the visceral loop continued downward, surrounding the posterior portion of the right buccal cartilage, and finally terminated in the right pleural ganglion.

Sense organs

Lepetella sierrai had two short lateral cephalic tentacles. The right tentacle was modified with a ciliated seminal groove. The cephalic tentacles were seemingly smooth, but showed small papillae with ciliary tufts at the tips in the sections using SEM (Fig. 2C,D). A single thick nerve innervated them.

Eyes, paired epipodial tentacles, and subradular organ were lacking. Statocysts were located directly dorsally to the pedal ganglia and included several small statoconia, which were in contact with very thin fibers within the statocyst's capsule. There was no distinct osphradial epithelium, but a single osphradial ganglion was present in the left anterior mantle roof.

Metazoan parasites

An unidentified parasite was observed in the body of the serially sectioned specimen of *L. sierrai*. Between the body wall and mantle tissue a round mass of foreign tissue was found (Fig. 6A). A narrow piece of tissue from this mass penetrated the body wall and internally gave rise to a highly branched structure within the limpet's body. No diagnostic appendages or distinct organs associated with this mass were observed in the histological sections, so the identity of this parasite is unknown. However, it is possible that this instance of parasitism is an early stage of the parasitic chitonophilid copepods that have been observed by other researchers in this species (Dantart & Luque 1994; Huys et al. 2002), similar to the "vermiform endosome" stage seen in lepetodrilid limpets by Tunnicliffe et al. (2008). Although the sections are not clear, there does appear to be a "rootlet system" (*sensu* Huys et al. 2002) and what may be a small female body on the outside of the body wall.

Microbial symbiosis

A dense network of bacteriocytes in the mantle margin and pedal tissue of the *L. sierrai* extended in a horseshoe shape that almost completely surrounded the entire animal (Fig. 1). Observations by TEM (Fig. 8; Michalak & Haszprunar, pers. obs.) of *Lepetella* "from Banyuls"

showed that the bacteriocytes form an epithelial network with a common but narrow lumen. However, connections to the alimentary tract, other organs, or to body sinuses were not observed in the serial sections. These bacteriocytes have previously been identified as "glandular" areas, but TEM confirmed their identity as microbial host cells (Fig. 8).

Discussion

Discrimination of species

As shown in Table 1 all putative species available were uniquely characterized by a combination of various characters and thus may represent distinct lineages. However, nothing is known about the variability of lepetellid soft bodies, and only three putative species show clear anatomical autapomorphies. Species C had complex papillae at the mantle margin, cartilages consisting of many small cells rather than large ones, reduced gill-leaflets, and huge oesophageal glands. *Lepetella* "from Banyuls" showed unique oesophageal pouches with vesicles. *Tectisumen clypidellaeformis* had cartilages consisting of medium-sized cells, and an optic nerve but no eyes. As there are many undescribed species of lepetellids, further systematic work will benefit greatly from the examination of molecular data.

Comparison to other members of Lepetelloidea

The most distinctive morphological and ecological differences that have been observed among members of the Lepetelloidea are presented in Table 2. Comparisons including more traits and higher level taxa have been made and discussed elsewhere (Haszprunar 1988a,1998; Haszprunar & McLean 1996). Preliminary molecular phylogenetic data presented by Kano et al. (2013) indicates that the tube-dwelling Lepetellidae discussed here are monophyletic and quite derived within the Lepetelloidea, indicating a comparatively recent specialization to polychaete tubes as a substrate in this group. Here we focus on particular life history and ecological hypotheses for *Lepetella*.

Feeding ecology and symbiosis.

Lepetella species inhabit the inner and outer walls of empty tubes of polychaete worms from the genus Hyalinoecia. The tubes are composed of a sugar-phosphate polymer called onuphic acid (Graham et al. 1965). Most of the Lepetella specimens we examined were collected from tubes brought up in dredges of muddy continental shelf/slope sediment. The microhabitat of the tube provides a semi-hard substrate for the limpet, and is also a likely source of nourishment. Other cocculiniform limpets inhabiting similar biogenic microhabitats, such as *Tentaoculus*, which lives on crab carapaces and shows a typical vetigastropod gut, are presumed to be feeding on the microbial and/or fungal community on the substrate. Lepetella specimens have been observed on both the outside and inside of Hyalinoecia tubes, and it has been noted by A. Warén (pers. comm.) that shell shape differs depending on whether they are situated on the inside or outside of the tube. Thus, shell characters are not particularly useful for taxonomy in this group. Lepetella has extensive modifications to the alimentary tract including a characteristic radula (Dantart & Luque 1994), large oesophageal pouches, and an extensively folded and multi-lobed midgut rather than a typical stomach with gastric shield. Additionally, round depressions where tube material has been removed were clearly evident when limpets were removed (G. Haszprunar, pers. obs.). We hypothesize that the alimentary tract has been highly modified to cope with the digestion of the sugar-phosphate polymer tube. The lepetellid radula, which is a synapomorphy for the family Lepetellidae, is specialized to work as a coarse file on the tubes

rather than simply as a scratching brush as in most other members of Lepetelloidea, which have radulae modified to scrape off surface film. Yet, we doubt that lepetellids can subsist on the nutrition it receives from the onuphic acid tube alone, and it is possible that they also ingest the biofilm that is assumed to be decomposing the tube.

The presence and location of an extensive system of bacteriocytes suggests the possibility of chemosynthetic pathways as well. Despite an extensive search we could not find any connection of the bacteriocyte network to the gut; its major part is situated in the subpallial sinus. This suggests a likely exchange with the environment within the *Hyalinoecia* tube or directly surrounding it. Although studies of the oxygen and sulfide profiles inside and surrounding the tube have not been performed, studies on the burrows of the lugworm, *Arenicola marina*, show a steep shift from an oxygenated microenvironment near the surface of the burrow to an anoxic one with high but frequently fluctuating levels of free hydrogen sulfide a few millimeters further into the burrow (Wetzel et al. 1995). If a similar oxygen-sulfide gradient exists within the tubes of *Hyalinoecia*, this would be the ideal environment for sulfide oxidizing chemoautotrophic symbionts. Confirmation of bacteria in the mantle rim tissue by TEM has only been done in *Lepetella* "from Banyuls". However, all other *Lepetella* specimens examined by histology have the same "glandular" tissue that has now been recognized as a bacteriocyte system in the mantle rim. Additional research is needed to better understand the mode of nourishment and the role of this symbiosis in the biology of this limpet group.

Reproductive behavior.

All examined *Lepetella* specimens possess an ovotestis with spatially distinct areas of testis and ovary. They are designated as simultaneous hermaphrodites, except for Species B for which there is evidence for protandric hermaphroditism. Given limited samples it is difficult to determine the mode of reproductive development, and the hypothesis of protandry in Species B is based on only three specimens varying in size, the smallest having mostly testis and little ovary, the medium sized specimen having nearly equally sized testis and ovary, and the largest one having a much larger ovary and reduced testis. It is possible that protandry is a common reproductive strategy amongst lepetellid limpets, however further study of a wider range of sizes in each species is needed.

Given the high yolk content of the eggs, it is very likely that larval development is lecithotrophic, as in all other studied Vetigastropoda. The ciliated seminal groove on the right cephalic tentacle, which is observed in all specimens, is evidence that the cephalic tentacle has been modified as a copulatory organ for internal fertilization. Further evidence for internal fertilization is the presence of a distinct seminal receptacle containing only mature spermatozoa, suggesting these are allosperm. The testis and ovary show continuous gametogenesis, suggesting that these animals have non-seasonal reproduction. Hermaphroditism, internal fertilization, and non-seasonal, continuous spawning are all traits that increase fertilization probability given the meeting of two conspecifics. These traits are also present in several other deep-sea vetigastropod groups (see Kano 2008). Given the patchiness and small size of *Hyalinoecia* tube substrata, and the low densities of limpet populations on the tubes, it is not surprising to find these traits in *Lepetella* as well.

Parasitism

The first recognized parasites in *Lepetella* were chitonophilid copepods whose eggs were originally identified as brooded limpet eggs by Dantart & Luque (1994) and subsequently

investigated in detail and identified as copepods by Huys et al. (2002). Distinct, round white eggs can be seen in the mantle cavity when this parasite is present. The female copepod's body is external to the host's body but it attaches by a rootlet system. Dwarf males subsequently attach to the females and fertilized eggs are produced. It is possible that while we did not observe the distinctive eggs, we may have observed an early stage of this parasitism with just a small female body with rootlets extending into the limpet. This parasitism does not seem to be uncommon in *Lepetella* specimens, and has also been recognized and beautifully photographed in lepetodrilid limpets (Tunnicliffe et al. 2008). However, we cannot exclude the possibility that the parasitic copepods we observed are members of another family, the Splanchnotrophidae (see e.g. Anton & Schrödl 2013). Preservation was not good enough to confirm the identity of the parasite, and further study of this parasitic relationship will require additional well-preserved specimens, preferably for molecular data.

Acknowledgments

We are most grateful to Anders Warén (Swedish Museum of Natural History, Stockholm) for providing most the material studied. Further material was generously provided by the Australian Museum Sydney, Louis Dantart (Universidad de Barcelona) and Angel Luque (Universidad Autónoma de Madrid), and Constantine Mifsud (Malta). We thank the staff of the Malacology Department of the Museum für Naturkunde, Berlin, for the loan of Thiele's sections. For sectioning and photographing, we thank Bernhard Ruthensteiner (ZSM), Eva Lodde (ZSM), and Heidemarie Gensler (LMU Munich). Bernhard Ruthensteiner also trained JJ concerning 3Dreconstruction and visualization. We thank David Lindberg for helpful comments on the manuscript. We also thank editors Louise Page and Bruno Pernet, Anders Warén, and an anonymous reviewer for improving the quality of the manuscript with their constructive reviews.

References

- Aktipis SW & Giribet G 2010. A phylogeny of Vetigastropoda and other "archaeogastropods": re-organizing old gastropod clades. Invertebr. Biol. 129:220-240.
- Aktipis SW & Giribet G 2012. Testing relationships among the vetigastropod taxa: a molecular approach. J. Mollus. Stud. 78:12-27.
- Anton RF & Schrödl M 2013. The gastropod–crustacean connection: towards the phylogeny and evolution of the parasitic copepod family Splanchnotrophidae. Zool. J. Linn. Soc. 167:473-500.
- Dall WH 1882. On certain limpets and chitons from the deep waters off the eastern coast of the United States. Proc. U.S. Nat. Mus. 81:400-426.
- Dantart L & Luque A 1994. Cocculiniformia and Lepetidae (Gastropoda: Archaeogastropoda) from Iberian waters. J. Mollus. Stud. 60:277-313.
- Dell RK 1956. The archibenthal Mollusca of New Zealand. Dominian Mus. Bull. 18.
- Finlay HJ 1927. A further commentary on New Zealand molluscan systematics. Trans. Proc. N. Zeal. Inst. Wellington 57:pp. 320-485.
- Graham GN, Kelly PG, Pautard FGE & Wilson R 1965. Onuphic Acid: A sugar phosphate polymer from the tube of *Hyalinoecia tubicola*. Nature 206:1256-1257.
- Hasprunar G 1985. On the innervation of gastropod shell muscles. J. Moll. Stud. 51: 309-314.
- Haszprunar G 1987. The anatomy of *Addisonia* (Mollusca, Gastropoda). Zoomorphology (Berlin) 106:269-278.

Haszprunar G 1988a. Comparative anatomy of cocculiniform gastropods and its bearing on archaeogastropod systematics. Malacological Review 4:64-84.

- Haszprunar G 1988b. Anatomy and affinities of pseudococculinid limpets (Mollusca, Archaeogastropoda). Zool. Scr. 17:161-179.
- Haszprunar G 1988c. Anatomy and relationships of the bone-feeding limpets, *Cocculinella minutissima* (Smith) and *Osteopelta mirabilis* Marshall (Archaeogastropoda). J. Mollus. Stud. 54:1-20.
- Haszprunar G 1988d. On the origin and evolution of major gastropod groups, with special reference to the Streptoneura. J. Moll. Stud. 54: 367-441
- Haszprunar G 1989. Anatomy and relationships of the Choristellidae (Archaeogastropoda, Lepetelloidea). 1. Proceedings of the International Malacological Congress 10:509-510.
- Haszprunar G 1998. Superorder Cocculiniformia. In: Mollusca: The Southern Synthesis. Fauna of Australia. Beesley PL, Ross G.J.B, & Wells A., ed. pp. 653-664. CSIRO Publishing, Melbourne.
- Haszprunar G & McLean JH 1996. Anatomy and systematics of bathyphytophilid limpets (Mollusca, Archaeogastropoda) from the northeastern Pacific. Zool. Scr. 25:35-49.
- Hickman CS 1983. Radular patterns, systematics, diversity, and ecology of deep-sea limpets. Veliger 26:73-92.
- Huys R, Lopez-Gonzalez PJ, Roldan E & Luque A 2002. Brooding in cocculiniform limpets (Gastropoda) and familial distinctiveness of the Nucellicolidae (Copepoda): Misconceptions reviewed from a chitonophilid perspective. Biol. J. Linn. Soc. 75:187-217.
- Kano Y 2008. Vetigastropod phylogeny and a new concept of Seguenzioidea: independent evolution of copulatory organs in the deep-sea habitats. Zool. Scr. 37:1-21.
- Kano Y, Judge J, Takano T, Marshall BA & Warén A 2013. Illuminating relationships and habitat shifts in the lepetelloidean limpet radiation into deep-sea organic and chemosynhetic habitats: a molecular approach. Abstract T1.P3 presented at the 2013 World Congress of Malacology, Ponta Delgada, Azores, July 22-28.
- Kiel S 2010. The vent and seep biota. Springer: New York.
- Lesicki A 1998. Checklist of gastropod species referred to the order Cocculiniformia Haszprunar, 1987 (Gastropoda: Cocculinoidea et Lepetelloidea) with some remarks on their food preferences. Folia Malacologica 6:47-62.
- Marshall BA 1983. The family Cocculinellidae (Mollusca: Gastropoda) in New Zealand. National Museum of New Zealand Records 2:139-143.
- Marshall BA 1987. Osteopeltidae (Mollusca: Gastropoda): a new family of limpets associated with whale bone in the deep-sea. J. Mollus. Stud. 53:121-127.
- McLean JH 1985. The archaeogastropod family Addisoniidae Dall, 1882: life habit and review of species. Veliger 28:99-108.
- McLean JH & Haszprunar G 1987. Pyropeltidae, a new family of cocculiniform limpets from hydrothermal vents. Veliger 30:196-205.
- Mifsud C 1996. Living Mollusca from circalittoral coastal muds, off Western Malta. La Conchiglia:23-41.
- Moskalev LI 1978. Lepetellidae (Gastropoda, Prosobranchia) and related forms. Trudy Instituta Okeanologii Akademii Nauk SSSR 113:132-146.
- Ponder WF & Lindberg DR 1997. Towards a phylogeny of gastropod molluscs: An analysis using morphological characters. Zool. J. Linn. Soc. Lond. 119:83-265.

Ramil, F. & Vervoort W 1992. Report on the Hydroida collected by the "BALGIM" expedition in and around the Strait of Gibraltar. Zool. Verh. Leiden 277: 1-262.

Romeis B 1989. Mikroskopische Technik 17th Edition Urban & Schwarzenberg: München-Wien-Baltimore.

- Ruthensteiner B 2008. Soft part 3D visualization by serial sectioning and computer reconstruction. Zoosymposia 1:63-100.
- Ruthensteiner B & Heß M 2008. Embedding 3D Models of biological specimens in PDF publications. Microsc. Res. Techniq. 71:778-76.
- Salas C 1996. Marine bivalves from off the Southern Iberian Peninsula collected by the Balgim and Fauna 1 expeditions. Haliotis 25: 33-100.
- Suter H 1908. Additions to the marine molluscan fauna of New Zealand, with description of new species. Proc. Malac. Soc. Lond. 8:22-42.
- Thiele J 1908. Ueber die Anatomie und systematische Stellung von *Bathysciadium*, *Lepetella* und *Addisonia*. Bull. Mus. Comp. Zool. Harvard 52:81-90.
- Thiele J 1909. Cocculinoidea und die Gattungen *Phenacolepas* und *Titiscania*. Systematisches Conchylien Cabinet von Martini & Chemnitz 2:1-48.
- Tunnicliffe V, Rose JM, Bates AE, & Kelly AE 2008. Parasitization of a hydrothermal vent limpet (Lepetodrilidae, Vetigastropoda) by a highly modified copepod (Chitonophilidae, Cyclopoida). Parasitology 135:1281-93.
- Verrill AE 1880. Notice on the remarkable marine fauna occupying the outer banks off the southern coast of New England. Am. J. Sci. 3:115-120.
- Verrill AE 1881. Notice of recent additions of the marine invertebrates of the northeastern coast of America with descriptions of new genera and critical remarks on others. II. Mollusca with notes on Annelida, Echinodermata etc., collected by the US Fish Commission. Proc. U.S. Nat. Mus. 3.
- Warén A 1972. On the systematic position of *Fissurisepta granulosa* Jeffreys, 1882 and *Patella laterocompressa* de Rayneval & Ponzi, 1854 (Gastropoda, Prosobranchia). Sarsia 51:17-24.
- Warén A 1991. New and little known Mollusca from Iceland and Scandinavia. Sarsia 76:53-124.
- Wetzel MA, Jensen P & Giere O 1995. Oxygen/sulfide regime and nematode fauna associated with *Arenicola marina* burrows: new insights in the thiobios case. Mar. Biol. 124:301-312.
- Zharkova IS 1978. Reduction of the organs of vision in some representatives of the order Diotocardia. Zool. Zh. 57:1637-1640

Table 1. Distribution of soft-body anatomical characters known to be useful for the identification of lepetellid species. Character coding. 1. Oral lappets (0 = well developed, 1 = small, 2 = absent); 2. Mantle margin (0 = smooth, 1 = small papillae, 2 = complex papillae); 3. Mantle margin (0 = nonglandular, 1 = glandular); 4. Epipodial tentacles (0 = absent, 1 = single, median, 2 = paired); 5. Shell muscle (0 = distinct bundles, 1 = solid mass); 6. Gill leaflet position (0 = right side only, 1 = also at left side, 2 = also at central mantle roof); 7. Gill leaflet size (0 = long, 1 = medium length, 2 = short, 3 = reduced); 8. Gill base (0 = regular, 1 = glandular); 9. Right kidney (0 = elongated, 1 = compact); 10. Hermaphroditism (0 = simultaneous, 1 = protandric); 11. Cartilages (0 = many small cells, 1 = several cells of medium size, 2 = few large cells); 12. Esophageal gland (0 = normal, 1 = large, 2 = huge, 3 = vesicles); 13. Eyes (0 = present, 1 = absent, but optical nerve retained, 2 = complete loss). A question mark indicates the character state is unknown, and "x" indicates the character is not applicable for that species.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
Lepetella tubicola	0	?	?	?	?	0	1	?	?	?	?	?	0
L. espinosae	2	0	1	1	1	0	1	0	1	0	2	0	2
L. sierrai	0	1	1	1	0	0	1	1	0	0	2	0	2
L. barrajoni	1	1	?	1	?	0	1	x	?	?	?	?	2
<i>L</i> . "from Banyuls"	1	1	1	1	0	0	1	x	1	0	2	3	2
Sp. A	0	1	1	2	1	0	1	X	0	0	2	0	2
Sp. B	2	0	1	1	1	0	1	X	0	1	2	0	2
Sp. C	1	2	0	0	0	2	3	x	0	0	0	2	2
Sp. D	2	0	1	2	0	1	2	x	0	0	2	0	2
Sp. E	1	0	0	2	1	1	2	x	0	1	2	0	2
Sp. F	2	0	1	1	0	0	2	X	0	0	2	0	2
Tectisumen clypidellaeformis	2	0	0	0	1	2	0	х	1	0	1	1	1

Jaws	absent	present	present	absent	present	absent
Oral lappets	present or absent	present	absent	absent	absent	absent
Common urinogenital opening	present	present	separate openings	separate openings	present	present
Shell muscles	symmetrical	symmetrical	symmetrical	asymmetrical	symmetrical	asymmetrical
Bursicles	yes	yes	оп	оц	yes	yes
Gonoduct	hermaphroditic	hermaphroditic	gonochoristic	gonochoristic	hermaphroditic	hermaphroditic
Rectum penetrates heart	yes	yes	yes	ou	yes	yes
Seminal receptacle	present	absent	absent	present	absent	absent
Symbiosis evidence	bacteriocytes in mantle rim and periphery of foot	unknown	unknown	suspect "glandular" tissue of gills (GH obs.)	unknown	"grape-like" structures on posterior oesophagus
Midgut	large, multi-lobed	2 lobed, common opening	2 lobed, 2 openings	no stomach, enlarged intestine	unmodified	2 large glands
Gastric shield	absent	present	present	absent	present	present
Oesophageal pouches	large	present	glands with duct	glands with duct	present	large
Substrate	<i>Hyalinoecia</i> tubes	poom	Whale bone	Elasmobranch egg cases	hydrothermal vents	Seagrass rhizomes
Genus	Lepetella	Pseudococculina	Osteopelta	Addisonia	Pyropelta	Bathyphytophilus

Table 2. Comparison of representative lepetelloidean genera from different substrates for select variable traits.

Figure legends

Fig. 1. Three-dimensional reconstruction of *Lepetella sierrai*. The interactive model can be accessed by clicking on the figure (Adobe Reader Version 7 or higher required). Rotate model by clicking and dragging, zoom by scrolling, select/deselect components of the model in the model tree, and change view/surface visualization in pull down menus. **A**. External morphology, right side. **B**. Alimentary system, dorsal view. **C**. Reproductive system and right kidney, dorsal view. **D**. Heart, kidneys, glandular cells of the mantle margin, and gill, dorsal view. **E**. Nervous system and shell muscle, frontal view. **F**. Extension of system of bacteriocytes, dorsal view. au, auricle of heart; bac, bacteriocytes; bc, buccal cartilage; cg, cerebral ganglia; ct, cephalic tentacle; gd, gonoduct; gi, gill; i, intestine; lk, left kidney; lsm, left shell muscle; mg, midgut; mgc, mantle gland cells; ml, mouth (oral) lappet; mo, mouth opening; oe, oesophagus; oep, oesophageal pouch; og, osphradial ganglion; ov, ovary; pc, pericardium; pg, pedal ganglion; ra, radula; re, rectum; rk, right kidney; rs, seminal receptacle; rsm, right shell muscle; sg, seminal groove; st, stomach region; te, testis; ve, ventricle of heart.

Fig. 2. Histology of various parts of *Lepetella sierrai*. A. Longitudinal section of posterior epipodial tentacle, bacteriocytes, glandular cells of the mantle margin, and posterior shell muscle. B. Mantle cavity with left kidney, rectum and heart (from *Lepetella laterocompressa*).
C. Cephalic tentacle with ciliary tufts. D. Seminal groove. E. Gill leaflets with bursicle. b, bursicle; bac, bacteriocyte; cg, cerebral ganglion; ct, ciliary tuft; ept, epipodial tentacle; gc, glandular cell of the mantle margin; gl, gill leaflet; lk, left kidney; mg, midgut gland; ov, ovary; pc, pericardium; r, rectum; sg, seminal groove; sm, shell muscle; ve, ventricle.

Fig. 3. Mantle cavity and coelomic system of Species C, in dorsal view. au, auricle; gd, gonoduct; gl, gill leaflet; hr, attachment zone of head retractor; lk, left kidney; mc, posterior end of mantle cavity; ms, mantle sinus; o, ovary; os, osphradial ganglion; pc, pericardium; r, rectum; rc, seminal receptacle; rk, right kidney; sm, attachment zone of shell muscle; te, testis; ugo, urogenital opening; ve, ventricle.

Fig. 4. Mantle cavity and coelomic system of *Tectisumen clypidellaeformis*, in dorsal view. **A.** Late-stage adult specimen. **B.** Early-stage adult specimen. au, auricle; gl, gill leaflet; gd, gonoduct; lk, left kidney; mc, posterior end of mantle cavity; ms, mantle sinus; o, ovary; pc, pericardium; r, rectum; rc, receptaculum seminis; rk, right kidney; sm, attachment zone of shell muscle; te, testis; ugo, urogenital opening; ve, ventricle.

Fig. 5. Histological details of the reproductive system of *Lepetella sierrai*. **A.** Transverse section of entire animal showing anterior gonads, gill leaflets, and posterior buccal cartilage. **B.** Detail of an egg with vitelline layer. **C.** Transverse section of entire animal showing posterior gonads, seminal receptacle, and midgut. bac, bacteriocyte; bc, buccal cartilage; gc, glandular cell of the mantle margin; gl, gill leaflet; i, intestine; mg, midgut gland; nu, nucleus; o, ovary; sm, shell muscle; sr, seminal receptacle; te, testis; vi, vitelline layer; y, yolk.

Fig. 6. Histological details of the buccal apparatus of *Lepetella sierrai*. **A.** Overview of buccal apparatus showing part of parasite. **B.** Close-up of radula and oesophageal pouches with

oesophagus. bac, bacteriocyte; bc, buccal cartilage; bm, buccal muscle; mo, mouth opening; oe, oesophagus; oep, oesophageal pouch; p, parasite; ra, radula; te, testis.

Fig. 7. Nervous system of Species C, in dorsal view. bg, buccal ganglion; cg, cerebral ganglion; g, genital nerve; ma, mantle nerve; mo, nerves supplying the region of the mouth opening; ol, nerve of oral fold; og, osphradial ganglion; pg, pedal ganglion; p, pedal nerves; pl, pleural ganglion; ppc, parapedal commissure; sb, suboesophageal ganglion; sm, shell muscle nerve; sp, supraoesophageal ganglion; st, statocyst; t, tentacular nerve; vg, visceral ganglion; vi, visceral nerve.

Fig. 8. Transmission electron micrographs showing bacteriocytes in the mantle rim tissue of *Lepetella* "from Banyuls". **A.** Duct system of bacteriocytes with ciliated lumen. **B.** Bacteria within vacuole system of bacteriocytes in cross and longitudinal section planes. **C.** Mantle rim (bottom left is free environment, top right is lumen of the sinus). **D.** Bacteriocyte duct system with ciliated lumen between lobes of midgut gland. ci, cilia; gc, glandular cell of mantle epitheliaum; lu, lumen; mdd, midgut gland cell; mv, microvilli.













Figure 2:









Figure 5:



55

Figure 6:



Figure 7:



Figure 8:



Chapter 3

Macroinvertebrate Community Assembly on Deep-sea Wood Falls in Monterey Bay is Strongly Influenced by Wood Type

Jenna Judge and Jim Barry (Monterey Bay Aquarium Research Institute)

Abstract

Factors influencing patterns and processes in deep-sea wood fall communities are poorly known. In this study, we investigated the role of wood type in the assembly of deep-sea wood fall communities. Ten different wood types representing a wide range of structure from solid logs to bundles of branches with leaves were sunk to a depth of 3,100 m depth near Monterey Bay, CA. In total, 28 wood substrates were deployed on the deep-sea bed. After 2 years, the wood substrates were recovered, and returned to the surface with over 7,000 attached or colonizing macroinvertebrates. All macroinvertebrates were identified to the lowest taxonomic level possible, including several species new to science. Diversity indices, multivariate analyses of variance, and indicator species analysis indicated that; 1) there were significant variation in the colonizing community assemblages among different wood types and 2) that wood type accounted for approximately 70% of the variation. Patterns of variation across replicates and between wood types with different levels of complexity, physical, and chemical characteristics are discussed. Although trends linking wood properties and community structure were apparent, this is a complex system. Wood structures ranging in complexity fostered the colonization of certain taxa while limiting others. The initial complexity of a woody substrate may influence the overall successional pattern and cumulative diversity attained over the course of a wood fall's existence in the deep sea.

Keywords: sunken wood, community diversity, invertebrates, Xylophaga, deep-sea, wood-fall.

Introduction

Specialist invertebrate communities that colonize sunken wood in the deep sea have been known for decades (Turner, 1973; Wolff, 1979; Maser and Sedell, 1994). The source of this wood is the world's forests, which exhibit high variation in composition, cover, and proximity to rivers and coastlines (Maser and Sedell, 1994). This variation is thus reflected in the plant material that makes its way to the bottom of the ocean. Although little is known about patterns of wood deposition on the seafloor, the last decade or so has seen an increase in interest in the ecology of wood falls, especially as they relate to other reducing habitats like whale falls, hydrothermal vents, and hydrocarbon seeps (Bienhold et al., 2013; Cunha et al., 2013; Distel et al., 2000; Samadi et al., 2010). Sunken wood, like large vertebrate falls, has several successional stages exhibiting decreasing oxygen and increasing sulfide levels with each stage, increasingly supporting chemoautotrophic microbes and associated fauna (Bernardino et al., 2010; Bienhold et al., 2013; Laurent et al., 2013; Nishimoto et al., 2009). Wood falls tend to recruit characteristic fauna representing (1) specialists that consume the wood (e.g., wood-boring bivalves of genus *Xylophaga*) (Haga and Kase, 2013; Tyler et al., 2007; Voight, 2009; 2008; 2007; Voight and Segonzac, 2012; Young et al., 2013); (2) grazers of the biofilm that degrades the wood (e.g., lepetelloidean limpets) (Haszprunar 1988a); (3) opportunists that are drawn to patches of increased biomass (e.g., the squat lobster Munidopsis sp.)(Hoyoux et al., 2009; Williams and
Turner, 1986); and (4) filter-feeders seeking a hard substrate and position above the seafloor, such as crinoids and anemones (Samadi et al., 2010; Wolff, 1979). Each of these colonist categories utilizes wood falls in a unique way; thus variation in wood properties may promote the formation of distinct communities on different types of wood falls.

It has been noted anecdotally that different kinds of plant material (e.g. wood, seagrass, kelp, coconuts) possess different properties that influence both the composition of the colonizing assemblage, and the rate of colonization and consumption (Wolff 1979; Bouchet et al. 2001; Pailleret et al. 2007; Schwabe et al. 2015). Consequently, different sources of woody terrestrial inputs may influence the pattern and rate of community assembly in the deep sea. The composition of global forests has changed continually since the Carboniferous, when the first trees emerged (Willis and McElwain 2002). For instance, forests were dominated by gymnosperms by the early Jurassic (206-180 Ma), but angiosperms radiated in the Cretaceous (~70 MYA), shifting the make-up of most forests. Angiosperms continue to dominate most forests today (Willis and McElwain 2002). The composition of North American west coast forests has undergone major shifts over the last 60 Ma. In the Eocene (60-50 Ma), the west coast flora consisted of evergreen and deciduous dicot angiosperm trees, shrubs, and Gnetales. The Oligocene (~30 Ma) brought a shift toward a larger mix of both deciduous and evergreen dicots and conifers, plus ferns. And in the Miocene (11.2-5.3 Ma), the west coast became characterized by three biomes organized from north to south representing cool temperate, warm temperate, and winter wet communities. This translates to a gradual shift from forests with more conifers and ferns in Canada to fewer conifers and ferns with the addition of angiosperm shrubs starting near northern California (Willis and McElwain 2002). While these major shifts have occurred over millennia, on human timescales the localized effects of logging and dams have also had an influence on coastal forests and streams used to transport logs even 100 years after the cessation of "river-driving" (Sedell et al. 1991). These floral shifts have implications for specialist wood fall communities in the deep-sea when they enter the marine environment via streams and open coastlines. For instance, a dense forest near rivers unobstructed by dams would be expected to have more regular and larger inputs of wood with seasonal increases in flow and periodical floods than a heavily logged forest or one whose rivers are blocked by dams (Andrus et al. 1988; Ower and Arker 2008). Additionally, the diversity and composition of the forest, history of disturbance (e.g. fires, logging) will influence the types and proportions of woody material that may eventually make their way to the ocean (Ralph et al. 1994; Hyatt and Naiman 2001; Montgomery and Piégay 2003). Questions pertaining to the source, quantity, and eventual fate of wood falls are beyond the scope of this study, but it is important to consider wood falls as a connection between terrestrial and marine realms, and to consider the many implications that watershed dynamics have for marine ecosystems.

Several studies have deployed wood parcels to the deep-sea and/or collected natural wood falls in temperate and tropical regions, but none have directly tested the influence of wood type on macrofaunal community composition, and those that deployed experimental substrates typically standardized the wood as blocks or boards, removing the natural features (Turner 1973, 2002; Lichlyter 2004; Pailleret et al. 2007; Voight 2007; Bernardino et al. 2010b; Samadi et al. 2010; Schwabe et al. 2015). The goal of this study was to compare the macroinvertebrate communities that colonized a diverse array of wood types, retaining the features that they would likely retain if they sunk naturally, thus not having standard shape, weight, or volume, but capturing the

diversity of woody material that might attract a deep-sea organism as a source of food, shelter, or hard substrate.

The aim of this study was to experimentally examine the influence of wood type on rate of colonization, and the composition of the macroinvertebrate colonising assemblage in the deepsea by sinking woody material near Monterey Bay, CA. Several different wood types were chosen to maximize phylogenetic diversity within vascular plants, with samples ranging widely in physical properties such as hardness, branch thickness, presence of leaves or bark, and overall complexity as a substrate. Our null hypothesis was that the colonizing assemblage would be similar in both rate and composition among all wood types, including a similar assortment of wood specialists, opportunists, and hard-substrate seekers. Owing to known differences in wood properties (e.g. hardness, secondary chemical compounds), however, we expected to observe significant patterns of colonization among general wood types.

Methods

Substrate acquisition

Ten phylogenetically diverse plant species, common to California coastal forests, were selected as experimental substrates (Fig. 1, Table 1). Material was obtained in the form of logs and pruned branches from the Regional Parks Botanic Garden in Tilden Park (Berkeley, CA), the City of Berkeley (Sacramento Street median), UC Berkeley campus, and a tree fern enthusiast in El Cerrito, CA (ferntastic.com). The acquired materials include the base of a tree fern of the genus Cvathea; solid trunk pieces from the gymnosperms Ginkgo biloba, Pinus pinea, and Sequoiadendron sempervirens; bundles of branches with needle-like leaves from the yew, Torreva californica; fronds from the monocot palm, Washingtonia filifera; bundles of small branches from the early eudicot tree poppy, *Dendromecon rigida;* bundles of leafy branches from the spice bush, Calycanthus occidentalis; bundles of branches from island ironwood, Lyonothamnus floribundus; and bark covered logs of the coast live oak, Quercus agrifolia (Table 1). All samples were collected as fresh cuttings from live trees except for the *Ginkgo* which had been sitting as cut chunks in a forested area of UC Berkeley for three months, and the tree fern, which had recently died and was being stored in a plastic bag for approximately two weeks. The lag time between collection and deployment was approximately eight weeks, during which time wood was kept outside in a dry place and was not submerged until deployment to the study site.

Experimental deployments and sample recovery

Depending on the nature of the material, three logs or three bundles of fronds or branches from each wood type was bundled separately in 3mm nylon mesh, tagged with a numbered polyurethane tag, weighed, and measured for dimensions (Table 2). Only one piece of *Cyathea* tree fern was available for deployment, otherwise all other wood types had three replicate bundles. Polyurethane rope was used to tie around each bundle and make a handle for ROV manipulations. Plant bundles were deployed at 3100m at the "Deadwood 2" site (36° 15.6768' N, 122° 40.6790' W) by a benthic elevator and ROV *Doc Ricketts* on an MBARI cruise aboard the *R/V Western Flyer* on October 18, 2011 (Fig. 2). Bundles were placed arbitrarily every 3m in three rows, each row separated by 5m. After 2 years of undisturbed and unobserved bottom time, the R/V *Western Flyer* and ROV returned and all 28 bundles were recovered. Video documenting the surrounding sediment and all aspects of each bundle was recorded before retrieval. Specially made recovery devices consisting of a metal ring with attached fine mesh bag

and closing mechanism, were used to contain bundles and prevent loss of material during the ascent of the benthic elevator (Fig. 2D). All bundles were recovered and placed in the benthic elevator during three separate ROV dives on October 26, 27, and 28, 2013.

Sample processing

Onboard the R/V *Western Flyer*, each bundle was opened and photographed, and loose material was rinsed into a fine mesh bag for preservation in 90% ethanol. All animals and pieces of wood containing animals were preserved in 90% ethanol onboard the ship. Some pieces of wood were too large and too solid to break apart on the ship, so they were frozen in the minus 80 freezer onboard. On shore, frozen wood was broken with a wedge and splitting maul and then cut into smaller pieces with a table saw. Cut pieces containing visible animals were placed in 90% ethanol. All preserved material was shipped to UC Berkeley for further processing and identification. Subsequently, all animals were removed from the wood, and sorted to the lowest possible taxonomic level, or morphotype.

Statistical analyses

All analyses were completed in the R statistical platform (R Core Team 2014). A Mantel test was used to test whether the spatial arrangement of wood bundles affected the community composition on each wood bundle. Species richness, Simpson's diversity index, and Pielou's evenness were calculated for each wood type in the R package "vegan". The function "adonis" in "vegan" was used to perform a permutation-based, multivariate analysis of variance to determine the percent of variation between communities that can be attributed to wood type (Oksanen 2013). Three versions of the data were tested: (1) taxa identified to the lowest possible taxonomic level (species, genus, or family level depending on group) (2) taxa binned according to larger taxonomic levels, and (3) taxa binned by guild as either "Xylophagous", "Grazers", "Predators/Scavengers", "Detritivores", "Filter feeders", "Deposit feeders", or "Multiple feeding modes." No difference was found by reducing the taxonomic resolution, so further analyses were limited to taxonomically and guild-binned data (Table 3). Adonis operates on many dimensions of the multidimensional space, so cannot produce a graphical output to display the data. Thus, we used the function "cca" in R package "vegan" to do a constrained correspondence analysis on taxonomic and guild level datasets with wood type as the constraining factor. To test whether taxonomic groups or guilds had a significant association to a particular wood type, a species indicator test was applied to both datasets. The function "signassoc" in R package "indicspecies" returned the wood type with the tightest association to each taxonomic bin and guild (based on relative abundances) and whether it is was significant compared to a random distribution of taxa across wood types (De Caceres and Legendre 2009).

Results

All 28 wood bundles were successfully retrieved and a total of 7661 individuals were identified to at least the family level (Table 3). Due to variable preservation quality, species-level determination was not possible for all specimens. Several taxonomists assisted with family level identification, and in some cases lower rank identifications could be made. Material will be made available to experts for further identification and description if requested. For purposes of diversity and community assembly analyses, each taxon was placed in a taxonomic bin and a guild (see Table 3 for designations). Major patterns of invertebrate associations to wood type remained largely consistent despite binning, so most analyses used binned data to simplify

interpretation and visualization of patterns. A Mantel test confirmed that that spatial arrangement of wood bundles did not affect the resulting community compositions (p=0.299, r=0.031).

Abundance was highly variable between wood types with palm having the most specimens (n=2098, on three replicates) and tree fern having the least (n=25, on one sample) (Fig. 3). Richness, Simpson's diversity index, and Pielou's evenness were also quite variable between wood types, with some wood showing high variance among replicates (Fig. 4).

Analyses of variance with adonis indicated that wood type explains 68.40% of the variance between community assemblages when full taxonomic resolution is considered, 68.31% when taxa are binned into larger taxonomic groups, and 75.95% when binned by guild (Table 4, *p*-values <0.0001). This was strong evidence that wood type was a major factor in community assembly. Results of the CCA were displayed in ordination space with 95% confidence ellipses for each wood type (Figs. 5, 6). Both taxonomically (Fig. 5) and guild-binned (Fig. 6) data returned similar patterns reflecting variation in the consistency of replicates and associations of certain taxa with different types of wood. Palm, tree poppy, ironwood, and yew had relatively small confidence ellipses indicating consistency between replicates in community assemblages. However, *Ginkgo*, pine, redwood, and spicebush had much larger ellipses, indicating higher variation across the three replicates. Oak had intermediate variation between replicates. Colonizing taxa were clustered in ordination space with arthropods and snails grouped most tightly, while bivalves were the most isolated taxon.

When the community data was plotted as guilds in a CCA, the pattern is similar to that shown in the taxonomic group data display (Fig. 6). The Xylophagous guild is composed of boring bivalves and limnorid isopods and is oriented near the Oak, Redwood, Pine, and *Ginkgo* ellipses. The Grazers, Filter feeders, and taxa with multiple feeding modes cluster together with the most wood types overlapping them. The Deposit feeders and Detritivores cluster together near the intercept and within the Spicebush ellipse. The Predators and Scavengers are clutered nearest to the Palm ellipse and are also within the Spicebush ellipse.

Indicator species analysis further showed associations of certain invertebrate taxa and guilds with certain wood types. The analysis returned the "indicator species" as the wood type most associated with each taxonomic bin or guild (Table 5).

Discussion

The macroinvertebrate community that colonized sunken wood over the course of 2 years varied significantly based on the type of wood. A key factor in determining species richness is habitat heterogeneity in both terrestrial and marine systems (Barry and Dayton 1991; Stein et al. 2014). Most wood fall colonists likely arrived at the study site as larvae and patterns of colonization could either be due to differential settling choices made by the larvae or by differential success in establishing themselves on particular substrates. Although all wood bundles were enclosed in 3mm mesh, there were likely effects on flow at the microscale that may have influenced the likelihood of larvae being entrained and guided to a suitable substrate to establish themselves. For instance, there was more pre-existing structure in the bundles composed of palm fronds and branches of yew or tree poppy compared to the relatively large smooth surface presented by a log of oak, pine, or *Ginkgo*. Thus, depending on the life strategy of the animal, one might expect

wood-borers such as *Xylophaga* to have an easier time colonizing and boring into a larger piece of wood in which their bore-hole can continue to expand. However, some bivalves were found boring down the middle of thin branches, their size constrained by the diameter of the branch. On the other hand, organisms like amphipods, tanaids, and polychaetes, that cannot create their own shelter through boring, likely had better success colonizing and establishing in a wood bundle with pre-existing cavities such as the groove of a palm frond or the spaces amongst branches and leaves of spicebush or yew. The limpets were able to take advantage of the highest variety of substrates, perhaps due to their small size. They were found on the bark of oak logs, the surface of branches, on ironwood leaves, and even on the needle-like leaves of the yew. In fact, there were 509 individuals of the limpet Amphiplica gordensis found in one ironwood bundle and fewer than 70 on any oak log, perhaps due to the greater surface area and complexity of surfaces in a bundle of branches with leaves compared to a solid oak log. These expectations based on life habit are reflected by the patterns in the data and are supported by the analyses (Figs. 5, 6). Thus, there appears to be a relationship between the structural complexity of the woody substrate and the life strategy of the colonists, whether they require a surface to bore into, a place to attach, or a pre-existing cavity in which to reside.

An aspect not captured by this study is time. Other studies have shown that sunken wood goes through several successional stages, beginning as a solid log or block and becoming increasingly bored through and complex due to the work of Xylophagaid bivalves (Turner 1973; Voight 2007; Bienhold et al. 2008; Fagervold et al. 2014; McClain and Barry 2014). With only two years on the bottom, the oak, pine, Ginkgo and redwood logs from this study were not bored to the point of having many unoccupied cavities for mobile animals to colonize because the bivalves themselves were still occupying those spaces. However, in the case of wood falls that had five to seven years of bottom time at a neighboring site, McClain and Barry (2014) found that several Acacia logs had reached a later successional stage in which many vacant bore holes were occupied by gastropods, polychaetes and tanaids. Thus, wood-boring bivalves literally carved out space for other animals to occupy once they vacated that space themselves by dying natural or predator-mediated deaths. Without Xylophagaids to create bore holes, it would be difficult for many wood-associated taxa to utilize a large log. Thus, principally bivalves and limpets colonized the coast live oak and pine logs used in this study. On the other hand, woody substrates that were already complex in structure, such as the palm, yew, and spicebush in this study, supported many taxa that would be excluded from logs until bore-holes were available. In one sense, these "complex" substrates facilitated the diversity of the colonizing community without the need for bivalves to create the complexity, in a sense skipping aspects of succession. However, there were many fewer boring bivalves on these structurally complex substrates and those that were found were constrained by the diameter of available branches and lower volume of wood. Therefore, in another sense, originally complex substrates are limited in the cumulative diversity they can attain over their lifespan and limit the action of bivalves, which are key members of the community making carbon available to microbes and sediment-associated organisms (Bienhold et al. 2008; Fagervold et al. 2014).

Another aspect that likely constrains wood fall communities is the chemical composition and physical structure of the wood because these properties affect the decay rates of wood on land (Scheffer 1966). For instance, tree fern and redwood had the lowest abundance of macroinvertebrates compared to all other wood types. The physical structure of these wood types

is very different, but they are likely both deterring colonization through secondary compounds that prevent herbivory in their terrestrial environments. Redwood is known to be high in phenolic compounds, among other bioactive phytochemicals, and it seems to be effective against marine invertebrates as well (Scheffer 1966; Davies et al. 2014). Although the defense strategies of tree ferns has not been investigated in detail. Pteridophytes are known to have many phytochemical defenses that deter herbivory (Page 2002). Additionally, there is anecdotal evidence that sunken tree fern material is typically devoid of fauna when collected by trawl in the tropical Pacific (pers. comm. Philippe Bouchet, July 2012). Many of the plants used in this study have chemical compounds in their wood, leaves, or fruit that are deterrent to herbivores, but their role in deepsea colonization is unclear and understudied (Table 1, Scheffer 1966). Bark, which protects living trees, may also have affected colonization patterns (Pearce 1996). For instance, all *Xylophaga* on oak colonized from the cut ends of the log, while none had bored through the bark. A similar pattern was observed in the *Xylophaga* that bored redwood and pine. Wood hardness has previously been suggested by other researchers (McClain and Barry 2014) as an important factor in Xylophaga colonization and has even been shown to influence variation within one species, *Xylophaga washingtona*, on a variety of wood boards (Turner 2002). The present study observed a pattern of many small individuals of X. zierenbergi on Ginkgo only penetrating a small distance. On oak, there were slightly larger, and densely packed individuals penetrating a few centimeters into the cut ends. Fewer, but much larger individuals with deeper excavations were observed in pine, having softer wood compared to Ginkgo and oak. The size variation observed in the same species of boring bivalve on different wood types could be due to wood hardness, difference in timing of colonization, or competition between recruits. Without sampling more time points, it would be difficult to differentiate between these potential causes of size and abundance patterns. Beyond the broad diversity patterns presented here, it is difficult to assess the influence of particular substrate characteristics for the invertebrate colonizing assemblage, as all contribute to the overall complexity and attractiveness of a substrate to different members of the community, each of whom have their own requirements and habitat preferences.

Conclusions

Both variation in the overall complexity of woody substrates and their structural and chemical characteristics are potential factors that shape the macroinvertebrate communities that colonize them. Taxa associated with wood falls have different habitat requirements, so the type and complexity of wood available may constrain the pool of taxa that can utilize it. For instance, palm fronds represent a morphologically complex substrate that supported a high abundance of arthropod and polychaete colonists, but the number of species was limited and did not include *Xylophaga*, a key member of the typical wood fall fauna. On the other hand, wood logs (e.g. oak) were colonized less by arthropods and polychaetes, but did support *Xylophaga* and given time would likely have reached a successional state able to provide cavities for the mobile taxa (McClain and Barry 2014). This research represents evidence that wood type matters in community assembly of macroinvertebrates on woody substrates in the deep-sea. These preliminary patterns should be further investigated by testing wood substrate complexity and chemical factors explicitly in a more controlled experiment. Additionally, the nature and distribution of woody substrates on the benthos at larger spatial and time scales needs is required to predict how large a role wood plays in contributing to deep-sea diversity.

Acknowledgements

We would like to thank employees of the Tilden Park Regional Parks Botanical Garden, the City of Berkeley, UC Berkeley, and local tree service businesses and community members that donated wood and tree cuttings for this project. Deployment and retrieval would not have been possible without the crews of the RV Western Flver and ROV Doc Ricketts and without the logistics expertise of Patrick Whaling and Kurt Buck. The help of Rosemary Romero and Craig McClain with processing samples on board is appreciated. The help of the following UC Berkeley undergraduates was instrumental in extracting, sorting, and counting thousands of animals: Priscilla Chen, Connie Martin, Allegra Nottoli, Chris Castaneda, Jennifer Yu, Jessica Kendall-Bar, and Alyssa Kehlenbach. We extend heartfelt thanks to Janet Voight for her feedback at every stage of this project and the use of her taxonomic expertise to identify the Xylophagaid bivalves. We would also like to thank Camilla Souto, Adrian Glover, Tammy Horton, Stefanie Kaiser, Saskia Brix, Anders Warén, and Yasunori Kano for their assistance with identification of the other taxa. We thank Juan Guevara and Meagan Oldfather for help with analyses in R. Funding for this project was provided in the form of student research grants from the UCMP, COA, AMS, and AMNH Lerner Gray. We thank David Lindberg for his feedback on the manuscript and every stage of this project.

References

- Bernardino, A.F., Smith, C.R., Baco, A., Altamira, I., Sumida, P.Y.G., 2010. Macrofaunal succession in sediments around kelp and wood falls in the deep NE Pacific and community overlap with other reducing habitats. Deep Sea Research Part I: Oceanographic Research Papers 57, 708–723. doi:10.1016/j.dsr.2010.03.004
- Bienhold, C., Pop Ristova, P., Wenzhöfer, F., Dittmar, T., Boetius, A., 2013. How Deep-Sea Wood Falls Sustain Chemosynthetic Life. PLoS ONE 8, e53590. doi:10.1371/journal.pone.0053590.s013
- Cunha, M.R., Matos, F.B.L., Gà nio, L., Hilà rio, A., Moura, C.J., Ravara, A.O., Rodrigues, C.F., 2013. Are Organic Falls Bridging Reduced Environments in the Deep Sea? - Results from Colonization Experiments in the Gulf of CÃ₁diz. PLoS ONE 8, e76688. doi:10.1371/journal.pone.0076688.s005
- Distel, D.L., Baco, A.R., Chuang, E., Morrill, W., Cavanaugh, C., Smith, C.R., 2000. Do mussels take wooden steps to deep-sea vents? Nature 403, 1–2.
- Haga, T., Kase, T., 2013. Progenetic dwarf males in the deep-sea wood-boring genus Xylophaga (Bivalvia: Pholadoidea). Journal of Molluscan Studies 79, 90–94. doi:10.1093/mollus/eys037
- Hoyoux, C., Zbinden, M., Samadi, S., Gaill, F., Compère, P., 2009. Wood-based diet and gut microflora of a galatheid crab associated with Pacific deep-sea wood falls. Mar Biol 156, 2421–2439. doi:10.1007/s00227-009-1266-2
- Kindt R and Coe R. 2005. Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies. Nairobi: World Agroforestry Centre (ICRAF).
- Laurent, M.C.Z., Le Bris, N., Gaill, F., Gros, O., 2013. Dynamics of wood fall colonization in relation to sulfide concentration in a mangrove swamp. Marine Environmental Research 87-88, 85–95. doi:10.1016/j.marenvres.2013.03.007
- Maser, C., Sedell, J.R., 1994. From the forest to the sea: the ecology of wood in streams, rivers, estuaries, and oceans.

- Nishimoto, A., Mito, S., Shirayama, Y., 2009. Organic carbon and nitrogen source of sunken wood communities on continental shelves around Japan inferred from stable isotope ratios. Deep-Sea Research Part II 56, 1683–1688. doi:10.1016/j.dsr2.2009.05.032
- Pailleret, M., Haga, T., Petit, P., Privé-Gill, C., Saedlou, N., Gaill, F., Zbinden, M., 2007. Sunken wood from the Vanuatu Islands: identification of wood substrates and preliminary description of associated fauna. Marine Ecology 28, 233–241. doi:10.1111/j.1439-0485.2006.00149.x
- Samadi, S., Corbari, L., Lorion, J., Hourdez, S., Haga, T., Dupont, J., Boisselier, M.-C., Richer de Forges, B., 2010. Biodiversity of deep-sea organisms associated with sunken-wood or other organic remains sampled in the tropical Indo-Pacific. Cah. Biol. Mar. 51, 459–466.
- Turner, R.D., 1973. Wood-Boring Bivalves, Opportunistic Species in the Deep Sea. Science 180, 1377–1379.
- Tyler, P.A., Young, C.M., Dove, F., 2007. Settlement, growth and reproduction in the deep-sea wood-boring bivalve mollusc Xylophaga depalmai. Mar. Ecol. Prog. Ser. 343, 151–159. doi:10.3354/meps06832
- Voight, J.R., 2007. Experimental deep-sea deployments reveal diverse Northeast Pacific woodboring bivalves of Xylophagainae (Myoida: Pholadidae). Journal of Molluscan Studies 73, 377–391. doi:10.1093/mollus/eym034
- Voight, J.R., 2008. Deep-sea wood-boring bivalves of Xylophaga (Myoida: Pholadidae) on the Continental Shelf: a new species described. J. Mar. Biol. Ass. 88, 1459. doi:10.1017/S0025315408002117
- Voight, J.R., 2009. Diversity and reproduction of near-shore vs offshore wood-boring bivalves (Pholadidae: Xylophagainae) of the deep eastern Pacific ocean, with three new species. Journal of Molluscan Studies 75, 167–174. doi:10.1093/mollus/eyp012
- Voight, J.R., Segonzac, M., 2012. At the bottom of the deep blue sea: a new wood-boring bivalve (Mollusca, Pholadidae, Xylophaga) from the Cape Verde Abyssal Plain (subtropical Atlantic). Zoosystema 34, 171–180. doi:10.5252/z2012n1a8
- Williams, A.B., Turner, R.D., 1986. Squat Lobsters (Galatheidae: Munidopsis) Associated with Mesh-Enclosed Wood Panels Submerged in the Deep Sea. Journal of Crustacean Biology 6, 617–624.
- Wolff, T., 1979. Macrofaunal Utilization of Plant Remains in the Deep Sea. Sarsia 64, 117–136.
- Young, C.M., Emson, R.H., Rice, M.E., Tyler, P.A., 2013. A paradoxical mismatch of fecundity and recruitment in deep-sea opportunists_ Cocculinid and pseudococculinid limpets colonizing vascular plant remains on the Bahamian Slope. Deep-Sea Research Part II 92, 36–45. doi:10.1016/j.dsr2.2013.01.027

Scientific name	Common name	Part Used	Hardness	CA native	Toxicity
Cyathea sp.	Tree Fern	"trunk" base (fibrous mass)	fibrous	no*	Phytochemical defenses across ferns
Ginkgo biloba	Ginkgo	trunk	soft	no	resistant to insect damage
Pinus pinea	Stone pine	trunk	intermediate	no	Turpentine in trunk resin
Sequoiadendron sempervirens	Redwood	thick branch	intermediate	yes	resistant to decay, high phenols
Torreya californica	Yew	thin branches w/ needles	soft	yes, endemic	resistant to decay
Washingtonia filifera	California Fan Palm	fronds	dense fibers	yes	disease and pest resistant
Dendromecon rigida	Tree Poppy	thin branches	intermediate	yes	none known
Calycanthus occidentalis	Spicebush	branches w/ leaves	hard	yes	contains calycanthine, toxic to humans and livestock
Lyonothamnus floribundus	Island Ironwood	variably sized branches	hard	yes, endemic	none known
Quercus agrifolia.	Coast Live Oak	thick branch	hard	yes	none known

Table 1: Properties of wood species used in this study. *There are no CA native tree ferns alive today, but there were historically.

Number	Taxon	Length (cm)	Width (cm)	Mass (kg)
WB10	Palm	68.58	16.51	1.36
WB11	Palm	73.66	12.7	1.13
WB12	Palm	73.025	15.24	1.25
WB13	Redwood	41.275	16.51	5.44
WB14	Redwood	34.29	20.32	9.98
WB15	Redwood	40.64	15.24	6.35
WB16	Pine	30.48	12.7	4.31
WB17	Pine	29.21	17.145	4.31
WB18	Pine	34.925	16.51	5.22
WB19	Spicebush	62.23	13.335	2.04
WB20	Spicebush	53.975	14.605	2.04
WB21	Spicebush	69.215	15.24	2.72
WB22	Oak	31.115	19.685	9.30
WB23	Oak	34.925	20.955	10.89
WB24	Oak	40.64	22.86	13.15
WB25	Ginkgo	40.64	25.4	2.27
WB26	Ginkgo	55.88	17.78	2.72
WB27	Ginkgo	43.18	25.4	3.18
WB28	Tree Poppy	58.42	15.875	1.13
WB29	Tree Poppy	49.53	14.605	0.91
WB30	Tree Poppy	57.15	12.7	1.13
WB31	Ironwood	66.04	15.24	1.36
WB32	Ironwood	57.15	15.24	1.36
WB33	Ironwood	56.515	17.145	1.59
WB34	Yew	71.12	13.97	1.13
WB35	Yew	63.5	14.605	1.13
WB36	Yew	64.77	19.05	0.91
WB37	Tree Fern	22.86	11.43	0.68

 Table 2: Dimensions of wood bundles before deployment.

MALLFunctionFunctionMOLLUSCABIVALVIAXylophaga zierenbergibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga zierenbergibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga cf. coronabivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga heterosiphonbivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga muraokaibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xyloredo sp.bivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xyloredo sp.bivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xyloredo sp. novbivalveXylophagous(Turner 1973; Distel and Roberts 1997)Kyloredo sp. novsnailGrazer (biofilm)(Maclanal 1985)Amphiplica gordensislimpetGrazer (biofilm)(Maclanal 1985)NecturingsnailGrazer (biofilm)(Johnson et al
Nichle Sex BiVALVIAXylopholas crookibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga zierenbergibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga cf. coronabivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga heterosiphonbivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga muraokaibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga muraokaibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xyloredo sp.bivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xyloredo sp. novbivalveXylophagous(Turner 1973; Distel and Roberts 1997)GASTROPODACaymanabyssia vandoveraelimpetGrazer (biofilm)(Marshall 1985)Amphiplica gordensislimpetGrazer (biofilm)(Macdonald et al. 2010)Nyloskenea sp.(Johnson et al. 2010)(McClain and Barry 2014)snailGrazer (biofilm)Provanna sp. 1(Johnson et al. 2010)(McClain and Barry 2014)snailGrazer (biofilm)Provanna cf. pacificasnailGrazer (biofilm)(Johnson et al. 2010)ARTHROPODAHunidopsisgalatheidGrazer(Larsen 2007)MunidopsisgalatheidGrazer (biofilm)(Cunha et al. 2013)
Dry RiverDry RatvingSylopholas crookibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga zierenbergibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga f. coronabivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga heterosiphonbivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga muraokaibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xyloredo sp.bivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xyloredo sp.novbivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xyloredo sp.novbivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xyloredo sp.novbivalveXylophagous(Turner 1973; Distel and Roberts 1997)Agamanbyssia vandoveraelimpetGrazer (biofilm)(Maclana 1985)Amphipica gordensislimpetGrazer (biofilm)(Maclana 1985)(M
Aytophalous croomOranteAytophalous(Turner 1973; Distel and Roberts 1997)Xylophaga zierenbergibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga differencebivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga heterosiphonbivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga muraokaibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga muraokaibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xyloredo sp.bivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga differenceKylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga differenceKylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga differenceKylophagous(Turner 1973; Distel and Roberts 1997)XylophagousGrazer (biofilm)(Marshall 1985)Amphipica gordensislimpetGrazer (biofilm)Mectan and Barry 2014)snailGrazer (biofilm)Provanna sp. 1(Johnson et al. 2010)McClain and Barry 20
Nytophaga DevelopingDrawerNytophagoaaNytophagoaaNytophagoaaNytophagoaaNytophagoaaNytophagoaaNytophagoaaNytophagoaaNytophagoaaNytophagoaaNytophagoaaaNytophagoaaaNytophagoaaNytophagoaaa </td
Aytophaga beterosiphonbivalveAytophagous(Turner 1973; Distel and Roberts 1997)Xylophaga heterosiphonbivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xylophaga muraokaibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xyloredo sp.bivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xyloredo sp.bivalveXylophagous(Turner 1973; Distel and Roberts 1997)GASTROPODACaymanabyssia vandoveraelimpetGrazer (biofilm)Caymanabyssia vandoveraelimpetGrazer (biofilm)(Marshall 1985)Amphiplica gordensislimpetGrazer (biofilm)(Macdonald et al. 2010)Neptunea amiantasnailPredator(Macdonald et al. 2010)Xyloskenea sp.(Marshall 1988)(McClain and Barry 2014)snailGrazer (biofilm)Provanna sp. 1(Johnson et al. 2010)(McClain and Barry 2014)snailGrazer (biofilm)Provanna cf. pacificasnailGrazer (biofilm)(Johnson et al. 2010)ARTHROPODAAMPHIPODAAmphipodGrazer (biofilm)AmphipiodGrazer (biofilm)(Cunha et al. 2013)Paronesimoides voightaeamphipodGrazer (biofilm)(Cunha et al. 2013)Paronesimoides voightaegalatheidGrazer (biofilm & wood)(Hoyoux et al. 2009)
Nytophaga nuraokaibivalveNytophagous(Turner 1973; Distel and Roberts 1997)Xylophaga muraokaibivalveXylophagous(Turner 1973; Distel and Roberts 1997)Xyloredo sp.bivalveXylophagous(Turner 1973; Distel and Roberts 1997)GASTROPODACaymanabyssia vandoveraelimpetGrazer (biofilm)(Marshall 1985)Amphiplica gordensislimpetGrazer (biofilm)(Mclean 1991)Neptunea amiantasnailPredator(Macdonald et al. 2010)Xyloskenea sp.(McClain and Barry 2014)snailGrazer (biofilm)Provanna sp. 1(Johnson et al. 2010)(McClain and Barry 2014)SnailGrazer (biofilm)(Johnson et al. 2010)ARTHROPODAsnailGrazer (biofilm)AmpHiPODAsnailGrazer (biofilm)Bathyceradocus sp. nov.amphipodGrazer (biofilm)Provanna cf. pacificasnailGrazer (biofilm)AMPHIPODAsnailGrazer (biofilm)Bathyceradocus sp. nov.amphipodGrazer (biofilm)Paronesimoides voightaeamphipodGrazer (biofilm)Paronesimoides voightaeamphipodGrazer (biofilm)MunidopsisgalatheidGrazer (biofilm & wood)MunidopsisgalatheidGrazer (biofilm & wood)
Aytophaga maraokarbivalveAytophagous(Turner 1973; Dister and Roberts 1997)Xyloredo sp.bivalveXylophagous(Turner 1973; Dister and Roberts 1997)Xyloredo sp. novbivalveXylophagous(Turner 1973; Dister and Roberts 1997)GASTROPODACaymanabyssia vandoveraelimpetGrazer (biofilm)(Marshall 1985)Amphiplica gordensislimpetGrazer (biofilm)(Mclean 1991)Neptunea amiantasnailPredator(Macdonald et al. 2010)Xyloskenea sp.(Marshall 1988)(McClain and Barry 2014)snailGrazer (biofilm)Grazer (biofilm)(Johnson et al. 2010)(McClain and Barry 2014)snailGrazer (biofilm)Provanna sp. 1(Johnson et al. 2010)(McClain and Barry 2014)snailGrazer (biofilm)Provanna cf. pacificasnailGrazer (biofilm)AmpHIPODAAmpHipodGrazer (biofilm)Bathyceradocus sp. nov.amphipodGrazer (biofilm)Bathyceradocus sp. nov.amphipodGrazer (biofilm)MunidopsisgalatheidGrazer (biofilm)Curner 1973; Wolff 1979)Seba bathybiaSeba bathybiaamphipodGrazer (biofilm)MunidopsisgalatheidGrazer (biofilm & wood)MunidopsisgalatheidGrazer (biofilm & wood)
Ayloredo sp.bivalveAylophagous(Turner 1973; Dister and Roberts 1997)Xyloredo sp. novbivalveXylophagous(Turner 1973; Dister and Roberts 1997)GASTROPODACaymanabyssia vandoveraelimpetGrazer (biofilm)(Marshall 1985)Amphiplica gordensislimpetGrazer (biofilm)(Mclean 1991)Neptunea amiantasnailPredator(Macdonald et al. 2010)Xyloskenea sp.(Marshall 1988)(McClain and Barry 2014)snailGrazer (biofilm)Provanna sp. 1(Johnson et al. 2010)(McClain and Barry 2014)snailGrazer (biofilm)Provanna cf. pacificasnailGrazer (biofilm)AmpHiPODAAMPHIPODABathyceradocus sp. nov.amphipodGrazer (biofilm)Grazer (biofilm)(Cunha et al. 2013)Paronesimoides voightaeamphipodGrazerGrazer (biofilm)(Cunha et al. 2013)Paronesimoides voightaeamphipodGrazer (biofilm)(Hoyoux et al. 2009)
Any for due of print of the order of the problem o
Caymanabyssia vandoveraelimpetGrazer (biofilm)(Marshall 1985)Amphiplica gordensislimpetGrazer (biofilm)(Mclean 1991)Neptunea amiantasnailPredator(Macdonald et al. 2010)Xyloskenea sp.(Marshall 1988)(McClain and Barry 2014)snailGrazer (biofilm)Provanna sp. 1(Johnson et al. 2010)(McClain and Barry 2014)snailGrazer (biofilm)Provanna cf. pacificasnailGrazer (biofilm)ARTHROPODAGrazer (biofilm)(Johnson et al. 2010)ARTHROPODAState (biofilm)(Johnson et al. 2010)AmphipodGrazer (biofilm)(Cunha et al. 2010)ArthropodaGrazer (biofilm)(Cunha et al. 2013)Paronesimoides voightaeamphipodGrazergalatheidGrazer (biofilm)(Larsen 2007)MunidopsisgalatheidGrazer (biofilm & wood)
Amphiplica gordensislimpetGrazer (biofilm)(Matshall 1965)Amphiplica gordensislimpetGrazer (biofilm)(Mclean 1991)Neptunea amiantasnailPredator(Macdonald et al. 2010)Xyloskenea sp.(Marshall 1988)(McClain and Barry 2014)snailGrazer (biofilm)Provanna sp. 1(Johnson et al. 2010)(McClain and Barry 2014)snailGrazer (biofilm)Provanna cf. pacificasnailGrazer (biofilm)AMPHIPODAARTHROPODAAMPHIPODAState (biofilm)Bathyceradocus sp. nov.amphipodGrazer (biofilm)(Cunha et al. 2013)Paronesimoides voightaeamphipodGrazer (biofilm)GrazerMunidopsisgalatheidGrazer (biofilm & wood)(Hoyoux et al. 2009)
Implified gordensisImperOrazer (brownin)(Mercula 1994)Neptunea amiantasnailPredator(Macdonald et al. 2010)Xyloskenea sp.(Marshall 1988)(McClain and Barry 2014)snailGrazer (biofilm)Provanna sp. 1(Johnson et al. 2010)(McClain and Barry 2014)snailGrazer (biofilm)Provanna cf. pacificasnailGrazer (biofilm)Provanna cf. pacificasnailGrazer (biofilm)ARTHROPODAARTHROPODABathyceradocus sp. nov.amphipodGrazerGrazer (biofilm)(Cunha et al. 2013)Paronesimoides voightaeamphipodGrazer (biofilm & wood)(Hoyoux et al. 2009)
Noplance and and a bindFreedeof(Macdonaid et al. 2010)Xyloskenea sp.(Marshall 1988)(McClain and Barry 2014)snailGrazer (biofilm)Provanna sp. 1(Johnson et al. 2010)(McClain and Barry 2014)snailGrazer (biofilm)Provanna cf. pacificasnailGrazer (biofilm)Provanna cf. pacificasnailGrazer (biofilm)ARTHROPODABathyceradocus sp. nov.amphipodGrazer (biofilm)Seba bathybiaamphipodGrazer (biofilm)Paronesimoides voightaeamphipodGrazerMunidopsisgalatheidGrazer (biofilm & wood)
Aytosterical sp.(Infustion 1900)(McClain and Barry 2014)snailGrazer (biofilm)Provanna sp. 1(Johnson et al. 2010)(McClain and Barry 2014)snailGrazer (biofilm)Provanna cf. pacificasnailGrazer (biofilm)ARTHROPODAGrazer (biofilm)(Johnson et al. 2010)AMPHIPODABathyceradocus sp. nov.amphipodGrazer (biofilm)(Cunha et al. 2013)Paronesimoides voightaeamphipodGrazer (biofilm)Grazer (Larsen 2007)MunidopsisgalatheidGrazer (biofilm & wood)(Hoyoux et al. 2009)
Provanna sp. 1(Johnson et al. 2010)(McClain and Barry 2014)snailGrazer (biofilm)Provanna cf. pacificasnailGrazer (biofilm)ARTHROPODAAMPHIPODABathyceradocus sp. nov.amphipodGrazer (biofilm)Seba bathybiaamphipodGrazer (biofilm)Paronesimoides voightaeamphipodGrazerMunidopsisgalatheidGrazer (biofilm & wood)
Informula sp. 1Grazer (biofilm)(McClain and Barry 2014)snailGrazer (biofilm)Provanna cf. pacificasnailGrazer (biofilm)ARTHROPODAGrazer (biofilm)(Johnson et al. 2010)ARTHROPODABathyceradocus sp. nov.amphipodBathyceradocus sp. nov.amphipodGrazer (biofilm)Seba bathybiaamphipodGrazer (biofilm)Paronesimoides voightaeamphipodGrazerMunidopsisgalatheidGrazer (biofilm & wood)
Provanna cf. pacificasnailGrazer (biofilm)ARTHROPODAAMPHIPODABathyceradocus sp. nov.amphipodGrazer (biofilm)(Turner 1973; Wolff 1979)Seba bathybiaamphipodGrazer (biofilm)(Cunha et al. 2013)Paronesimoides voightaeamphipodGrazer (biofilm)Grazer (Larsen 2007)MunidopsisgalatheidGrazer (biofilm & wood)(Hoyoux et al. 2009)
ARTHROPODAAMPHIPODABathyceradocus sp. nov.amphipodGrazer(Turner 1973; Wolff 1979)Seba bathybiaamphipodGrazer (biofilm)(Cunha et al. 2013)Paronesimoides voightaeamphipodGrazer (biofilm & wood)(Hoyoux et al. 2009)
AMPHIPODABathyceradocus sp. nov.amphipodGrazer(Turner 1973; Wolff 1979)Seba bathybiaamphipodGrazer (biofilm)(Cunha et al. 2013)Paronesimoides voightaeamphipodGrazer(Larsen 2007)MunidopsisgalatheidGrazer (biofilm & wood)(Hoyoux et al. 2009)
Bathyceradocus sp. nov.amphipodGrazer(Turner 1973; Wolff 1979)Seba bathybiaamphipodGrazer (biofilm)(Cunha et al. 2013)Paronesimoides voightaeamphipodGrazer(Larsen 2007)MunidopsisgalatheidGrazer (biofilm & wood)(Hoyoux et al. 2009)
Seba bathybiaamphipodGrazer (biofilm)(Cunha et al. 2013)Paronesimoides voightaeamphipodGrazer(Larsen 2007)MunidopsisgalatheidGrazer (biofilm & wood)(Hoyoux et al. 2009)
Paronesimoides voightaeamphipodGrazer(Larsen 2007)MunidopsisgalatheidGrazer (biofilm & wood) (Hoyoux et al. 2009)
MunidopsisgalatheidGrazer (biofilm & wood) (Hoyoux et al. 2009)
Guidelie Gluzer (blothin & wood) (hoyoux et ul. 2009)
Copened A copened Grazer (Hentner and Ivanenko 2002)
ISOPODA
Janthura sp isopod Detritivore (Cunha et al. 2013)
Disconectes sp isopod Detritivore (Cunha et al. 2013)
Munna sp isopod Detritivore (Cunha et al. 2013)
Acanthaspidia sp isopod Detritivore (Cunha et al. 2013)
Hebefustis sp isopod Detritivore (Calina et al. 2013)
Haploniscidae isopod Detritivore (Nürzberg et al 2011)
Limnoridae gen. nov. Boring isopod Xvlophagous (King et al. 2010)
TANAIDACEA
Protanais sp. nov.
(McClain and Barry 2014) tanaid Detritivore (Larsen 2006; Cunha et al. 2013)
Pvcnogonidae pvcnogonid Predator (Macdonald et al. 2010)
ECHINODERMATA
OPHIUROIDEA
Ophiambix cf. aculeatus brittle star Grazer (biofilm & wood) (Paterson and Baker 1988)
(Stohr and Segonzac 2006; Amon
Ophiacantha bathybia brittle star Predators/Scavengers 2014)
Amphiura carchara brittle star Detritivore (Macdonald et al. 2010)
CRINOIDEA
Antedonidae crinoid Filter feeder (Leonard 1989)
CNIDARIA
Anemone anemone Filter feeder (Macdonald et al. 2010)
ANNELIDA
POLYCHAETA
AmpharetidaewormDeposit feeder(Fauchald and Jumars 1979)
Capitellidae worm Deposit feeder (Fauchald and Jumars 1979)

Table 3: Taxa found on wood bundles with taxonomic group and feeding guild designations.

Dorvilleidae	worm	Multiple	(Fauchald and Jumars 1979)
Hesionidae	worm	Predators/Scavengers	(Fauchald and Jumars 1979)
Maldanidae	worm	Deposit feeder	(Fauchald and Jumars 1979)
Nephtidae	worm	Predators/Scavengers	(Fauchald and Jumars 1979)
Nereidae	worm	Predators/Scavengers	(Fauchald and Jumars 1979)
Opheliidae	worm	Deposit feeder	(Fauchald and Jumars 1979)
Phyllodocidae	worm	Predators/Scavengers	(Fauchald and Jumars 1979)
Polynoidae	worm	Predators/Scavengers	(Fauchald and Jumars 1979)
Raricirrus sp.	worm	Deposit feeders	(Fauchald and Jumars 1979)
Terebellidae	worm	Deposit feeders	(Fauchald and Jumars 1979)
OLIGOCHAETA			
Oligochaete	worm	Multiple	(Macdonald et al. 2010)
NEMERTEA		_	
Heteronemertea/Lineidae	worm	Predator	(Macdonald et al. 2010)

Table 4: Results obtained from multivariate analysis of variance with wood type as the source of variation in adonis.

Dataset	R ²	<i>p</i> -value
Full taxonomic resolution	0.6840	1.00E-04
Taxonomic groups	0.6831	1.00E-04
Feeding guilds	0.7295	1.00E-04

Taxon	Wood Type	<i>p</i> -value
Bivalves	Ginkgo	0.039
Amphipods	Palm	0.03
Galatheids	Palm	0.408
Copepods	Tree Poppy	0.473
Isopods	Palm	0.086
Tanaids	Palm	0.02
Pycnogonids	Ironwood	0.2
Brittle stars	Yew	0.02
Crinoids	Ironwood	0.444
Limpets	Ironwood	0.01
Snails	Spicebush	0.086
Anemone	Ironwood	0.699
Worms	Spicebush	0.551
Guild	Wood Type	<i>p</i> -value
Xylophagous	Ginkgo	0.04
Grazers	Ironwood	0.02
Predators/Scavengers	Palm	0.02
Detritivores	Spicebush	0.001
Filter feeders	Ironwood	0.444
Deposit feeders	Spicebush	0.068
Multiple modes	Ironwood	0.556

Table 5: Indicator species analysis results of best matches between taxa and guilds with wood type. Significant *p*-values indicated in bold-type.

Figure Legends:

- **Figure 1:** Plant phylogeny modified from (Palmer, Soltis et al. 2004). Green symbols indicate lineages that are represented in this study.
- Figure 2: Wood deployment and recovery. A. Arrangement of bundles in benthic elevator. B. Benthic elevator being deployed over side of ship. C. Wood bundle after 2 years on the bottom with galatheid crabs and crinoid attached to rope. D. Recovery of wood with ROV. E. *Torreya* bundle being processed on ship. F. Oak log with boring bivalves.
- **Figure 3:** Plot of summed abundances per wood type broken into taxonomic bin. All wood types had three replicates except for Tree Fern which had one sample.
- Figure 4: Species richness, Pielou's J (evenness), and Simpson's diversity index plotted for each wood type.
- Figure 5: CCA ordination plot of taxonomically binned community data. 95% confidence ellipses.
- Figure 6: CCA ordination of guild-binned community data. 95% confidence ellipses.



Figure 2:







Wood type



Figure 5:



CCA1



