UNIVERSITY OF CALIFORNIA SAN DIEGO

Four New Species of *Osedax* Bone-Worms from New Zealand and the Gulf of Mexico and Range Expansions for Pacific *Osedax* Species

A Thesis submitted in partial satisfaction of the requirements
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in

Marine Biology

by

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The Thesis of Gabriella Berman is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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DEDICATION

This thesis is dedicated to my parents for giving me all of their love and support my whole life.

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ABSTRACT OF THE THESIS

Range Expansions for Five Species of *Osedax* bone-worms and Four New *Osedax* (*Siboglinidae, Annelida*) Species from New Zealand and the Gulf of Mexico

by

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Master of Science in Marine Biology

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Osedax is a genus of siboglinid annelids that live on and consume bones in the ocean. Aided by symbiotic bacteria *Osedax* dissolve the bone matrix for habitat and consume the nutrients inside. Currently there are 27 described and 10 undescribed species of *Osedax* from 16 localities globally. Using molecular and morphological data we described four new species bringing the total number of *Osedax* species to 31 and localities to 19. Two species are the first records of *Osedax* from New Zealand where extensive species diversity is suspected. Two species are from the Gulf

of Mexico, one of which is the first species named from a reptile fall. We also expanded the ranges of five described species to Oregon, San Diego, and Costa Rica and conducted population structure analysis on nine species using the *COI* gene. We found shared haplotypes and evidence of genetic connectivity across broad ranges such as between California and Japan and along the Pacific coast of North and Central America.

CHAPTER 1:

INTRODUCTION

Osedax is a genus of bone-eating marine annelids belonging to *Siboglinidae* (Rouse, Goffredi, and Vrijenhoek 2004) along with other taxa such as *Vestimentifera*. *Osedax* is notable for exploiting the bones of sunken carcasses in the ocean with the aid of symbiotic bacteria (Goffredi et al. 2005; Goffredi, Johnson, and Vrijenhoek 2007). *Osedax* secretes acid that dissolves and opens up the bone matrix so that *Osedax* can root themselves to the bone and consume the nutrients inside (Tresguerres, Katz, and Rouse 2013). *Osedax* was first described in 2004 from a whalefall in Monterey Bay (Rouse et al. 2004) and since then has been found worldwide, with and 27 named species and 10 yet to be named species from 19 localities (Amon et al. 2014; Eilertsen, Dahlgren, and Rapp 2020; Fujikura, Fujiwara, and Kawato 2006; Fujiwara et al. 2019; Glover et al. 2005; Rouse et al. 2008, 2015, 2018; Schander, Rapp, and Dahlgren 2010; Taboada et al. 2015; Zhou et al. 2020). In this study we describe four new species, one of which was previously reported (McClain et al. 2019), bringing the total number of named species to 31, and expanding the range for *Osedax* to 19 localities. Two of new species were collected from an alligator carcass and cow bones, respectively, that were experimentally sunken in the Gulf of Mexico (McClain et al. 2019). The other two new species in the study were collected from a whale skull caught in a trawl off New Zealand. The evidence to support the establishment of the four species is primarily molecular since the available specimens were in poor morphological condition. Two mitochondrial genes (cytochrome-*c*-oxidase subunit I and 16S rRNA) and three nuclear genes (18S rRNA and 28S rRNA and Histone-H3) were sequenced for higher genomic resolution and used to place the species in the *Osedax* phylogeny. Due to the poor morphological condition we are following a

precedent set by descriptions of *Osedax crouchi*, *Osedax nordenskjoeldi*, and *Osedax rogersi* which used samples of the palps and relied heavily on molecular data (Amon et al. 2014).

MATERIALS AND METHODS

Sample collection

Samples were collected from an *Alligator mississippiensis* carcass and cow bones that were experimentally deployed for 51 days at ~2000m in the Gulf of Mexico in the Mississippi River Delta off of Louisiana by colleagues at the Louisiana Universities Marine Consortium (LUMCON) (McClain et al. 2019). Bones were recovered, fixed in ethanol, and sent to Scripps Institution of Oceanography where *Osedax* were dissected out and photographed. Samples were also collected from a whale skull recovered from 390m in a scientific trawl deployed on the Pukaki Rise in New Zealand by colleagues at the National Institute of Water and Atmospheric Research (NIWA) and then frozen. *Osedax* were scraped off the skull, fixed in ethanol, and sent to Scripps Institution of Oceanography. Either Leica S8Apo or Leica MZ12.5 stereomicroscopes were used to dissect specimens and photograph them via a Canon Rebel T7i camera. *Osedax* specimens and types have been lodged at Benthic Invertebrate Collection at Scripps Institution of Oceanography, La Jolla, California USA (SIO-BIC).

DNA extraction, amplification, and Sanger sequencing

Either whole *Osedax* specimens or pieces of root tissue had DNA extracted using either Zymo Research DNA-Tissue Miniprep Kit or Zymo Research Quick-DNA Microprep Plus Kits (Irvine, California, USA), following the protocols supplied by the manufacturer. Extractions were used to sequence mitochondrial (cytochrome c oxidase subunit I (*COI*) and 16S rRNA (*16S*)) and

nuclear (18S rRNA (*18S*), 28S rRNA (*28S*), and Histone H3 (*H3*)) genes. All specimens were sequenced for *COI* with a single representative of each species sequenced for the other genes. Amplification was carried out using a PCR mixture of 12.5 µl Apex 2.0x Taq Red DNA Polymerase Master Mix (Genesee Scientific, San Diego, California, USA) or 12.5 µl Conquest PCR 2.0x Master Mix 1 (Lamda Biotech, Ballwin, Missouri, USA), 1 µl each of the appropriate forward and reverse primers (10 µM), 8.5 µl of ddH_2O, and 2 µl of eluted DNA. DNA sequencing was completed with the following PCR primers and temperature profiles, performed in a thermal cycler (Eppendorf, Hamburg, Germany) (Table 1.1). *COI* was amplified using the primer set polyLCO/polyHCO (Carr et al. 2011) with the reaction protocol 95°C/180s – (95°C/40s – 42°C/45s – 72°C/50s) * 40 cycles – 72°C/300s; COIf/COIr (Nelson and Fisher 2000) with the reaction protocol 95°C/300s – (94°C/60s – 55°C/60s – 72°C/120s) * 35 cycles – 72°C/420s; or LCO1490/HCO2198 (Folmer et al. 1994) with the reaction protocol 94°C/180s – (94°C/30s – 47°C/45s – 72°C/60s) * 5 cycles – (94°C/30s – 52°C/45s – 72°C/60s) * 30 cycles – 72°C/300s. *16S* was amplified using the primer set 16SarL/16SbrH (Palumbi 1996) with the reaction protocol 95°C/180s – (95°C/40s – 50°C/40s – 72°C/50s) * 35 cycles – 72°C/300s. *18S* was amplified with the following primer sets and reaction protocols. 18S-1F/18S-5R (Giribet et al. 1996) and 18S-a2.0 (Whiting et al. 1997)/18S-9R (Giribet et al. 1996): 95°C/180s – (95°C/30s – 50°C/30s – 72°C/90s) * 40 cycles – 72°C/480s. 18S-3F (Giribet et al. 1996)/18S-bi (Whiting et al. 1997): 95°C/180s – (95°C/30s – 52°C/30s – 72°C/90s) * 40 cycles – 72°C/480s. *28S* was amplified using the primer set D1F/D3R (Brown et al. 1999) with the reaction protocol 94°C/180s – (94°C/60s – 55°C/30s – 72°C/110s) * 35 cycles – 72°C/240s. *H3* was amplified using the primer set H3F/H3R (Colgan et al. 1998) with the reaction protocol 95°C/180s – (95°C/30s – 53°C/45s – 72°C/45s) * 40 cycles – 72°C/300s. Final PCR products were purified with the ExoSAP-IT protocol (USB

Affymetrix, Ohio, USA), and Sanger sequencing was performed by Eurofins Genomics (Louisville, Kentucky, USA). Sequences were assembled using Geneious software v11.1 (©Biomatters Ltd.; http://www.geneious.com/, New Zealand) and the new DNA sequences obtained have been deposited in GenBank (Bethesda, Maryland, USA) (Table1.2).

Phylogenetic analyses

A representative terminal from each of previously published *Osedax* species (named or unnamed) was included in the analysis as well as one from each of the four new species. Not all species have the five targeted genes sequences available, some only have *COI* available or and others only have one to two other genes (see Table 1.1) Outgroups for the analysis were representative siboglinids from *Vestimentifera* and *Sclerolinum* that form the sister group to *Osedax* (Li et al. 2017). The individual genes were aligned in Mesquite (v3.61) (Maddison and Maddison 2019) using MAFFT (Katoh and Standley 2013; Rozewicki et al. 2019) with default settings for *COI* and *H3* and with the G-INS-I option for the RNA genes. The 5 gene partitions were concatenated using SequenceMatrix (Vaidya, Lohman, and Meier 2011). A maximum likelihood analysis was conducted with RAxML-NG (Kozlov et al. 2019) using RAxML GUI v.2.0 (Edler et al. 2021). Optimal models were chosen for each partition using ModelTest-NG v.0.1.7 (Darriba et al. 2020) as follows (based on AICc): COI= GTR+I+G4, 16S = TIM2+I+G4, 18S= GTR+I+G4, 28S= TIM3+I+G4, H3= TVMef+I+G4. Node support was assessed via thorough bootstrapping (with 1,000 pseudo replicates). Interspecific and intraspecific pairwise distances were calculated in PAUP* (v4.0a168) (Swofford 2002) using untrimmed alignments. TCS haplotype networks (Clement, Posada, and Crandall 2000) were constructed using PopArt (Leigh and Bryant 2015) using trimmed alignments.

RESULTS

Osedax bozoi n. sp.

Figure 1.2A, B, C

Material examined. Holotype: SIO-BIC A13918 (*COI*, GenBank Accession ON357631, Paratypes: A13920, A13922 (GenBank# ON357630 (*COI*), ON357686 (*COI*)). Photographs of paratypes are missing. All specimens fixed and preserved in 95% ethanol.

Diagnosis and description. Apinnulate palps are approximately 1.5mm long and less than 0.5mm wide (1.2A, B). Palps are contained inside of a tube (1.2A, B). Ends of palps are curled inside of tube (1.2A, B). Obvious demarcation between palps and trunk. Trunk is approximately 1mm long and 0.5mm wide (1.2A, B). Specimen is white (1.2A, B). Roots are broken (1.2A, B). Two root extensions are visible on either side of the trunk (1.2A, B). Oviduct wraps around the pinnules and stops at root extension (1.2A). Oviduct is visible in the root (1.2B). No males observed.

Distribution. The holotype and paratypes were recovered from cow bones (fig 1.2C) deployed at 1996m in the Mississippi River Delta region of the Gulf of Mexico south of New Orleans, Louisiana. The species has not yet been found anywhere else.

Etymology. *Osedax bozoi* n. sp. is named for Ms. Berman's late cat, Bozo (named for Bozo the Clown).

Remarks. *Osedax bozoi* belongs to clade II, an apinnulate clade. Specimens were not observed alive. Specimen A10278 was sequenced for *16S*, *18S*, *28S*, and *H3* as well as *COI*, but the specimens was completely used for the DNA extraction so A13918, which had a close *COI* sequence, was nominated as the holotype. Specimens A10277 and A10276 were also destroyed for sequencing. *Osedax bozoi* n. sp. had a 2.1% maximum pairwise distance among the six

available sequences (Table 1.2). The haplotype network for *Osedax bozoi* n. sp. had four unique haplotypes (Fig. 1.4). One is shared by three of the six sequences. There are three nucleotide substitutions between the most divergent haplotypes. *Osedax bozoi* n. sp. was recovered as the sister group to a clade within clade II that comprised *O. docricketts*, *O. westernflyer and O. knutei* and *O*. 'BioSuOr-1' (Fig. 1.1), though this was poorly supported. The first three of these taxa are all from the Pacific Ocean while O. 'BioSuOr-1' is from the western Atlantic. In terms of distance the nearest species was *Osedax docricketts*, an apinnulate species known from 1820m in Monterey Bay (California, USA) and in Sagami Bay (Japan), collected from cow and whale bones (Rouse et al. 2018). *Osedax bozoi* and *Osedax docricketts* share some morphological characteristics; both lack pigmentation on the trunk and palps and pinnules, both have a tube containing the palps. However, where *O. bozoi* has a distinct demarcation between the palps and the trunk, *O. docricketts* does not, and the ovisac and oviduct are distinctive on *O. bozoi. Osedax docricketts* is suspected to be a cryptic species complex (Rouse et al. 2018), but *O. bozoi* is most closely related to EU267676, an individual from Monterey Bay that is closely related to the *O. docricketts* holotype. The minimum interspecific distance between the two species is 13.7% (Table 1.3).

Osedax craigmcclaini n. sp.

Figure 1.2D, E, F

Material examined. Holotype: A13910 (GenBank# ON211944 *(COI*)). Specimen fixed in 95% ethanol.

Diagnosis and description. Palps are pinnulated, white, and less than 1mm long and approximately 0.33mm wide (fig. 1.2D). No other parts of the body were observed. No males observed.

Distribution. The holotype was recovered from *A. mississippiensis* bones (fig. 1.2F) deployed at 2034m in the Mississippi River Delta region of the Gulf of Mexico south of New Orleans, Louisiana. The species has not yet been found anywhere else.

Etymology. *Osedax craigmcclaini* n. sp. is named for Dr. Craig McClain, an esteemed deep sea biologist, who led the experimental alligator fall project (McClain et al. 2019) and provided the *Osedax* specimens for this study.

Remarks. *Osedax craigmcclaini* belongs to clade V, a pinnulate clade. Evidence for this species was originally published in McClain (2019) with *COI* only (GenBank Accession number MN258704), but the specimen, A10731, was destroyed for the DNA extraction. *16S* (ON217799), *18S* (ON220153**),** *28S* (ON226742), and *H3* (ON254807) were sequenced from the A10731 DNA extraction. The specimen A13910 has been designated here as the holotype based on its *COI* sequence (ON211944) closely matching MN258704 (1.2% uncorrected distance) (Table 1.2). The nearest sister species is *Osedax fenrisi*, a pinnulate species collected from 2341m on the Arctic mid-Ocean Ridge (Eilertsen et al. 2020). The minimum interspecific distance between the two species was 14.6% (Table 1.3). *Osedax craigmcclaini* n. sp. showed two unique haplotypes with seven nucleotide substitutions between them (fig. 1.4). Specimens were not observed alive, however in situ images of the alligator *O. craigmcclaini* was collected from show red *Osedax* coating the jawbone and spine (fig. 1.2E, F), suggesting that living *O. craigmcclaini* may have red, hemoglobin-containing palps. As is likely also the case for *O. bozoi* n. sp., ethanol preservation may have caused the palps to turn white.

Osedax estcourti n. sp.

Figure 1.3A, C

Material examined. Holotype: A13925 (GenBank# ON211943 (*COI*), ON217536 (*16S*), ON220129 (*18S*), ON220739 (*28S*), ON254809 (*H3*)). Specimen fixed in 95% ethanol.

Diagnosis and description. Recovered material consisted of desiccated roots and palps (fig. 1.3A). Trunk not visible (fig. 1.3A). Apinnulate palps are brown, approximately 3mm long and 1mm wide (fig. 1.3A). Three palps contained inside translucent tube (fig. 1.3A). Roots approximately 3mm long, 1.5mm wide, white colored (fig. 1.3A). No males observed.

Distribution. The species was recovered from 390-393m on the Pukaki Rise off New Zealand.

Etymology. *Osedax estcourti* n. sp. is named in remembrance of Ivan Neil Estcourt (1938-1981), benthic ecologist and the first polychaetologist researcher at the former New Zealand Oceanographic Institute (now NIWA).

Remarks. *Osedax estcourti* belongs to clade II, an apinnulate clade. All other vouchers were destroyed for sequencing (ON211941, ON211942). The species had a 1.5% maximum intraspecific pairwise distance between the three available sequences (Table 1.2). *Osedax estcourti* is a sister species to *Osedax ventana*, an apinnulate species known from 2898m in Monterey Bay (California, USA). The minimum interspecific distance between *O. estcourti* and *O. ventana* is 14.6% (Table 1.3). The haplotype network for *O. estcourti* showed three distinct haplotypes, one for each sequence with a maximum of 10 nucleotide substitutions (fig. 1.5). Specimens were not observed alive, however images of the whale skull at the time it was collected show red *Osedax* coating the surface (fig. 1.3C), suggesting that living *O. estcourti* may have red, hemoglobin-containing palps

Osedax traceyae n. sp.

Figure 1.3B, C

Material examined. Holotype: A13924 (GenBank# ON211990 (*COI*), ON212680 (*16S*) ON210988 (*18S*), ON220740 (*28S*), ON254808 (*H3*)). Paratypes: A13926, A13927, A13928, A13929 (GenBank# ON211991 (*COI*), ON211992 (*COI*), ON211987 (*COI*) ON211988 (*COI*)). All specimens fixed in 95% ethanol.

Diagnosis and description. Recovered physical material consisted only of desiccated palps in ethanol (fig. 1.3B). Apinnulate palps are brown, approximately 4mm in length and 1mm wide (fig. 1.3B). Palps contained inside translucent membrane (fig. 1.3B). Palp tips curled up inside membrane (fig. 1.3B). No males observed.

Paratypes. Females fixed in ethanol and stored at SIO-BIC. A13926: Palps, trunk, and roots. Palps are brown, approximately 3.5mm long and approximately 0.5mm wide. Trunk is approximately 1mm long and approximately 0.5mm wide. Roots are approximately 2mm long and approximately 1mm wide. Two root projections on either side. Roots and trunk are white. A13927: Trunk and palps extend out of bone fragment. Palps are orange. Trunk is approximately 1mm long and approximately 0.33mm wide. Palps are approximately 2mm long and approximately 0.33mm wide. A13928: Palps and trunk. Visible demarcation between palps and trunk. Palps are brown. Trunk is white. Palps are approximately 2mm long and 0.5mm wide. Trunk is approximately 2mm long and 1mm wide. A13929: Palps and trunk. Palps are approximately 2mm long and 0.5mm wide. Trunk is approximately 1mm long and 0.5mm wide. Palps and trunk are brown and white.

Distribution. The species was recovered from 390-393m on the Pukaki Rise off New Zealand.

Etymology. *Osedax traceyae* n. sp. is named in appreciation of Dianne (Di) M. Tracey, outstanding deep-sea fisheries and coral researcher, of the National Institute of Water and

Atmospheric Research (NIWA), Wellington, New Zealand, whose shipboard initiatives secured the whale skull and worms for this study.

Remarks. *Osedax traceyae* belongs to clade II, an apinnulate clade. The species had a 0.5% maximum intraspecific pairwise distance among the eleven sequences analyzed (Table 1.2). The haplotype network for this species revealed two haplotypes, one of which was shared by ten of the eleven sequences (fig. 1.5). *Osedax traceyae* is the sister group to the rest of Clade II but the support value for this grouping is very low. *Osedax bozoi* n. sp. is sister species to *O. traceyae* and the minimum interspecific distance between the two species was 15.2% (Table 1.3). Specimens were not observed alive, however images of the whale skull at the time it was collected show red *Osedax* coating the surface (fig. 1.3C), suggesting that living *O. traceyae* may have red, hemoglobin-containing palps.

DISCUSSION

The addition of *O. bozoi* n. sp., *O. craigmcclaini* n. sp., *O. estcourti* n. sp., and *O. traceyae* species expands *Osedax's* range, these are the first species from New Zealand and the Gulf of Mexico, but low support values on the phylogenetic tree prevent rigorous evolutionary and biogeographical conclusions. Although the genus itself is well supported, many of the more inclusive nodes on the phylogenetic tree, namely clades II, III, and VI, and relationships within clades are also not well supported. The phylogenetic relationships within *Osedax* could be resolved with the addition of more loci. Including more genes in the phylogenetic analysis, such as *COI*, *16S, 18S, 28S,* and *H3* as was done for *O. bozoi* n. sp., *O. craigmcclaini* n. sp., *O. estcourti* n. sp., and *O. traceyae* in this study could also increase support values and resolve some of the current phylogenetic uncertainty. *Osedax antarcticus*, *O. crouchi*, *Osedax japonicus*, all *Osedax*

'BioSuOr' OTUs, and OTUs *Osedax* 'sagami-4' and *Osedax* 'sagami-5' were sequenced at three or fewer loci and have low support values on the tree (Table 1, fig. 1.1). Including the missing genes could clarify these species' places on the tree. Whole mitochondria or whole genome sequencing are techniques that could be applied to all *Osedax* species and would provide higher genetic coverage for biogeographic and evolutionary history interpretations.

Large depth ranges have been hypothesized to contribute in part to high species richness in Monterey Bay Canyon (Rouse et al. 2015, 2018; Vrijenhoek, Johnson, and Rouse 2009). Only five species of *Osedax* are known from above 400m, *Osedax deceptionensis, Osedax japonicus, Osedax* 'mediterranea', *Osedax mucofloris,* and *Osedax packardorum* (Rouse et al. 2018). The other 35 species and OTUs are known from deeper depths with the greatest proportion of species known between 1000m and 3000m (Rouse et al. 2018). *Osedax estcourti* n. sp. and *Osedax traceyae* n. sp. were collected at 390m from the Pukaki Rise feature on the Chatham Rise, a bathymetric feature that descends to 3000m. The depth range of more than 2500m on the Chatham Rise could in part drive high species richness in the region and the discovery of the species at such an unusually shallow depth suggests that there could be high species richness at deeper depths. The Mississippi River Delta has a similar depth range however, continuous sediment deposition from the Mississippi River means that carcasses are likely rapidly buried leaving little time for *Osedax* to colonize the bones. Species diversity could be low in this region, especially at shallower depths where sediment deposition may be more frequent and *Osedax* are less common (Rouse et al. 2018; Taboada et al. 2015). The alligator carcass was colonized by *Osedax* within 51 days, however the fate of the carcass was not followed beyond that period so it is not known whether it persisted or was buried by sediment (McClain et al. 2019) but sediment flux from the Mississippi River likely influences how long carcasses and bones persist on the seafloor. Sedimentation can

interrupt the degradation process and rapidly bury a carcass (Lundsten et al. 2010), so large depth ranges likely only contribute to species richness when sediment flux is not high.

Osedax is able to exploit diverse bone types and substrate versatility has likely helped the clade to spread around the globe (Danise and Higgs 2015; Kiel et al. 2010; Kiel, Kahl, and Goedert 2011, 2013; McClain et al. 2019; Rouse et al. 2015, 2018). Fossil evidence demonstrates that ancient *Osedax* were able to exploit fish, bird, reptile, and whale bones and even whale teeth (Danise and Higgs 2015; Kiel et al. 2010, 2011, 2013). Modern *Osedax* have been found on whale, dolphin, fish, pig, cow, turtle, turkey, fur seal, elephant seal, and alligator bones (McClain et al. 2019; Rouse et al. 2015, 2018). The discovery of these four species on three different bone types highlights *Osedax's* capacity to utilize a variety of bone substrate. Vertebrate bones across the North Pacific and around South America have been hypothesized to connect broadly distributed species (Rouse et al. 2018; Shimabukuro and Sumida 2019) but likely also provide substrate for undescribed species of *Osedax*.

With 31 species described in the 18 years since the genus was named (Amon et al. 2014; Eilertsen et al. 2020; Fujiwara et al. 2019; Glover et al. 2005; Rouse et al. 2008, 2015, 2018, 2004; Taboada et al. 2015; Vrijenhoek et al. 2009), *Osedax* is a rapidly growing genus and may contain high species richness. However, if support values in the phylogenetic tree remain low then it will not be possible to draw robust conclusions about *Osedax's* biogeography and evolutionary history. Phylogenetic resolution can be increased through more rigorous genetic sampling of known species and rigorous genetic sampling should be conducted with future species as well. Understanding *Osedax's* biogeography and evolutionary history should be as much of a priority as describing new species.

Table 1.1: Genes, primers, and reaction protocols used in this study.

Gene	Primer set	Authority	Reaction protocol
COI	polyLCO/polyHCO	Carr *et al.* 2011	95°C/180s – (95°C/40s – 42°C/45s – 72°C/50s) * 40 cycles – 72°C/300s
	COIf/COIr	Nelson & Fisher 2000	95°C/300s – (94°C/60s – 55°C/60s – 72°C/120s) * 35 cycles – 72°C/420s
	LCO1490/HCO2198	Folmer *et al.* 1994	94°C/180s – (94°C/30s – 47°C/45s – 72°C/60s) * 5 cycles – (94°C/30s – 52°C/45s – 72°C/60s) * 30 cycles – 72°C/300s
16S	16SarL/16SbrH	Palumbi 1996	95°C/180s – (95°C/40s – 50°C/40s – 72°C/50s) * 35 cycles – 72°C/300s
18S	18S-1F/18S-5R	Giribet *et al.* 1996	95°C/180s – (95°C/30s – 50°C/30s – 72°C/90s) * 40 cycles – 72°C/480s
	18S-a2.0/18S-9R	Giribet *et al.* 1996/ Whiting *et al.* 1997	95°C/180s – (95°C/30s – 50°C/30s – 72°C/90s) * 40 cycles – 72°C/480s
	18S-3F/18S-bi	Giribet *et al.* 1996/ Whiting *et al.* 1997	95°C/180s – (95°C/30s – 52°C/30s – 72°C/90s) * 40 cycles – 72°C/480s
28S	D1F/D3R	Brown *et al.* 1999	94°C/180s – (94°C/60s – 55°C/30s – 72°C/110s) * 35 cycles – 72°C/240s
H3	H3F/H3R	Colgan *et al.* 1998	95°C/180s – (95°C/30s – 53°C/45s – 72°C/45s) * 40 cycles – 72°C/300s

Table 1.2: Species and GenBank numbers for sequences in this study. New sequences are **bold**. Holotypes = *.

Species	Authority	*COI*	*16S*	*18S*	*28S*	*H3*
Lamellibrachia columna	Webb 1969	DQ996645	FJ347646	FJ347679	MG264417	FJ347696
Riftia pachyptila	Jones 1985	KP119562	KP119573	KP119591	KP119582	KP119555
Sclerolinum brattstromi	Webb 1964	FJ347644	FJ347644	FJ347680	FJ347677	FJ347697
Osedax antarcticus	(Glover *et al.* 2013)	KF444422	KF444418	KF444420	-	-
Osedax 'BioSuOr-1'	(Shimabukuro *et al.* 2019)	MH616036	-	-	-	-
Osedax 'BioSuOr-2'	(Shimabukuro *et al.* 2019)	MH616081	-	-	-	-
Osedax 'BioSuOr-3'	(Shimabukuro *et al.* 2019)	MH616075	-	-	-	-
Osedax 'BioSuOr-4'	(Shimabukuro *et al.* 2019)	MH616012	-	-	-	-
Osedax bozoi n. sp.	This study	**ON357627**	**ON261606**	**ON261611**	**ON261610**	**ON254806**
Osedax braziliensis	(Fujiwara *et al.*, 2019)	LC381421	-	LC381424	-	-
Osedax bryani	SIO-BIC A4619	KP119563	KP119574	KP119597	KP119584	KP119561
Osedax craigmcclaini n. sp.	This study	MN258704	**ON217799**	**ON220153**	**ON226742**	**ON254807**
Osedax crouchi	(Amon *et al.* 2014)	KJ598038	KJ598032	KJ598035	-	-

Table 1.2: Species and GenBank numbers for sequences in this study. New sequences are **bold**. Holotypes = *. Continued.

Osedax deceptionensis	(Taboada *et al.* 2015)	KF444428	KF444419	KF444421	MG264418	KT860546
Osedax docricketts	(Rouse *et al*., 2018)	FJ347626	FJ347650	FJ347688	FJ347666	FJ347710
Osedax estcourti n. sp.	This study	**ON211943***	**ON217536***	**ON220129***	**ON220739***	**ON254809***
Osedax fenrisi	(Eilersten *et al.* 2020)	MT556178	-	MT556473	-	-
Osedax frankpressi	(Rouse *et al.* 2004)	FJ347607	FJ347658	FJ347682	FJ347674	FJ347705
Osedax jabba	(Rouse *et al*., 2018)	FJ347638*	FJ347647	FJ347693	FJ347676	FJ347703
Osedax japonicus	(Fujikura *et al.* 2006)	FM998111	-	FM995535	-	-
Osedax knutei	(Rouse *et al*., 2018)	FJ347635*	FJ347648	FJ347692	FJ347664	FJ347700
Osedax lehmani	(Rouse *et al*., 2018)	DQ996634	FJ347660	FJ347689	FJ347672	FJ347706
Osedax lonnyi	(Rouse *et al*., 2018)	FJ347643*	FJ347651	FJ347695	FJ347663	FJ347699
Osedax 'MB16'	(Salathé & Vrijenhoek 2012)	JX280613	KP119581	KP119592	KP119588	KP119560
Osedax 'mediterranea'	(Taboada *et al.* 2015)	KT860548	KT860551	KT860550	KT860549	KT860547
Osedax mucofloris	(Glover *et al.* 2005)	AY827562	-	-	AY941263	-
Osedax nordenskjoeldi	(Amon *et al.* 2014)	KJ598039	KJ598033	KJ598036	-	-
Osedax packardorum	(Rouse *et al*., 2018)	FJ347629	FJ347661	FJ347690	FJ347673	FJ347707
Osedax priapus	(Rouse et al. 2015)	KP119564	KP119575	KP119594	KP119585	KP119556
Osedax randyi	(Rouse *et al*., 2018)	FJ347615*	FJ347659	FJ347684	FJ347675	FJ347712
Osedax rogersi	(Amon *et al.* 2014)	KJ598034	KJ598037	KJ598040	-	-
Osedax roseus	(Rouse *et al*., 2008)	FJ347609	FJ347657	FJ347683	FJ347670	FJ347709
Osedax rubiplumus	(Rouse *et al.* 2004)	EU852488	FJ347656	FJ347681	FJ347671	FJ347704
Osedax ryderi	(Rouse *et al*., 2018)	KP119563*	KP119574	KP119597	KP119584	KP119561
Osedax 'sagami-3'	(Pradillon *et al.* unpublished)	FM998081	-	FM995537	-	-
Osedax 'sagami-4'	(Pradillon *et al.* unpublished)	FM998082	-	FM995541	-	-
Osedax 'sagami-5'	(Pradillon *et al.* unpublished)	FM998083	-	FM995539	-	-
Osedax sigridae	(Rouse *et al*., 2018)	FJ347642	FJ347655	FJ347694	FJ347669	FJ347711

Table 1.2: Species and GenBank numbers for sequences in this study. New sequences are **bold**. Holotypes = *. Continued.

Osedax talkovici	(Rouse *et al*., 2018)	FJ347621	FJ347654	FJ347685	FJ347668	FJ347698
Osedax tiburon	(Rouse *et al*., 2018)	FJ347624	FJ347653	FJ347687	FJ347662	FJ347702
Osedax traceyae n. sp.	This study	**ON211990***	**ON212680***	**ON210988***	**ON220740***	**ON254808***
Osedax ventana	(Rouse *et al*., 2018)	EU236218*	FJ347652*	FJ347686*	FJ347665*	FJ347701*
Osedax westernflyer	(Rouse *et al*., 2018)	FJ347631	FJ347649	FJ347691	FJ347667	FJ347708

Table 1.3: Uncorrected intraspecific distances.

Species	Intraspecific Distance
Osedax bozoi n. sp.	0.02194
Osedax craigmcclaini n. sp.	0.01222
Osedax estcourti n. sp.	0.01459
Osedax traceyae n. sp.	0.00506

Table 1.4: Uncorrected interspecific distances between new species and their nearest sister species.

Species	Neighboring species	Interspecific Distance
Osedax bozoi n. sp.	*O. docricketts*	0.13682
Osedax craigmcclaini n. sp.	*O. fenrisi*	0.14579
Osedax estcourti n. sp.	*O. ventana*	0.13641
Osedax traceyae n. sp.	*O. bozoi* n. sp.	0.15198

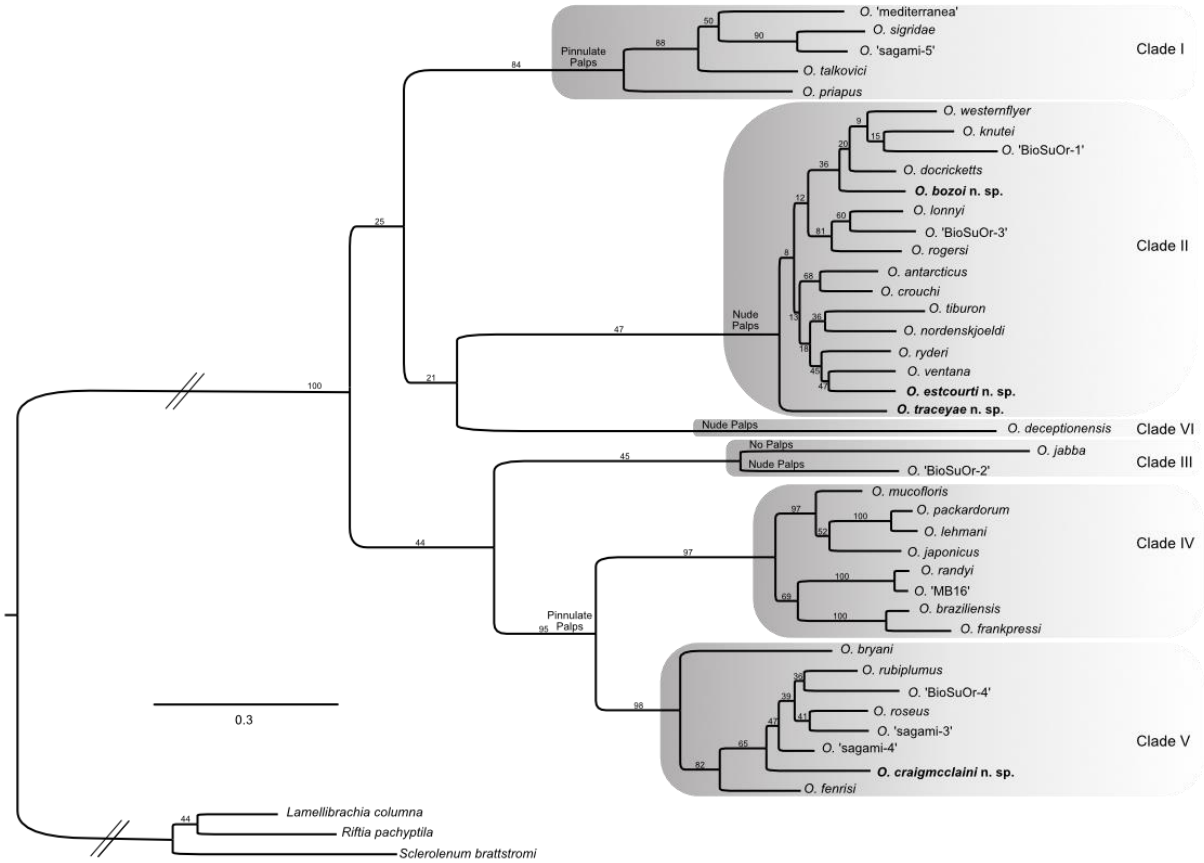

Figure 1.1: Maximum likelihood *Osedax* phylogenetic tree with *Monilifera* as the outgroup. The tree was made using the gene segments listed in table 1.2. Bootstrap support values are on each node. Presence or absence of pinnules is noted for each clade.

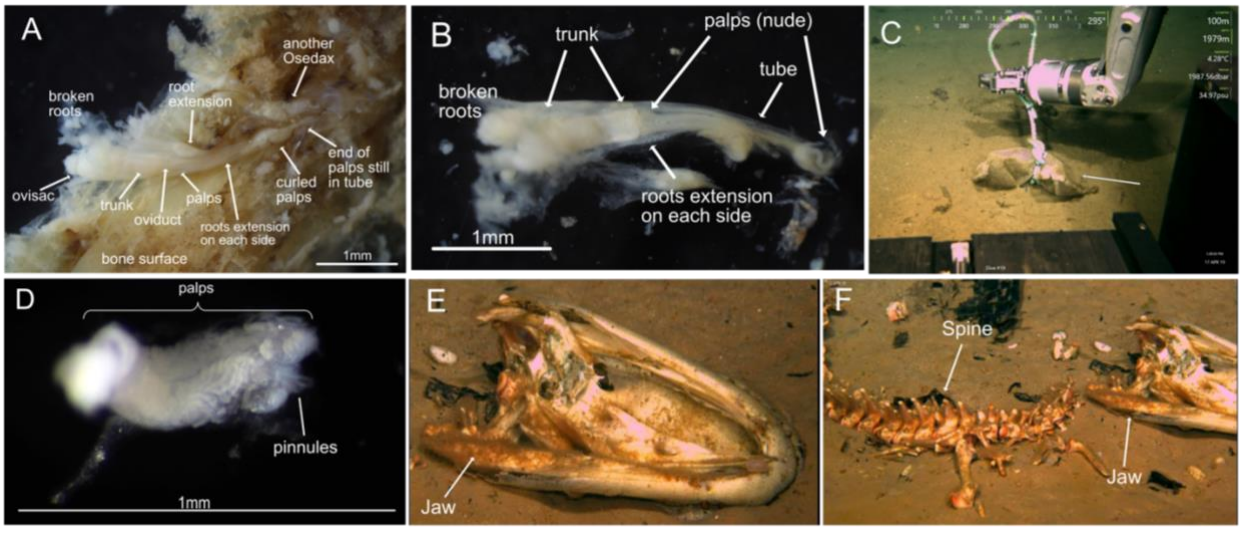

Figure 1.2: A. Osedax *bozoi* n. sp. dorsal view. B. Osedax *bozoi* n. sp. ventral view. C. Cow bones from which *Osedax bozoi* were collected, wrapped in mesh, and being recovered by ROV. D. Osedax *craigmcclaini* n. sp. palps E. *Alligator mississippiensis* skull with Osedax on jaw. F.

Spine and skull of *Alligator mississippiensis* with Osedax on vertebrae and jaw.

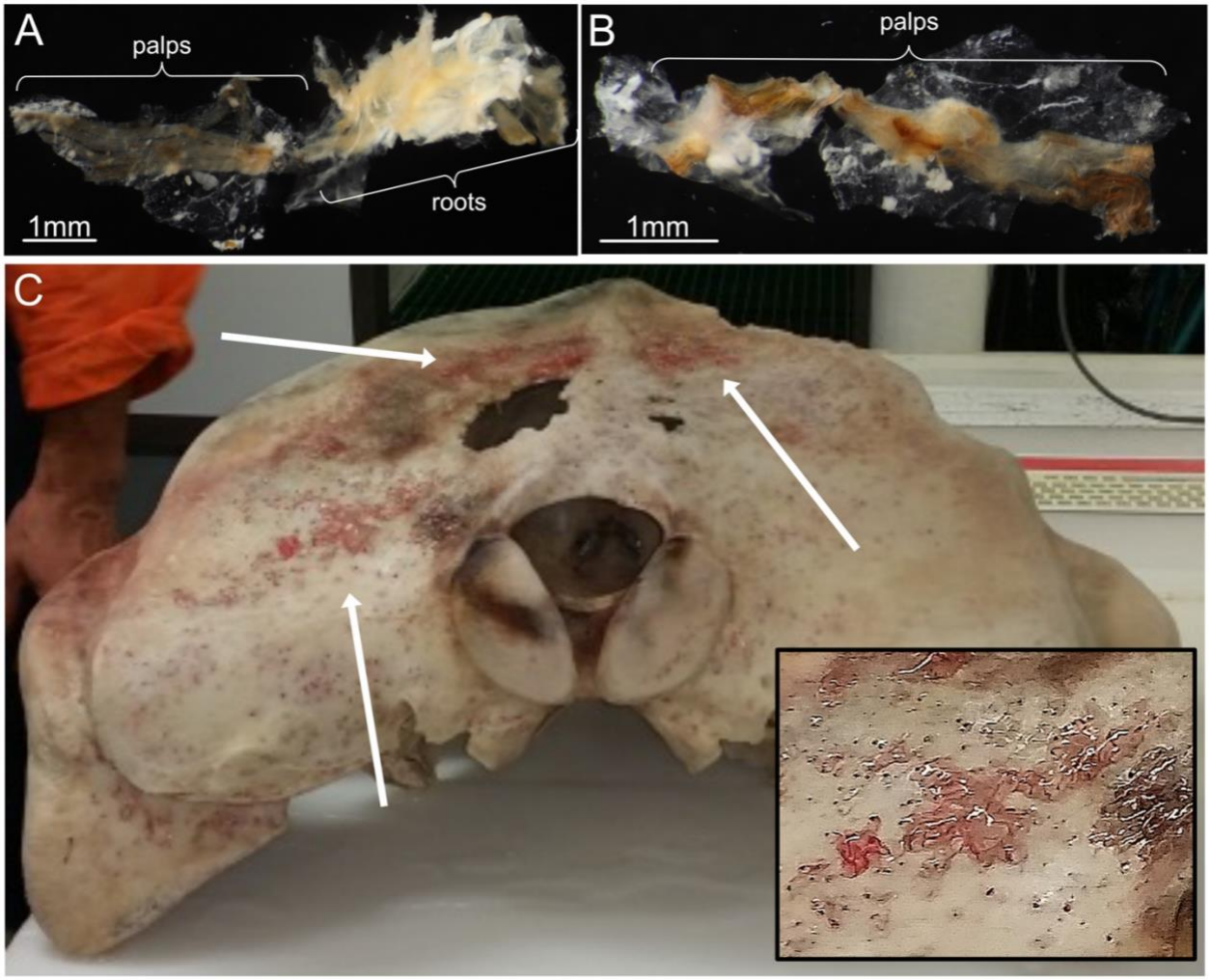

Figure 1.3: A. *Osedax estcourti* n. sp. B. *Osedax traceyae* n. sp. C. Whale skull with close up of *Osedax.* Arrows point at *Osedax* patches.

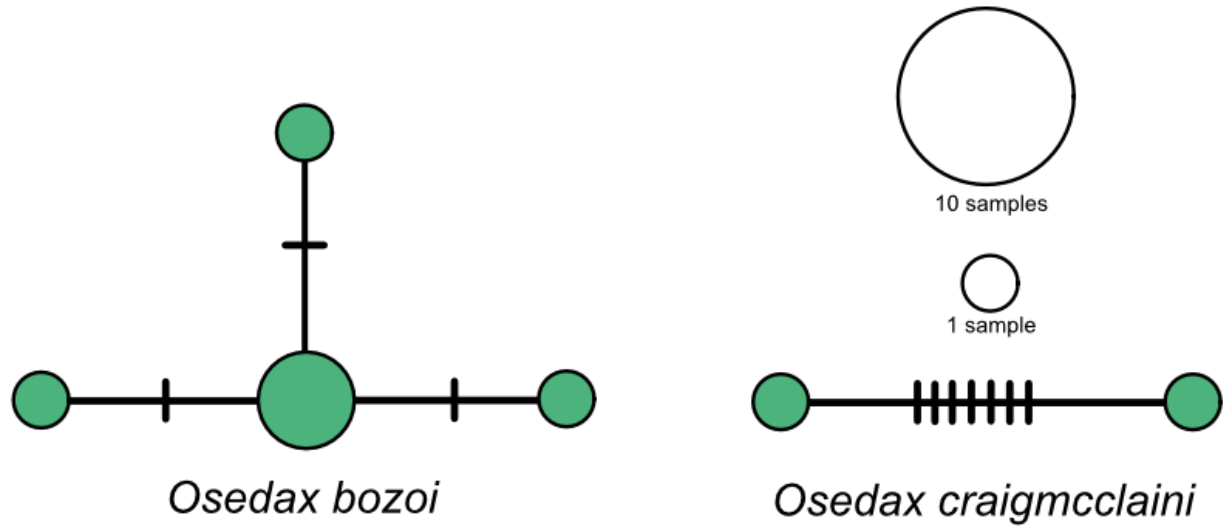

Figure 1.4: Haplotype networks for *O. bozoi* n. sp. and *O. craigmcclaini* n. sp. Circles are haplotypes, black circles and crosshatches are single nucleotide substitutions.

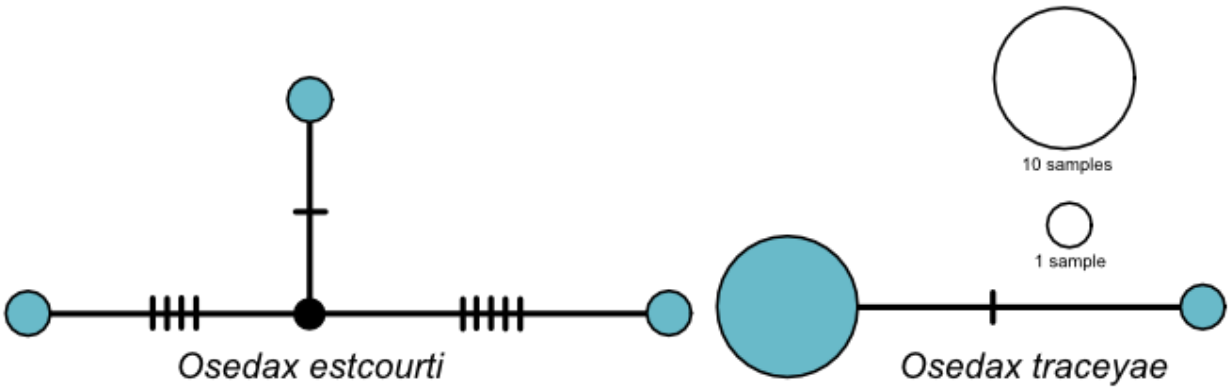

Figure 1.5: Haplotype networks for *O. estcourti* n. sp. and *O. traceyae* n. sp. Circles are haplotypes, black circles and crosshatches are single nucleotide substitutions.

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Chapter 1 is currently being prepared for submission for publication of the material. Berman, Gabriella H.; Hiley, Avery S.; Read, Geoffrey B.; Rouse, Greg W. The thesis author was the primary investigator and author of this material.

CHAPTER 2

INTRODUCTION

Osedax, part of *Siboglinidae*, secrete acid to dissolve sunken bone as a habitat and, aided by symbiotic bacteria, feed on the bones(Goffredi et al. 2005; Rouse et al. 2004; Tresguerres et al. 2013). *Osedax* can exploit bones of diverse vertebrates, from teleost fishes to mammals, which, together with high fecundity and lecithotrophic larvae may enable them to span extensive ranges (Rouse et al. 2018; Vrijenhoek et al. 2009; Zhou et al. 2020). To date, 26 *Osedax* species have been formally named with several others yet to be described (Eilertsen et al. 2020; Fujiwara et al. 2019; McClain et al. 2019; Rouse et al. 2018, 2004; Shimabukuro and Sumida 2019). Most *Osedax* species have only been collected from their type localities (Rouse et al. 2018), though there are a few exceptions. For example, *Osedax rubiplumus,* originally described from 3000m deep water from Monterey Bay in central California, has subsequently been found in the south Atlantic, western Pacific, and Indian Oceans, and off Antarctica (Zhou et al. 2020). *Osedax roseus*, *O. westernflyer,* and *O. docricketts* were all originally described from the eastern Pacific, but also are found in the western Pacific, in Japanese waters (Rouse et al. 2018). *Osedax priapus* was originally described from Monterey Bay but is also found in Oregon (Rouse et al. 2015). Finally, *Osedax frankpressi* is known from the eastern Pacific to the western Atlantic (Rouse et al. 2018; Shimabukuro and Sumida 2019). Much is still unknown about *Osedax* species distributions and the genetic structure across their ranges. In this study, we note expanded ranges for five *Osedax* species, most of which were previously only known from single localities. We used haplotype networks based on mitochondrial cytochrome oxidase subunit I (*COI*) to document range extensions and compare phylogeography among *Osedax* species.

MATERIALS AND METHODS

We aligned all available mitochondrial Cytochrome Oxidase subunit I (*COI*) sequence data for *Osedax* from GenBank with new sequences generated from specimens collected from naturally occurring animal falls and experimentally sunken bones off California and Oregon (USA) and the Pacific coast of Costa Rica (tables 2.1, 2.2). DNA extractions and PCR products were amplified, purified, and sequenced following previous protocols *Vrijenhoek et al. 2009, Vrijenhoek et al. 2008*.

Alignments for the *COI* data were made in Mesquite (v3.61) (Maddison and Maddison 2019) using MAFFT with default settings(Katoh and Standley 2013; Rozewicki et al. 2019). Uncorrected intraspecific pairwise distances were calculated in PAUP* (v4.0a168) (Swofford 2002) for each species with untrimmed alignments. Alignments were trimmed to allow for TCS haplotype networks (Clement et al. 2000) to be generated in with PopART (Goffredi et al. 2004; Leigh and Bryant 2015; Rouse et al. 2004). This resulted in alignments of 1005 basepairs for *O. docricketts*, 462 basepairs for *O. frankpressi*, 296 basepairs for *O. knutei*, 793 basepairs for *O. packardorum*, 891 basepairs for *O. priapus*, 1005 basepairs for *O. randyi*, 730 basepairs for *O. roseus*, 807 basepairs for *O. talkovici*, 983 basepairs for *O. westernflyer*. The published *O. roseus* sequences EU032471-EU032484 from Monterey were excluded from the *O. roseus* network because there was little overlap with the Japanese sequences. The published *O. roseus* sequence JF509949, and ON024292 were also excluded from the *O. roseus* network due to sequencing errors at the 5' ends of the sequences. We estimated Φ_{ST} values with Arlequin (v3.5.2.2) (Excoffier and Lischer 2010) for species with large enough sample sizes; *O. frankpressi*, *O. packardorum*, *O. priapus*, *O. roseus*, and *O. talkovici*.

DATA RESOURCES

All *COI* sequences in this paper are available on NCBI GenBank and vouchers are stored in the Benthic Invertebrate Collection at Scripps Institution of Oceanography. The sequences used are as follows: for *Osedax docricketts* we used FM998088-FM998103, FM998105-FM998107, FJ347625, FJ347626, EU267675, and EU267676. For *O. frankpressi* we used AY586486-AY586504, DQ996621, EU223312-EU223316, FJ347605-FJ347607, MH616017-MH616034, and OM994437-OM994445. For *O. knutei* we used FJ347632, FJ347634, FJ347635, MG262305-MG262307, JF509952-JF509955, and ON041066-ON041090. For *O. packardorum* we used DQ996639, DQ996641, DQ996642, EU223339-EU223346, EU223349-EU223355, FJ431198-FJ431200, FJ431202-FJ431204, FJ347628, FJ347629, and ON023592-ON023656. For *O. priapus* we used GQ504740, GQ504741, KP119564-KP119571, and OM988386-OM988399. For *O. randyi* we used FM998108, FM998109, FJ347610-FJ347615, and OM734777. For *O. roseus* we used DQ996625-DQ996628, EU223317-EU223319, EU032469-EU032484, EU164760-EU164773, FM998064-FM998077, FJ347609, JF509949, and ON024260-ON024309. For *O. talkovici* we used FJ431196, FJ431197, FJ431201, FJ431205, FJ347616-FJ347621, JF509950, JF509951, MG262310-MG262313, and ON024160-ON024259. For *O. westernflyer* we used FM998110, FJ347630, FJ347631, and MG262302-MG262304.

RESULTS

We extended the ranges for *O. frankpressi*, *O. packardorum*, *O. knutei*, *O. roseus*, and *O. talkovici* and confirmed the ranges for *O. docricketts*, *O. priapus*, *O, randyi*, and *O. westernflyer*. The known range for *Osedax knutei* was extended southwards from Monterey Bay

(California) to off San Diego (California) and Costa Rica. A record of *O. knutei* at 845m was found in Monterey, expanding the depth range 173 meters shallower. *Osedax packardorum* and *O. talkovici* were extended both north and south, from Monterey Bay to Oregon and San Diego (fig. 2.1). *Osedax packardorum's* range was extended 7 meters shallower to 382m (fig. 2.1). *Osedax roseus,* previously known from Sagami Bay (Japan) and Monterey Bay, was extended southwards to off San Diego, California. A record of *O. roseus* was found at 1844m, expanding *O. roseus's* depth range 24 meters deeper (fig. 2.1). *Osedax frankpressi,* previously recorded from Monterey Bay and Brazil, was found off Oregon and Costa Rica. Records of *O. frankpressi's* were found at 1018m, expanding the species depth range 800m shallower and a total depth range of 1880m (Rouse et al. 2018; Shimabukuro and Sumida 2019). No other depth range expansions were recorded. *Osedax docricketts*, *O. randyi*, and *O. westernflyer* were confirmed as being trans-Pacific, with new records from the Monterey Bay, CA for *O. randyi*. *Osedax priapus* was confirmed in Oregon and Monterey Bay with new sequences from both localities.

Uncorrected intraspecific pairwise distances ranged from maximum values of 4.5% for *O. knutei and* 4% for *O. frankpressi* and to 0.9% for *O. randyi Table 3*. *Osedax talkovici*, *O. roseus*, and *O. packardorum* had the largest sample sizes but not the largest intraspecific pairwise distances *Table 3*. Within-species pairwise distances for *O. frankpressi* were 1% for samples from the Pacific and 1.7% within Brazil (table 2.3). *Osedax randyi* and *O. westernflyer* had the smallest sample sizes and the smallest pairwise distances (table 2.3).

We used TCS haplotype networks to visualize the diversity and biogeography of the nine species of *Osedax* bone worms. The geographical distribution of *O. frankpressi* was the largest examined, spanning from the Pacific to Atlantic Oceans. The network for *O. frankpressi* revealed

two divergent haplotype clusters, one from Brazil and the other from Oregon, California, and Costa Rica (fig. 2.2). *Osedax frankpressi* from differed across its range by nearly 4% (uncorrected pairwise distance) and by a minimum of 3% between the Pacific and Brazilian sequences (fig. 2.2, table 2.3). In the eastern Pacific, one *O. frankpressi* haplotype was shared from Oregon to Costa Rica and the maximum intraspecific distance was less than 1% (fig. 2.2). *Osedax roseus* had the second largest distribution and traversed the Pacific Ocean. *Osedax roseus* had high levels of intraspecific diversity but little geographic variability with three distinct subnetworks (fig. 2.3). Although we found several shared haplotypes between Japan (Sagami Bay) and California, we also found a distinct subnetwork in Sagami Bay (fig. 2.3). *Osedax docricketts, O. randyi* and *O. westernflyer,* also both had trans-Pacific distributions, but relatively small sample sizes were available, and none showed haplotypes shared across the Pacific (fig. 2.4, 2.5, 2.6). Of these *Osedax docricketts* showed marked haplotype diversity in the eastern Pacific compared to Japan and most were quite divergent from the Japanese sequences (fig. 2.4). *Osedax knutei* ranged from central California to Costa Rica (fig. 2.7) and *O. priapus* occurred from Oregon to central California (fig. 2.8). *Osedax knutei* and *O. priapus* both had similar network topologies where a few individuals shared one haplotype, but many singleton haplotypes were somewhat divergent (fig. 2.7, 2.8). *Osedax packardorum* and *O. talkovici* were distributed from Oregon to San Diego, California. The networks. Both species had many individual haplotypes as well as several haplotypes shared among several localities (fig. 2.9, 2.10). Each showed some dominant haplotypes shared across most localities (fig. 2.9, 2.10). *Osedax talkovici* had the largest sample size with 116 sequences and the highest levels of haplotype variability along the eastern Pacific (fig. 2.10).

ΦST values along the eastern Pacific coast were close to zero, indicating well-mixed populations with high rates of gene flow for all species. The only significant ΦST estimates among localities were between Pacific and Atlantic specimens and between California and Costa Rica (ΦST=0.22) for O. frankpressi, although there were only four sequences from Costa Rica, and between Japan and California for *O. roseus* (table 2.4).

DISCUSSION

The data added in this study suggests that *Osedax* species tend to have larger maximum intraspecific distances than siboglinid relatives with large ranges, large sample sizes, or both. *Osedax's* intraspecific distances were larger than *Lamellibrachia* which has large ranges (McCowin, Rowden, and Rouse 2019), *Riftia* which has large sample sizes (Hurtado et al. 2002), *Sclerolinum* which has both large ranges and large sample sizes (Georgieva et al. 2015), *Tevnia* which has large sample sizes (Hurtado et al. 2002), and *Escarpia* which has large ranges and large sample sizes (Cowart et al. 2013). For example, *Lamellibrachia columna* and *Lamellibrachia juni* have maximum intraspecific distances of 1.24% and 1.39% respectively (McCowin et al. 2019). *Riftia pachyptila* has a maximum intraspecific distance of 0.15% (Hurtado et al. 2002). *Sclerolinum contortum* has a maximum intraspecific distance of 1.4% (Georgieva et al. 2015). *Tevnia jerichonana* has a maximum intraspecific distance of 1.3% (Hurtado et al. 2002). Pairwise comparisons of *Escarpia laminata*, *Escarpia spicata*, and *Escarpia southwardae* showed very little differentiation between the Gulf of Mexico and the west coast of Africa, although intraspecific pairwise distances were not calculated with *COI* (Cowart et al. 2013). While some *Osedax* species that have been well sampled, such as *O. rubiplumus* and *O. priapus* have similar relatively small distances, this is at the lower end of intraspecific distances for *Osedax* (Rouse et

al. 2018; Zhou et al. 2020). Intraspecific distances in *Osedax* can be up to 4.5% for *O. knutei* and eight out of nine of the species in the study had distances as large as or greater than 1.4%, the maximum for their siboglinid relatives.

Intraspecific and interspecific distances for *Osedax* are in the ranges of interspecific distances for *Vestimentifera* but are similar to distances in other annelids. Interspecific distances for *Vestimentifera* can be as low as 2.5% between *Lamellibrachia barhami* and *Lamellibrachia anaximandri* or *Lamellibrachia donwalshi* and *L. anaximandri* (McCowin and Rouse 2018; McCowin et al. 2019). *Vestimentifera* appears to be an extreme case of low interspecific distances within annelids; in one extreme example *Escarpia laminata*, *Escarpia spicata*, and *Escarpia southwardae* actually share a *COI* haplotype but gene flow was clearly extremely limited between the localities inhabited by the species but panmixia was apparent within the localities (Cowart et al. 2013). The majority of *Osedax* species in this study had intraspecific distances greater than 2.5% while the minimum interspecific distances in *Osedax* are 6-7% between *O. randyi* and *Osedax* 'MB16', and 7.4% between *Osedax lehmani* and *O. packardorum* (Rouse et al. 2018). Other annelids show similar values; for example, the amphinomid polychaete *Hermodice caranculata* has a maximum intraspecific distance of 2.92%, a value that was determined to be too small to warrant splitting up the species (Ahrens et al. 2013). Other genera and species with comparable interspecific distances include the dorvilleid polychaete *Parougia* which has minimum interspecific distances of 7% or more (Yen and Rouse 2020), the phyllodocid *Eumida sanguinea* with minimum interspecific distance of 6.5%, and the amphinomid *Eurythoe complanata* cryptic species complex, with an interspecific distance of 10% in the Atlantic (Barroso et al. 2010). However, there is no standard when it comes to species delimitations in annelids; a 5% intraspecific distance was sufficient to split the dorvilleids *Ophryotroca*

japonica and *Ophryotroca glandulata* into their respective species based on reproductive isolation (Paxton and Åkesson 2010), but a 5% interspecific divergence between *Streblospio gynobranchiata* and a *Streblospio benedict* population was enough to group the population inside of *S. gynobranchiata* (Schulze et al. 2000). Minimum interspecific distances of ~2-23% have been used to delineate cryptic annelid species and intraspecific distances of up to 7% have been recorded for annelids (Nygren 2014), putting *Osedax* well within normal minimum interspecific ranges for annelids and making *Vestimentifera* somewhat exceptional.

Large geographic ranges in *Osedax* are not always correlated with large intraspecific values. For example, *Osedax knutei* has an intraspecific distance of 4.5% within a geographical range between Monterey and Costa Rica. On the other hand, *O. frankpressi's* has a smaller intraspecific distance of 4% despite a range that encompasses the west coast of the United States, Costa Rica, and Brazil. *Osedax packardorum* (~3%), *O. priapus* (~2%), and *O. talkovici* (~2.3%) all have intraspecific distances between 2% and 3% but their ranges are confined to the west coast of the United States. Meanwhile, *O. docricketts* (3.5%) and *O. roseus* (2.4%) both have intraspecific distances comparable to *O. packardorum*, *O. priapus*, and *O. talkovici* and trans-Pacific ranges. *Osedax randyi*, and *O. westernflyer* all have trans-Pacific ranges and intraspecific distances close to or less than 1%. *Osedax rubiplumus* has the largest geographical range of any *Osedax*, spanning from Antarctica, across the Pacific, and the Indian Ocean, however, it has a very small intraspecific distance at 0.91% (Shimabukuro and Sumida 2019; Zhou et al. 2020). The evidence from multiple species suggests that intraspecific distance is not indicative of range size, however species with small sample sizes, such as *O. docricketts*, *O. randyi*, and *O. westernflyer* may not provide reliable data.

Despite large geographical distances some *Osedax* species experience genetic connectivity across their ranges. *Osedax packardorum*, *O. priapus*, *O. roseus*, and *O. talkovici* are genetically well connected across their ranges, which for *O. roseus* is more than 8000km between Sagami Bay and Monterey Bay, as demonstrated by Φ_{ST} values, equal to or less than 0.191. *Osedax roseus* has very high genetic connectivity between Monterey Bay and San Diego with a Φ_{ST} value of 0.00 which suggests that the populations could be panmictic. In contrast to *S. contortum*, which has a large range and large sample sizes like *O. packardorum*, *O. priapus*, *O. roseus*, and *O. talkovici* but no hared haplotypes between populations (Eilertsen et al. 2018), all four species had haplotypes shared across multiple localities further indicating connectivity across their ranges.

In contrast to *O. packardorum, O. priapus, O. roseus,* and *O. talkovici, O. docricketts, O. knutei, O. randyi,* and *O. westernflyer* had no haplotypes shared across multiple localities. In the case of *O. randyi* and *O. westernflyer* this is likely due to a small sample size. However, *O. docricketts* and *O. knutei* could be cryptic species complexes. The inclusion of 9 of the 13 Monterey Bay sequences in *O. docricketts* is potentially unjustified based on a large number of nucleotide substitutions between the holotype and the most divergent sequence (55 nucelotide substitutions) and a higher-than-expected intraspecific value (Rouse et al. 2018). *Osedax docricketts* also has an intraspecific distance of ~3.5%, this is larger than expected considering there are only 24 sequences in the species, *Osedax talkovici* in contrast has 116 sequences and an intraspecific distance of 2.3%. This evidence suggests that *O. docricketts* could actually be a cryptic species complex. *Osedax knutei* has the largest intraspecific distance of any Osedax species at 4.5%. The haplotype network for *O. knutei* shows that many individuals share a haplotype in Monterey but there are also divergent haplotypes in Monterey Bay, San Diego, and Costa Rica. The large intraspecific distance, the presence of one haplotype shared by most of

the individuals in the species, and the abscense of shared haplotypes among the three localities suggests that *O. knutei* could be a cryptic species complex.

Osedax frankpressi had the largest geographic distribution of the species in this study, the second largest depth range of any *Osedax* species with a range of ~1880m, the highest reported Φ_{ST} values in this study, and one of the largest intraspecific distance of any *Osedax* species. We did not find any shared haplotypes between ocean basins for *O. frankpressi*, but we did find a shared haplotype between Oregon, Monterey Bay, and Costa Rica. A prior study found a divergence of ~3% between the Atlantic (Brazil) and the Pacific (California to Costa Rica), maximum distances of 0.7% within Brazil and 0.3% in the Pacific (Shimabukuro and Sumida 2019). We added new sequences from Oregon, California, and Costa Rica and found that the intraspecific pairwise distance for the species was up to 4% though the minimum distance between Brazil and the Pacific was still ~3%. A Φ_{ST} value of 0.86 demonstrated clear evidence of population subdivision for *O. frankpressi* between Pacific, and Atlantic populations that was reinforced by the divergent haplotype network between the Pacific and Atlantic. We also found that one haplotype is shared by *O. frankpressi* individuals from Oregon to Costa Rica, a distance of over 6,000 kilometers. Further sampling around South America may show better evidence of connectivity, and bones on the seafloor around South America have been hypothesized to connect *O. frankpressi* populations (Shimabukuro and Sumida 2019), but present data suggests gene flow is very low between the Pacific and Brazil as demonstrated by the large Φ_{ST} value of 0.86 between Monterey and Brazil. Although the intraspecific distance of 4% is still lower than the smallest interspecific distance for neighboring *Osedax* which is 6-7% between *O. randyi* and *O.* 'MB16', the apparent population subdivision between the Pacific and Atlantic populations could warrant splitting *O. frankpressi* into two species.

The large ranges for these *Osedax* species are not unusual among deep sea invertebrates; broad geographic ranges have been documented for other deep sea benthic animals based on DNA data (Eilertsen et al. 2018; Ekimova et al. 2021; Georgieva et al. 2015; Kobayashi and Araya 2018; McCowin and Rouse 2018; McCowin et al. 2019; Shimabukuro and Sumida 2019; Signorelli, Sellanes, and Asorey 2021; Yen and Rouse 2020). For example, nudibranch mollusks *Dendronotus patricki* and *Dendronotus dalli* have latitudinal transpacific distributions similar to *O. docricketts*, *O. randyi*, *O. roseus*, and *O. westernflyer* (Ekimova et al. 2021). The bathymodiolin mussel *Adipicola leticiae* has been found in the Pacific and the Atlantic similar to *O. frankpressi* (Signorelli et al. 2021). And siboglinids *Sclerolinum contortum*, *Nicomache lokii*, *Lamellibrachia barhami*, and *Escarpia spicata* and Dorvilleids *Parougia batia*, and *Parougia billiemiroae* have longitudinal transpacific distributions similar to *O. knutei*, *O. packardorum*, *O. priapus*, *O. talkovici* and the Eastern Pacific distributions of *O. frankpressi* and *O. roseus* (Eilertsen et al. 2018; Georgieva et al. 2015; Kobayashi and Araya 2018; McCowin and Rouse 2018; Yen and Rouse 2020). In the case of *Osedax* large ranges may be possible due to the presence of additional bone habitat and life history traits such as high fecundity and lecithotrophic larvae (Miyamoto et al. 2013; Rouse et al. 2009; Shimabukuro and Sumida 2019). Although many *Osedax* species are known to be widely dispersed, many are concentrated in Monterey Bay (Rouse et al. 2015, 2018, 2004; Vrijenhoek et al. 2009); *Osedax's* life history traits make them well suited to wide dispersal and ecological success. This study demonstrates that some *Osedax* species are as widely distributed as some other deep-sea invertebrates and often experience little population subdivision over large ranges.

Table 2.1: Number of unpublished and published COI sequences of *Osedax* used in this study and number of samples from each locality. Range extension = *.

Species	Total	Sagami Bay	Oregon	Monterey Bay	San Diego	Costa Rica	Brazil
O. docricketts	15	11	0	4	0	0	0
O. frankpressi	56	0	1*	33	0	4*	18
O. knutei	35	0	0	33	1*	1*	0
O. packardorum	92	0	22*	38	32*	0	0
O. priapus	24	0	9	15	0	0	0
O. randyi	9	2	0	7	0	0	0
O. roseus	85	14	0	19	52*	0	0
O. talkovici	116	0	13*	41	62*	0	0
O. westernflyer	6	1	0	5	0	0	0

Table 2.2: GenBank accession numbers used for the *Osedax* species in this study. Alternative names listed on GenBank are also listed. New sequences are in **bold**.

Species	GenBank number	Other GenBank names
O. docricketts	FM998088-FM998103, FM998105-FM998107, FJ347625, FJ347626, EU267675, EU267676	Nude-palp C, Sagami-6
O. frankpressi	AY586486-AY586504, DQ996621, EU223312-EU223316, FJ347605-FJ347607, MH616017-MH616034, **OM994437-OM994445**	-
O. knutei	FJ347632, FJ347634, FJ347635, MG262305-MG262307, JF509952-JF509955, **ON041066-ON041090**	Nude-palp E
O. packardorum	DQ996639, DQ996641, DQ996642, EU223339-EU223346, EU223349-EU223355, FJ431198-FJ431200, FJ431202-FJ431204, FJ347628, FJ347629, **ON023592-ON023656**	Orange collar, Sp. 4 SBJ-2006
O. priapus	GQ504740, GQ504741, KP119564-KP119571, **OM988386-OM988399**	Pinnules, Sp. 16
O. randyi	FM998108, FM998109, FJ347610-FJ347615, **OM734777**	White collar, Sagami-7
O. roseus	DQ996625-DQ996628, EU223317-EU223319, EU032469-EU032484, EU164760-EU164773, FM998064-FM998077, FJ347609, JF509949, **ON024260-ON024309**	SBJ-2007a, Sp. 2 SBJ-2006, Rosy, Roseus (Japan)
O. talkovici	FJ431196, FJ431197, FJ431201, FJ431205, FJ347616-FJ347621, JF509950, JF509951, MG262310-MG262313, **ON024160-ON024259**	Yellow patch, Pinnules
O. westernflyer	FM998110, FJ347630, FJ347631, MG262302-MG262304	Nude-palp D, Sagami-8

Table 2.3: Uncorrected maximum intraspecific *COI* pairwise distance matrices for *Osedax*.

Species	Uncorrected pairwise distances
Osedax docricketts	0.03484
Osedax frankpressi	0.03927
Osedax knutei	0.04466
Osedax packardorum	0.02991
Osedax priapus	0.02021
Osedax randyi	0.00897
Osedax roseus	0.02392
Osedax talkovici	0.02283
Osedax westernflyer	0.01393

Table 2.4: Φ_{ST} values between localities of *Osedax* species worldwide. Bold values indicate significant differentiation.

Species	Oregon Monterey Bay	Oregon San Diego	Monterey Bay Sagami Bay	Monterey Bay San Diego	Monterey Bay Costa Rica	Monterey Bay Brazil	Sagami Bay San Diego
O. frankpressi	-	-	-	-	**0.22**	**0.860**	-
O. packardorum	0.074	0.007	-	0.071	-	-	-
O. priapus	0.075	-	-	-	-	-	-
O. roseus	-	-	**0.171**	0.00	-	-	**0.191**
O. talkovici	0.051	0.024	-	0.039	-	-	-

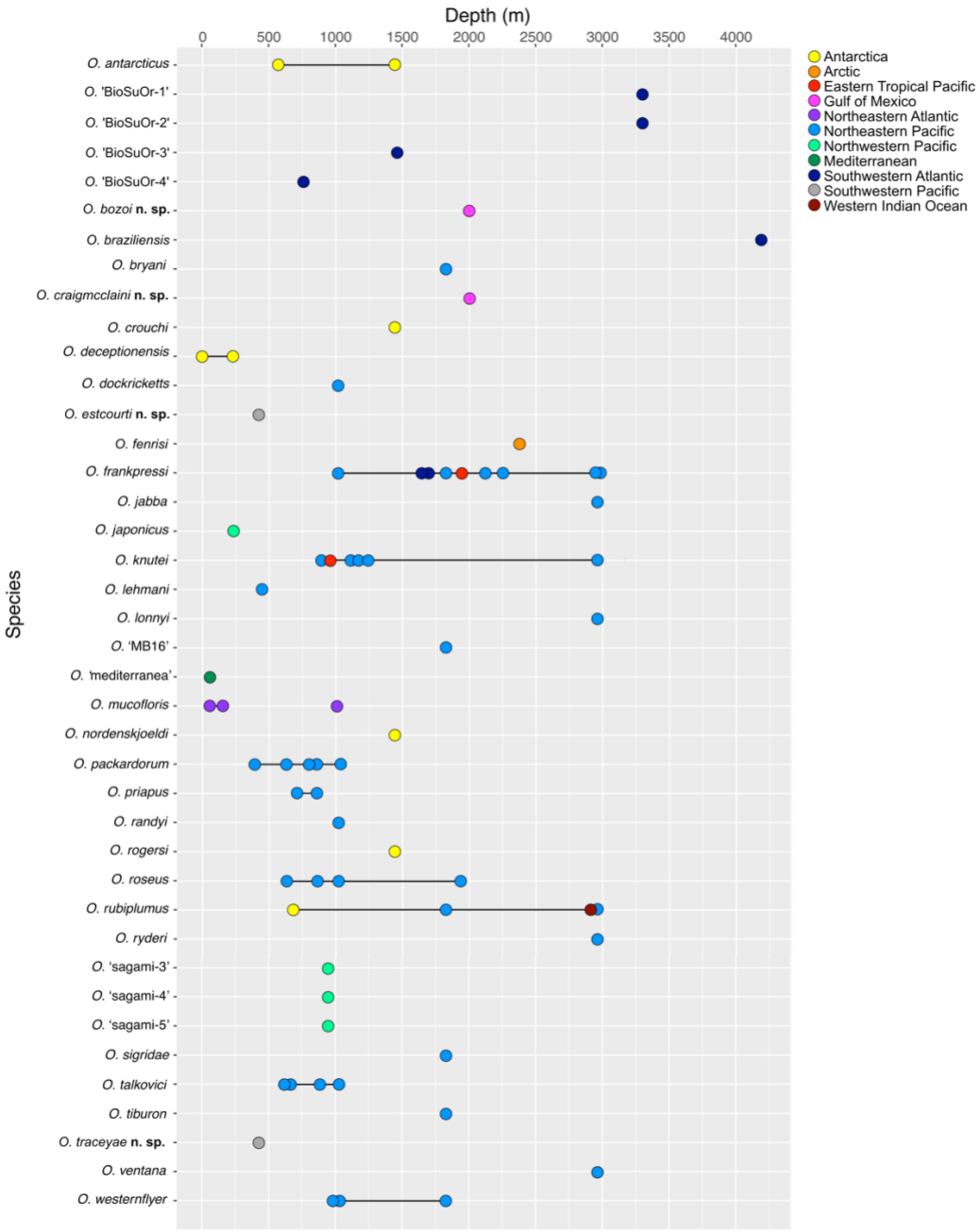

Figure 2.1: Regions and depths of sample collection and depth ranges. Depths are unknown for samples collected in Japan unless listed in this figure.

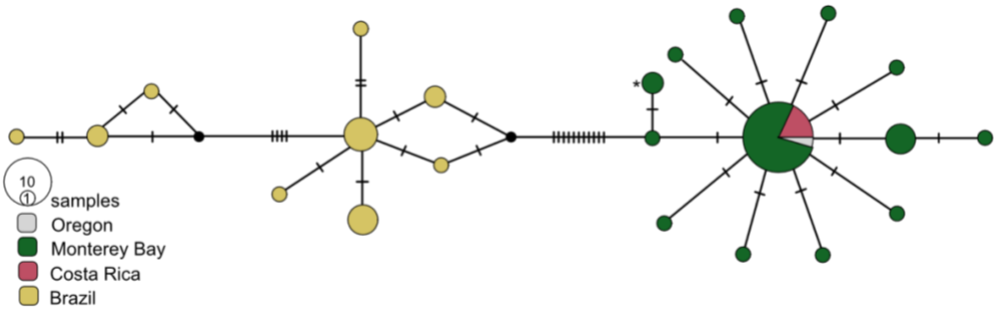

Figure 2.2: *Osedax frankpressi* haplotype network colored by sampling locality. Crosshatches and black circles represent missing mutations. Holotype = *.

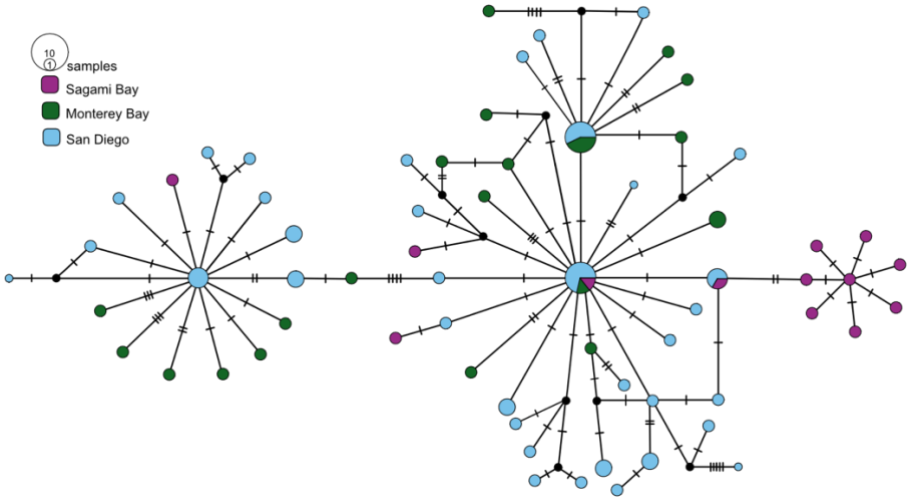

Figure 2.3: *Osedax roseus* haplotype network colored by sampling locality. Crosshatches and black circles represent missing mutations.

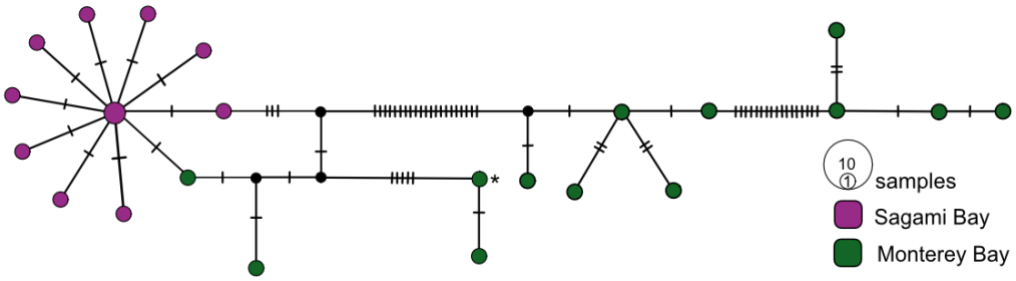

Figure 2.4: *Osedax docricketts* haplotype network colored by sampling locality. Crosshatches and black circles represent missing mutations. Holotype = *.

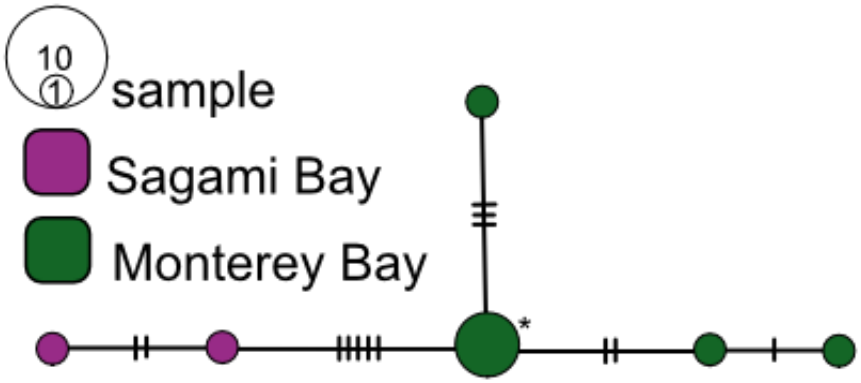

Figure 2.5: *Osedax randyi* haplotype network colored by sampling locality. Crosshatches and black circles represent missing mutations. Holotype = *.

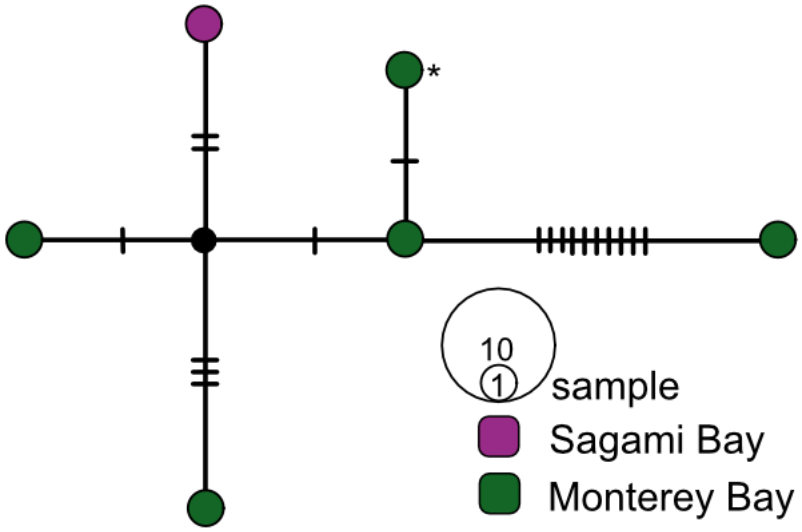

Figure 2.6: *Osedax westernflyer* haplotype network colored by sampling locality. Crosshatches and black circles represent missing mutations. Holotype = *.

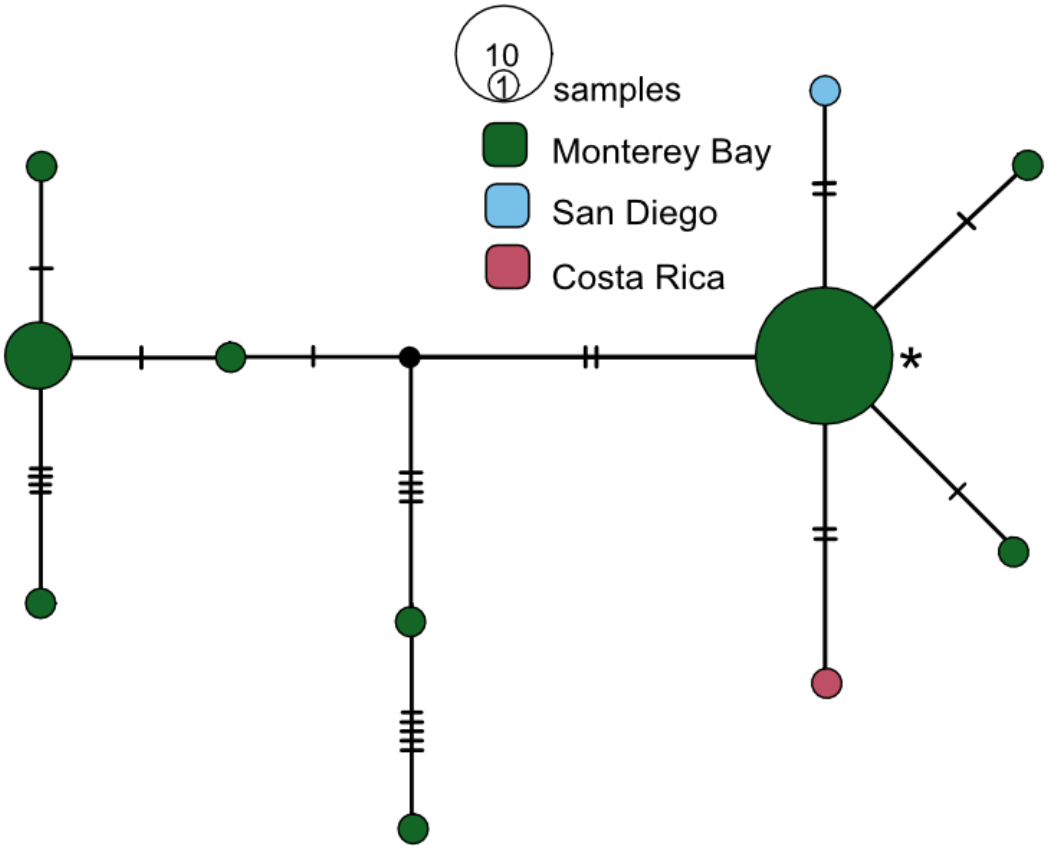

Figure 2.7: *Osedax knutei* haplotype network colored by sampling locality. Crosshatches and black circles represent missing mutations. Holotype = *.

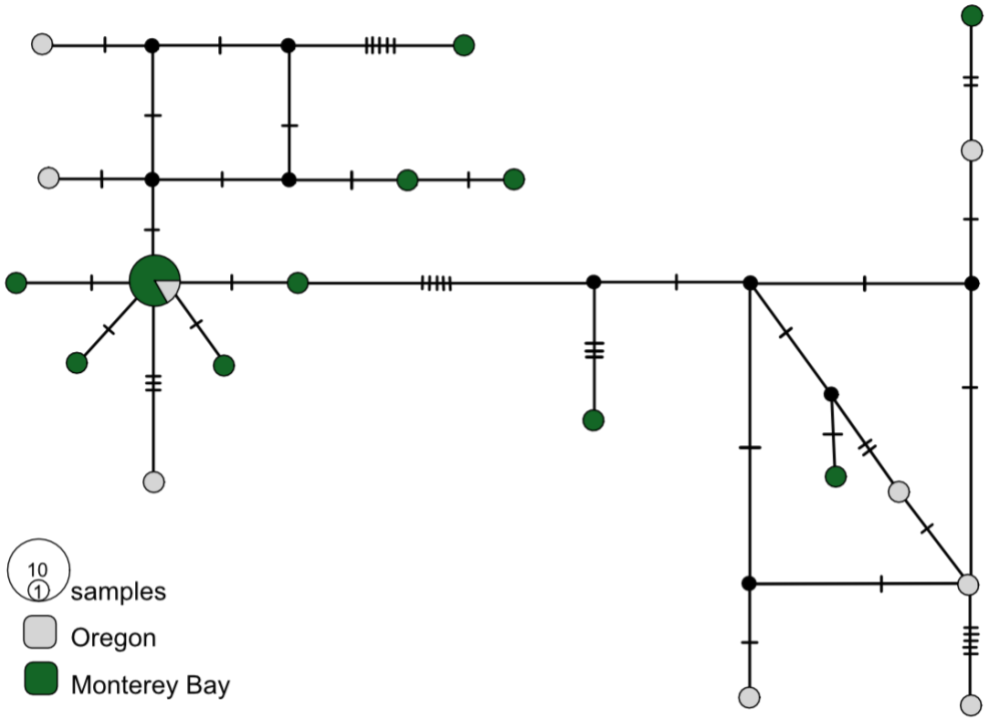

Figure 2.8: *Osedax priapus* haplotype network colored by sampling locality. Crosshatches and black circles represent missing mutations.

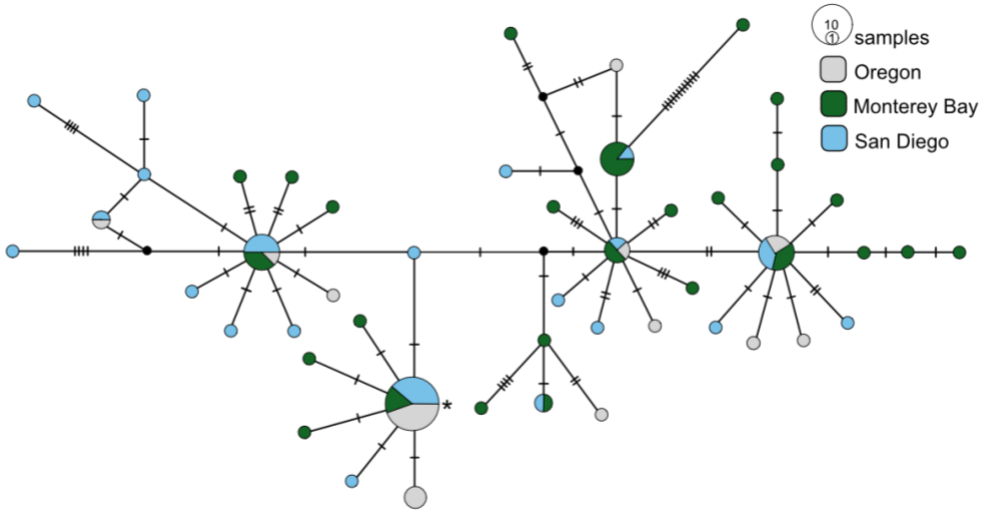

Figure 2.9: *Osedax packardorum* haplotype network colored by sampling locality. Crosshatches and black circles represent missing mutations. Holotype = *.

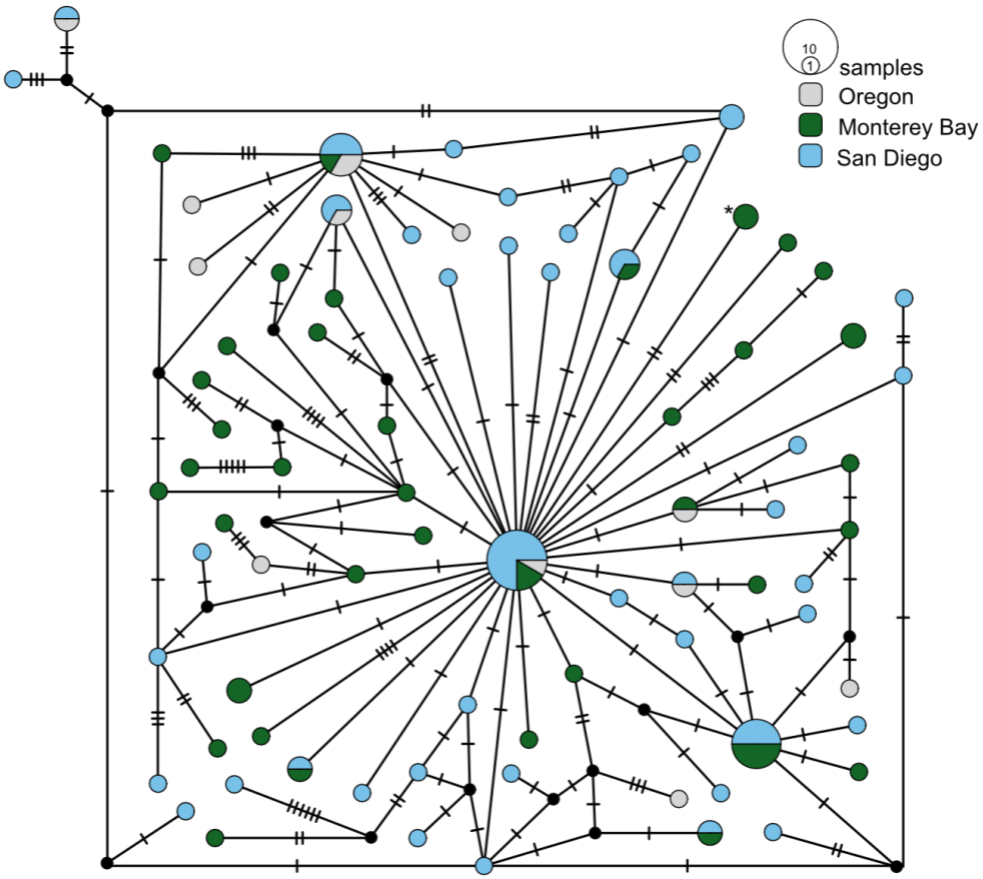

Figure 2.10: *Osedax talkovici* haplotype network colored by sampling locality. Crosshatches and black circles represent missing mutations. Holotype = *.

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