Confirmation of the shell-boring oyster parasite *Polydora websteri* (Polychaeta: Spionidae) in Washington State, USA

Julieta C. Martinelli[1†*], Heather M. Lopes[1†], Lorenz Hauser[1], Isadora Jimenez-Hidalgo[1], Teri L. King[2], Jacqueline L. Padilla-Gamiño[1], Paul Rawson[3], Laura H. Spencer[1], Jason D. Williams[4], Chelsea L. Wood[1]

[1]School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98105, USA

[2]Washington Sea Grant, University of Washington, Shelton, WA 98584

[3]School of Marine Sciences, University of Maine, Orono, ME, 04469, USA

[4]Department of Biology, Hofstra University, Hempstead, NY 11549, USA

Corresponding author: Julieta C. Martinelli

[*]E-mail address: julimar@uw.edu

ORCID ID: 0000-0003-4210-7642

[†]Shared first authorship

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

Invasions by shell-boring polychaetes such as *Polydora websteri* have resulted in the collapse of oyster aquaculture industries in Australia, New Zealand, and Hawaii. These worms burrow into bivalve shells, creating unsightly mud blisters that are unappealing to consumers and, when nicked during shucking, release mud and detritus that can foul oyster meats. Recent findings of mud blisters on the shells of Pacific oysters (*Crassostrea gigas*) in Washington State suggest a new spionid polychaete outbreak. To determine the identity of the polychaete causing these blisters, we obtained Pacific oysters from two locations in Puget Sound and examined them for blisters and burrows caused by polydorid worms. Specimens were also obtained from eastern oysters (*Crassostrea virginica*) collected in New York for morphological and molecular comparison. We extracted polychaetes, compared their morphology to original descriptions, and sequenced mitochondrial (cytochrome c oxidase I [mtCOI]) and nuclear (18S rRNA) genes to obtain species-level identifications for these worms. Our data show that *Polydora websteri* are present in mud blisters, constituting the first confirmed record of this species in Washington State. The presence of this notorious invader could threaten the sustainability of oyster aquaculture in Washington, which currently produces more farmed bivalves than any other US state.

Keywords: polychaete, *Polydora*, aquaculture, mud blister, *Crassostrea*

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

Introduction

In the global pantheon of invasive species, the most notorious invaders simultaneously compromise the function of native ecosystems and jeopardize the human livelihoods that depend upon those ecosystems. Among these invaders is the spionid polychaete *Polydora websteri,* commonly known as a "mud worm" or "mud blister worm"[1], which bores into the shells of molluscs[2]. By creating unsightly blisters on the shells of their commercially important hosts, these pests have led to significant economic losses for shellfish aquaculture[3]. *P. websteri* can infest a variety of mollusc hosts (see reviews[4-6]), including oysters[7-10], mussels[11-13], scallops[14-16], and abalone[17].

P. websteri and related polydorins (*sensu*[18]; a group of nine spionid genera with a modified fifth chaetiger) have compromised and collapsed oyster aquaculture industries around the world. In the late 1800s, the introduction of *P. websteri* with translocated oysters caused subtidal oyster beds in New South Wales, Australia to disappear [19-22, 8]. When oyster transplants from Kaneohe Bay brought *P. websteri* to Kakuku, Hawaii, the introduction caused extensive damage to shellfish production[23,24]. Oyster farms on the east coast of the United States have been plagued with *P. websteri* infestations since the 1940s, resulting in substantial oyster farm losses[25-27]. In addition, high mortalities of the Japanese scallop *Patinopecten yessoensis* in British Columbia, Canada were attributed to *P. websteri*[28]. These examples attest to the ability of *P. websteri* to successfully invade new locations and, once established, to significantly affect aquaculture production.

P. websteri infestations are detrimental to oyster aquaculture because the worms result in unsightly blisters on oyster valves, decreasing market value. The mud worm has a pelagic larval stage, after which the larvae settle onto the external side of a calcareous shell[22,29,30]. The worm

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

then forms a U-shaped burrow with two exterior openings[27,31]. As they grow, burrows breach the inner surface of the valve, causing the host to produce a brittle layer of nacre that walls off the burrow[4,10,32-34]. The worm continues to expand this burrow beneath the thin, calcareous layer produced by its host; as this space fills with detritus, mud, and worm feces, a "mud blister" is formed[33,35]. Blisters can be irregular in shape and darkly colored, compromising the presentation of oysters served on the half-shell[36]. Moreover, if a blister is nicked during oyster shucking, the mud and feces will foul the oyster meat, rendering it inedible[3]. This is particularly problematic for oyster-growing areas where a large proportion of production goes to the half-shell market.

In addition to their detrimental impact on aquaculture production, heavy mud worm infestations can also impact shell integrity, growth, and survivorship of mollusc hosts[37]. When infested with *Polydora ciliata*, the gastropod *Littorina littorea* has significantly reduced shell strength relative to uninfested individuals, making the infested gastropods more vulnerable to predation[38]. Pacific oysters (*Crassostrea gigas*) infested by the polydorids *P. hoplura*, *P. cornuta,* and *Boccardia semibranchiata* grow more slowly and have poorer body condition than do uninfested oysters[39]. Glycogen, protein, and lipid content relative to the shell cavity volume are lower in infested compared to uninfested *Crassostrea* spp. oysters[7,25,40]. Additionally, polydorins have been shown to increase mortality rates in Pacific oysters that are heavily infested[41,42]. These negative effects on growth and survivorship may be caused by the energetic demands of worm-induced nacre production[7,40,43-46]; that is, infested hosts may need to invest energy into isolating their tissue from the worm by building multiple costly shell layers instead of investing that energy into their own growth and reproduction[47]. Given these impacts on host vital rates, *P. websteri* outbreaks may affect more than just the bottom line of the shellfish

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

industry; they may also compromise the important ecosystem services provided by filter-feeding shellfish species[48].

Polydora websteri has been reported from locations all over the world (see reviews[10,49-52]), but due to its complex taxonomic history (see [53-55]), many records remain to be confirmed. Some historical reports of *P. ciliata* (a non-burrowing species) have been re-identified as other, shell-boring polydorin species, including *P. websteri*[9], and more such erroneous historical reports might exist. *Polydora websteri* is believed to be of Asian origin, and genetic homogeneity among North American, Hawaiian, and Asian specimens suggests that human-mediated transport produces high levels of connectivity among populations[10]. Although *P. websteri* has been predicted to be present in Washington, USA[56] based on records of its presence north in British Columbia[28,57] and south in Oregon and California (e.g.[29,58-60]), it has never before been described from Washington. Its potential absence is a fortunate circumstance; as the United States' leading producer of bivalve shellfish, Washington State's bivalve aquaculture brings in over $92 million dollars in revenue annually[61]. Of Washington State's cultured shellfish production, Pacific oysters (*Crassostrea gigas*) contribute 38% by weight and 38% by value[61]. Pacific oysters are also culturally important to local communities, Native American tribes, family-owned farms, and recreational farmers and collectors[62]. As the industry has evolved in recent years, producers have shifted to the lucrative half-shell market, where the shell is presented to the consumer[61]. Washington's oyster industry is therefore structured in such a way that a *P. websteri* outbreak could cause extensive damage.

Washington State oysters have long been prized for the consistent color of their inner valves, in contrast to the mud-blister-blemished valves of oysters grown in other parts of North America (T King, *personal communication*). However, in recent years, one of us (TK) began noticing

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

mud blisters on the valves of Pacific oysters (*Crassostrea gigas*) grown in Puget Sound (Fig. 1). Site visits with local oyster growers confirmed these observations, and suggested that Washington State – a globally important aquaculture region – may be experiencing a *P. websteri* outbreak. To confirm the species identity of the organisms causing these blisters, we sampled Pacific oysters from two bays in the Puget Sound, an estuary in Washington State with extensive commercial oyster beds. In addition, specimens of *P. websteri* in eastern oysters (*Crassostrea virginica*) from Long Island, New York (close to the type locality) were collected for morphological and molecular comparisons. Worms were recovered from shell blisters and burrows, and identified to species using morphological traits, as well as mitochondrial COI [mtCOI] and nuclear 18S [18S rRNA] gene sequences. Our results constitute the first formal report of a shell-boring polychaete from Puget Sound, and the first report of the notorious pest *Polydora websteri* in Washington State.

Results

Morphological identification. Specimens from both Washington (Fig. 3) and New York (Fig. 4) matched the taxonomically important features of *Polydora websteri* in the original description[27], redescription[54] and more recent reports[9,10,52,63]; see morphological description of specimens in Supplementary Text 1). In addition to *P. websteri*, some specimens of *Boccardiella hamata* were identified (see morphological description of specimens in Supplementary Text 1).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

Infestation prevalence. Of the 186 oysters, 41% (77 individuals) were infested with at least one blister or burrow. Among oysters from Oakland Bay, 53% were infested; among oysters from Totten Inlet, 34% were infested.

Molecular identification. Most worms collected from Oakland Bay were identified as *P. websteri* from both DNA sequences (Table 1). Analysis of 18S rRNAsequences of 12 worms from Oakland Bay clustered with *P. websteri* sequences obtained from GenBank and with the four sequences from Long Island (Fig. 5). Ten of the worms from Oakland Bay and the four from Long Island were also sequenced with mtCOI and also clustered with *P. websteri* in that tree (Fig. 6, Table 1). Worms collected from Totten Inlet were more diverse and formed a separate branch from Oakland Bay for both 18S rRNA and mtCOI genes. Eleven 18S rRNA sequences from Totten Inlet formed a sister group to *P. websteri* sequence entries obtained from GenBank (Fig. 5). The three individuals also sequenced for mtCOI formed their own group, which was a distant sister clade to *P. hoplura* and was very distant from *P. websteri*. Two individuals from Totten Inlet (TOT13 and TOT14) clustered separately from all known species in both 18S rRNA and mtCOI (Figs. 5 and 6). One individual from Oakland Bay and one from Totten Inlet also formed a distinct group, which clustered with *Boccardiella hamata* with 18S rRNA but not with mtCOI. In summary, both 18S rRNA and mtCOI sequences confirm the presence of *P. websteri*, but also suggest the presence of possibly three other, as yet unidentified, species. Sequences alignment statistics can be found in Table 3.

Discussion

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

Our findings constitute the first report of *Polydora websteri* in Washington State. In addition to *P. websteri,* our data suggest that several other shell-boring polydorin species may also be present in local oysters. This work indicates that Washington State waters host several cryptogenic, shell-boring polydorins, all of which may pose a danger to the region's valuable oyster aquaculture industry.

Nearly all worms from Oakland Bay were molecularly identified as *P. websteri* based on 18S rRNA and mtCOI (Table 1). The majority of worms from Totten Inlet remain unresolved as we were not able to molecularly identify them. Based on detailed morphological analysis, specimens of *P. websteri* from Oakland Bay (Fig. 3) matched previous descriptions and the newly collected material from Long Island, NY near the type locality (Fig. 4); the same specimens that we morphologically identified were also sequenced, and morphological and molecular diagnoses agreed. We therefore confirm the presence of *P. websteri,* a shell-boring mud worm, in the shells of Washington State Pacific oysters. *Polydora websteri* has never before been reported from Washington. This blister-forming species could endanger an aquaculture industry that provides both multi-million dollar revenues ($92 million in 2015) and valuable ecosystem services to Washington State.

The fact that *P. websteri* has never before been documented in Washington State oysters suggests a recent introduction, but it is also possible that the species has been present in the region for some time and has undergone a recent uptick in prevalence perhaps associated to the aquaculture industry or environmental changes. Extensive exchange of shell and live oysters among regions in Washington continues to the present day, and to such an extent that *P. websteri* populations are genetically homogenous across broad swathes of their contemporary range[10]. Washington State has a long history of exchange with other oyster-growing regions[64] and

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

171 polydorin pelagic larvae may also have been introduced through ballast water[65,66]. Although it is
172 likely that *P. websteri* is native to Asia and exotic to North America[10], we suggest that *P.*
173 *websteri* be considered cryptogenic in Washington State[67] until further research can resolve its
174 origins. It is possible that the species is native to Washington and that it has never before been
175 described because it was present only at very low prevalence until recently. The prevalence of *P.*
176 *websteri* is sensitive to environmental change. For example, increasing siltation can increase the
177 susceptibility of *Crassostrea virginica* to *P. websteri*[68]. In contrast, reducing pH actually
178 decreases susceptibility to infestation[69]. Because *P. websteri* can recruit to both live and dead
179 oyster shells[30], the expansion of the oyster aquaculture industry, oyster restoration, and increased
180 density of oysters in beds across the state might have promoted an increase in transmission and
181 prevalence if the polychaete was already present. Whatever their origin, the blister-forming
182 polychaetes we document here are a new challenge for Washington State oyster growers and the
183 government agencies charged with management of shellfish stocks.

184 Because *P. websteri* is a generalist pest[9,32,33], it may impact other shellfish species of
185 ecological, economic, and cultural importance to Washington State. An important example is the
186 Olympia oyster (*Ostrea lurida)*, an overexploited native species that is the focus of intensive
187 restoration efforts[70]. Mussels[11-13], scallops[14-16], and abalone ([17]; see review in [4]) are also at risk.
188 Given the important ecosystem services provided by filter-feeding shellfish species[48], a
189 polydorin outbreak could affect more than just the bottom line of the shellfish industry;
190 ecosystem functioning is also at risk.

191 In addition to *P. websteri,* our data suggest the presence of other unidentified polydorin
192 species such as the worms from Totten Inlet that were not resolved in the phylogenetic trees.
193 Polydorins have a long history of being misidentified, because the morphological differences

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

between some species are subtle or even absent[9,71]. For example, [72] found that specimens morphologically identified as *P. cornuta* actually represented three distinct species. For this reason, we relied both on morphological analysis and molecular sequencing to identify the worms we recovered. Given the weak association between our specimens and GenBank sequences for *P. hoplura, P. cornuta* and *B. hamata,* additional molecular and morphological analysis is needed to confirm the presence of these species in Washington State. The unresolved phylogeny of our sampled worms requires more analysis, especially because our as-yet-unidentified species are probably not yet represented by sequences in the GenBank online database. Primers for identifying polydorins were not developed until recently[73], so reference material may soon be available for resolving this phylogeny.

In this work, we positively identified the notorious shell-boring polydorin, *P. websteri,* in commercially farmed Pacific oysters, providing the first formal documentation of this globally distributed pest in Washington State. Of 186 oysters collected, 41% were infested. The pathology caused by shell-boring mud worms results in unsightly blisters that reduce the market value of infested oysters, especially those served on the half-shell. Washington's Pacific oyster industry is dominated by the half-shell market, and given the high prevalence of infestation found in this study, these pests have the potential to threaten the valuable Pacific oyster aquaculture operations in Washington. Past invasions by *P. websteri* have resulted in oyster aquaculture industry collapses. Given this history, *P. websteri* poses a substantial threat to marine ecosystems and human livelihoods in Washington State.

Methods

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

Oyster collections. To assess whether shell-boring polychaetes were present in Washington Pacific oysters (*Crassostrea gigas*) and to confirm the species identity of these worms, we purchased 186 commercially grown oysters from retail shellfish farms in Washington State, USA. Of these, 72 individuals came from Oakland Bay (47° 13' 45.93", -123° 3' 19.43", Fig. 2, Table 1), and 114 individuals were from Totten Inlet (47° 9' 43.09", -122° 59' 19.62", Fig. 2, Table 1). Both sites are in South Puget Sound, a region that yields 37% of the total mass and 58% of the value of shellfish produced annually by Washington State[61]. For comparison, we also collected commercially-grown eastern oysters (*Crassostrea virginica*) from North Sea Harbor, Long Island, New York, USA (40° 56' 24.13"N, 72° 25' 3.97"W, Table 1) – a region where the presence of *Polydora websteri* is well established.

Worm collections. All oysters were shucked, and the soft tissues removed. We observed right and left valves under a stereomicroscope for indications of mud worm infestation, such as burrows and blisters. All oysters (with or without infestation) were photographed. We removed any worms present in blisters or burrows with a probe or forceps, or by fracturing shells with a hammer to expose worms in their burrows. Once removed from the shell, we photographed the worms and fixed them whole in 95% ethanol for molecular analysis or, in some cases, sectioned worms such that molecular analysis of a worm (typically middle and posterior chaetigers) could be linked with morphological analysis of the same worm (typically anterior ends).

Morphological identification. For morphological examination, worms were fixed in 4% formalin/seawater overnight, washed in warm tap water, and transferred to 70% ethyl alcohol (EtOH) for storage. For examination with a scanning electron microscope (SEM), the specimens

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

were dehydrated in an ascending ethanol series through 100% EtOH. Drying was accomplished with a Samdri 795 Critical Point Dryer. Once dried, the specimens were mounted on aluminum stubs, coated with gold using an EMS-550 Sputter coater, and viewed with a FEI Quanta 250 SEM. Voucher specimens (Table 1) were deposited in the National Museum of Natural History, Smithsonian Institution, Washington DC, USA (USNM).

Infestation prevalence. We considered any oyster that had at least one blister or burrow to be infested. Prevalence was calculated as the proportion of infested oysters in each sample. We also calculated the number of blisters/burrows per oyster.

DNA extraction, PCR amplification, and sequencing. Within the family Spionidae, species display variable morphology, making it challenging to obtain an accurate species-level identification based solely on morphological traits[63,71,72]. A more fruitful approach is through nuclear 18S rRNA analysis[73]. We followed the protocol of[73] in using a molecular approach to identify worms recovered from blisters and burrows.

For a subset (n = 27) of the total number of worms vouchered (n = 107) and for four additional worms collected from Long Island, New York, we extracted DNA using DNeasy 96 Blood & Tissue Kit (Qiagen, Valencia, CA) following the manufacturers' instructions. We used two genes for molecular identification: the nuclear 18S rRNA [18S rRNA] and the mitochondrial cytochrome c oxidase I [mtCOI]. For the 18S rRNA gene, three regions were amplified: 18S-1F1/18S-1R632, 18S-2F576/18S-2R1209, and 18S-3F1129/18S-R1172[74]. For mtCOI, we amplified one region: Dorid_COI.3F/Dorid_COI.1R[73]. The expected length of the fragments was between 680 and 780 bp.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

We used polymerase chain reaction (PCR) to amplify DNA using a C1000 Touch (Bio-Rad, Hercules, CA) thermocycler. PCR reactions consisted of 2.5 µM of each primer, 2.0 µl of template DNA, 5 µl of 2X PCR buffer (Phusion® Hot Start Flex, Thermo Scientific, Foster City, CA), and 0.5 µl $MgSO_4$ in a 10-µl reaction. 18S rRNA was PCR-amplified with an initial activation step of three minutes at 98°C, followed by 35 cycles of denaturation (30 seconds at 98°C), annealing (30 seconds at 54°C), and extension (30 seconds at 72°C) with a final extension step (10 minutes at 72°C). Only the first of the three regions for 18S rRNA (18S-1F1/18S-1R632) was used for analysis because the other two did not amplify consistently. mtCOI was PCR-amplified with an initial activation step of 98°C, followed by 30 cycles of: denaturation (30 seconds at 98°C), annealing (30 seconds at 45°C), and extension (60 seconds at 72°C) with a final step of five minutes at 72°C. The size of the PCR amplicons was checked in a 1.5% agarose gel. All PCR products were sent for sequencing to Molecular Cloning Laboratories (San Francisco, CA).

Molecular identification. We combined forward and reverse complementary sequences of 18S rRNA and mtCOI genes using Geneious (version 11.0.5). Initially, the majority of 18S rRNA sequences were 660 bp in length, but we trimmed the sequence alignment to 614 bp for analysis. mtCOI sequences were initially 680 bp in length and were trimmed to 554 bp for analysis. After sequences were trimmed, we aligned partial sequences of 18S rRNA and mtCOI genes with sequences of related species from the *Polydora* and *Boccardiella* genera obtained from GenBank (Table 2). We reconstructed phylogenetic trees using the neighbor-joining method based on Kimura 2-parameter model with 1000 bootstrap replications. We used the

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

Molecular Evolutionary Genetics Analysis software (MEGA version 7.0.26), with *Pseudopolydora dayii* as an outgroup.

Acknowledgments

The authors thank Marissa Leatherman for assistance with field and lab work. Luke Tornabene provided help with sequences processing and GenBank submissions. CLW was supported by a Sloan Research Fellowship from the Alfred P. Sloan Foundation. This work was funded in part by a grant to Washington Sea Grant, University of Washington from the National Oceanic and Atmospheric Administration (Award No. NA14OAR4170078 AM12) to CLW and by a grant from the National Science Foundation (DBI-1337525) to JDW. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies.

Author Contributions Statement

JCM & HML carried out fieldwork, sample processing, molecular analysis, prepared figures and wrote the manuscript. LH, IJH and PR provided assistance and guidance with molecular analyses. JDW carried out morphological analysis and SEM imaging. TLK assisted oyster collections, and together with JLPG, LHS and CLW provided critical feedback and contributed to writing. All authors reviewed the manuscript and approved the final version of it.

Competing Interests

The authors declare no competing interests.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

References

1. Lauckner, G. Diseases of Mollusca: Bivalvia. In: O. Kinne, ed. Diseases of Marine Animals, Volume II, Bivalvia to Scaphopoda. Hamburg, Germany: Biologische Anstalt Helgoland (1983).
2. Blake, J.A. Reproduction and larval development of *Polydora* from Northern New England (Polychaeta: Spionidae). *Ophelia* **7**, 1–63 (1969).
3. Shinn, A., Partoomyot, J., Bron, J., Paladini, G., Brooker, E., Brooker, A. Economic costs of protistan and metazoan parasites to global mariculture. *Parasitology* **142**, 196–270 (2015).
4. Blake, J., Evans. J. *Polydora* and other related Polychaeta Spionidae as borers in mollusk shells and other calcareous substrates. *Veliger* **15**, 235–249 (1973).
5. Martin, D., Britayev, T.A. Symbiotic polychaetes: Review of known species. *Oceanography and Marine Biology: an Annual Review* **36**, 217–340 (1998).
6. Simon, C.A., Sato-Okoshi, W. Polydorid polychaetes on farmed molluscs: distribution, spread and factors contributing to their success. *Aquaculture Environment Interactions* **7**, 147–166 (2015).
7. Royer, J., Ropert, M., Mathieu, M., and Costil, K. Presence of spionid worms and other epibionts in Pacific oysters (*Crassostrea gigas*) cultured in Normandy, France. *Aquaculture* **253**, 461–474 (2006).
8. Ogburn, D.M., White, I., McPhee, D.P. The disappearance of oyster reefs from eastern Australian estuaries: Impact of colonial settlement or mudworm invasion? *Coastal Management* **35**, 271–287 (2007).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

328 9. Read, G.B. Comparison and history of *Polydora websteri* and *P. haswelli* (Polychaeta:
329 Spionidae) as mud-blister worms in New Zealand shellfish. New Zealand Journal of Marine
330 and Freshwater Research **44**, 83–100 (2010).
331 10. Rice, L.N., Lindsay, S., Rawson, P. Genetic homogeneity among geographically distant
332 populations of the blister worm *Polydora websteri*. *Aquaculture Environment Interactions*
333 **10**, 437–446 (2018).
334 11. Kent, R. The influence of heavy infestations of *Polydora ciliata* on the flesh content of
335 *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom* **59**, 289–
336 297 (1979).
337 12. Kent, R. The effect of *Polydora ciliata* on the shell strength of *Mytilus edulis*. *Journal du*
338 *Conseil International pour l'Exploration de la Mer* **39**, 252–255 (1981).
339 13. Read, G.B., Handley, S. New alien mudworm now becoming a pest in longline mussels.
340 *Water and Atmosphere* **12**, 30–31 (2004).
341 14. Evans, J.W. Borers in the shell of the sea scallop, *Placopecten magellanicus*. *American*
342 *Zoologist* **9**,775–782 (1969).
343 15. Bergman, K., Elner, R., Risk, M. The influence of *Polydora websteri* borings on the
344 strength of the shell of the sea scallop, *Placopecten magellanicus*. *Canadian Journal of*
345 *Zoology* **60**, 2551–2556 (1982).
346 16. Mori, K., Sato, W., Nomura, T., Imajima, M. Infestation of the Japanese scallop
347 *Patinopecten yessoensis* by the boring polychaetes, *Polydora*, on the Okhotsk Sea coast of
348 Hokkaido, especially in Abashiri waters. *Nippon Suisan Gakkaishi* **51**, 371–380 (1985).
349 17. Hahn, K. Handbook of Culture of Abalone and Other Marine Gastropods. Boca Ragon,
350 FL: CRC Press (1989).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

18. Radashevsky, V.I. Spionidae (Annelida) from shallow waters around the British Islands: an identification guide for the NMBAQC Scheme with an overview of spionid morphology and biology. *Zootaxa* **3152**, 1–35 (2012).

19. Whitelegge, T. Report on the worm disease affecting the oysters on the coast of New South Wales. *Records of the Australian Museum* **1**, 41–54 (1890).

20. Roughley, T. Oyster Culture on the George's River, New South Wales. Sydney, Australia: William Applegate Gullick, Government Printer (1922).

21. Roughley, T. The Story of the Oyster: Its History, Growth, Cultivation and Pests in New South Wales. Sydney, Australia: Alfred James Kent, Government Printer (1925).

22. Nell, J. Controlling mudworm in oysters. New South Wales, Australia: New South Wales Department of Primary Industries (2007).

23. Bailey-Brock, J., Ringwood, A. Methods for control of the mud blister worm, *Polydora websteri,* in Hawaiian oyster culture. *Sea Grant Quarterly* **4**, 1–6 (1982).

24. Bailey-Brock, J. Phylum Annelida. In: Reef and Shore Fauna of Hawaii. Honolulu, HI: *Bernice P Bishop Museum Special Publications*. pp. 213-454 (1987).

25. Lunz, G.R. The annelid worm, *Polydora,* as an oyster pest. *Science* **92**,310 (1940).

26. Lunz, G.R. *Polydora*, a pest in South Carolina oysters. *Journal of the Elisha Mitchell Scientific Society* **57**, 273–283 (1941).

27. Loosanoff, V.L., Engle, J.B. *Polydora* in oysters suspended in the water. *Biological Bulletin* **85**, 69–78 (1943).

28. Bower, S., Blackbourn, S., Meyer, G., Nishimura, D. Diseases of cultured Japanese scallops (*Patinopecten yessoensis*) in British Columbia, Canada. *Aquaculture* **107**, 201–210 (1992).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

29. Blake, J.A. The Annelida Part 3. Polychaeta: Orbiniidae to Cossuridae. In: Blake JA, Hilbig B, and Scott PH, eds. Taxonomic Atlas of the Benthic Fauna of the Santa Maria Basin and Western Santa Barbara Channel. Santa Barbara, CA: Santa Barbara Museum of Natural History (1996).

30. Clements, J.C., Bourque, D., McLaughlin, J., Stephenson, M., Comeau, L.A. Wanted dead or alive: *Polydora websteri* recruit to both live oysters and empty shells of the eastern oyster, *Crassostrea virginica*. *Journal of Fish Diseases* **41**, 855–858 (2018).

31. Hopkins, S.H. The planktonic larvae of *Polydora websteri* Hartman (Annelida, Polychaeta) and their settling on oysters. *Bulletin of Marine Science* **8**, 268–277 (1958).

32. Korringa, P. The shell of *Ostrea edulis* as a habitat. *Archives Néerlandaises de Zoologie* **10**, 32–146 (1954).

33. Haigler, S.A. Boring mechanism of *Polydora websteri* inhabiting *Crassostrea virginica*. *American Zoologist* **9**, 821–828 (1969).

34. Zottoli, R.A., Carriker, M.R. Burrow morphology, tube formation, and microarchitecture of shell dissolution by the spionid polychaete *Polydora websteri*. *Marine Biology* **27**, 307–316 (1974).

35. Handley, S.J., Bergquist, P.R. Spionid polychaete infestations of intertidal pacific oysters *Crassostrea gigas* (Thunberg), Mahurangi Harbour, northern New Zealand. *Aquaculture* **153**, 191–205 (1997).

36. Morse, D.L., Rawson, P.D., Kraeuter, J.N. Mud blister worms and oyster aquaculture. Orono, ME: *Maine Sea Grant and the University of Maine Cooperative Extension* (2015).

37. Sato-Okoshi, W., Abe, H., Nishitani, G., Simon, C.A. And then there was one: *Polydora uncinata* and *Polydora hoplura* (Annelida: Spionidae), the problematic polydorid pest

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

species represent a single species. *Journal of the Marine Biological Association of the United Kingdom* **97**, 1675–1684 (2017).

38. Buschbaum, C., Buschbaum, G., Schrey, I., Thieltges, D.W. Shell-boring polychaetes affect gastropod shell strength and crab predation. *Marine Ecology Progress Series* **329**, 123–130 (2007).

39. Chambon, C., Legeay, A., Durrieu, G., Gonzalez, P., Ciret, P., Massabuau, J. Influence of the parasite worm *Polydora* sp. on the behaviour of the oyster *Crassostrea gigas*: A study of the respiratory impact and associated oxidative stress. *Marine Biology* **152**, 329–338 (2007).

40. Wargo, R.N., Ford, S.E. The effect of shell infestation by *Polydora* sp. and infection by *Haplosporidium nelsoni*. *Estuaries* **16**, 229–234 (1993).

41. Owen, H. Etiological studies on oyster mortality. II. *Polydora websteri* Hartmann (Polychaeta: Spionidae). *Bulletin of Marine Science of the Gulf and Caribbean* **7**, 35–46 (1957).

42. Dinamani, P. Potential disease-causing organisms associated with mantle cavity of Pacific oyster *Crassostrea gigas* in northern New Zealand. *Diseases of Aquatic Organisms* **2**, 55–63 (1986).

43. Kojima, H., Imajima, M. Burrowing polychaetes in the shells of the abalone *Haliotis diversicolor aquatilis* chiefly on the species of *Polydora*. *Nippon Suisan Gakkaishi* **48**, 31–35 (1982).

44. Lleonart, M., Handlinger, J., Powell, M. Spionid mudworm infestation of farmed abalone (*Haliotis* spp.). *Aquaculture* **221**, 85–96 (2003).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

418 45. Simon, C.A. *Polydora* and *Dipolydora* (Polychaeta: Spionidae) associated with molluscs
419 on the south coast of South Africa, with descriptions of two new species. *African*
420 *Invertebrates* **52**, 39–50 (2011).
421 46. Boonzaaier, M., Neethling, S., Mouton, A., Simon, C. Polydorid polychaetes (Spionidae)
422 on farmed and wild abalone (*Haliotis midae*) in South Africa: An epidemiological survey.
423 *African Journal of Marine Science* **36**, 369–376 (2014).
424 47. Handley, S. Power to the oyster: Do spionid-induced shell blisters affect condition in
425 subtidal oysters? *Journal of Shellfish Research* **17**, 1093–1099 (1998).
426 48. Coen, L.D., Brumbaugh, R.D., Bushek, D., Grizzle, R., Luckenbach, M.W., Posey, M.H.,
427 Powers, S.P., Tolley, S.G. Ecosystem services related to oyster restoration. *Marine Ecology*
428 *Progress Series* **341**, 303–307 (2007).
429 49. Ruellet, T. Infestation des coquilles d'huîtres *Crassostrea gigas* par les polydores en
430 Basse-Normandie: Recommandations et mise au point d'un traitement pour réduire cette
431 nuisance. PhD dissertation, Université de Caen (2004).
432 50. Walker, L.M. A review of the current status of the *Polydora*-complex (Polychaeta:
433 Spionidae) in Australia and a checklist of recorded species. *Zootaxa* **2751**, 40–62 (2011).
434 51. Walker, L.M. A revision of the *Polydora*-complex (Annelida: Spionidae) fauna from
435 Australia. PhD dissertation, University of Queensland (2013).
436 52. Ye, L., Cao, C., Tang, B., Yao, T., Wang, R., Wang, J. Morphological and molecular
437 characterization of *Polydora websteri* (Annelida: Spionidae) with remarks on relationship of
438 adult worms and larvae using mitochondrial COI gene as a molecular marker. *Pakistan*
439 *Journal of Zoology* **49**, 699–710 (2017).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

53. Radashevsky, V.I., Williams, J.D. *Polydora websteri* Hartman in Loosanoff & Engle, 1943 (Annelida, Polychaeta): proposed conservation of the specific name by a ruling that it is not to be treated as a replacement name for *P. caeca* Webster, 1879, and designation of a lectotype for *P. websteri. Bulletin of Zoological Nomenclature* **55**, 212–216 (1998).

54. Radashevsky, V.I. Description of the proposed lectotype for *Polydora websteri* Hartman *In* Loosanoff & Engle, 1943. *Ophelia* **51**, 107–113 (1999).

55. ICZN. Opinion 1974. *Polydora websteri* Hartman in Loosanoff & Engle, 1943 (Annelida, Polychaeta): Specific name conserved by a ruling that it is not to be treated as a replacement for *P. caeca* Webster, 1879, and a lectotype designated for *P. websteri*. *Bulletin of Zoological Nomenclature* **58**, 152–153(2001).

56. Hobson, K.D., Banse, K. Sedentariate and archiannelid polychaetes of British Columbia and Washington. *Canadian Bulletin of Fisheries and Aquatic Sciences* **209**, 1–144 (1981).

57. Bower, S. Shellfish diseases on the west coast of Canada. *Bulletin of the Aquaculture Association of Canada* **90**, 19–22 (1990).

58. Hartman, O. The marine annelids of San Francisco Bay and its environs, California. *Occasional Papers of the Allan Hancock Foundation* **15**, 1–20 (1954).

59. Hartman, O. Polychaetous annelids from California. *Allan Hancock Pacific Expeditions* **25**, 226 (1961).

60. Hartman, O. Atlas of the Sedentariate Polychaetous Annelids from California. Los Angeles, CA: *Allan Hancock Foundation, University of Southern California* (1969).

61. Washington Sea Grant (2015) Shellfish aquaculture in Washington State. Final report to the Washington State Legislature, 84 p.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

62. Washington State Shellfish Initiative (2016) Washington: A Shellfish State. http://www.governor.wa.gov/sites/default/files/WSI%20factsheet.pdf

63. Sato-Okoshi, W., Abe, H. Morphology and molecular analysis of the 18S rRNA gene of oyster shell borers, *Polydora* species (Polychaeta: Spionidae), from Japan and Australia. *Journal of the Marine Biological Association of the United Kingdom* **9,** 1279–1286 (2013).

64. Fofonoff, P.W., Ruiz, G.M., Steves, B., Carlton, J.T. In ships or on ships? Mechanisms of transfer and invasion for non-native species to the coasts of North America. In: G. M. Ruiz and J. T. Carlton, eds. Invasive Species: Vectors and Management Strategies. Washington, DC: Island Press. pp. 152–182 (2003).

65. Carlton, J.T., Geller, J.B. Ecological roulette: the global transport of nonindigenous marine organisms. *Science* **261**, 78–82 (1993).

66. Drake, J.M., Lodge, D.M. Global hot spots of biological invasions: Evaluating options for ballast-water management. *Proceedings of the Royal Society B* **271**, 575–580 (2004).

67. Carlton, J.T. Biological invasions and cryptogenic species. *Ecology* **77**,1635–1655 (1996).

68. Clements, J.C., Bourque, D., McLaughlin, J., Stephenson, M., Comeau, L.A. Siltation increases the susceptibility of surface -cultured eastern oysters (*Crassostrea virginica*) to parasitism by the mudworm *Polydora websteri. Aquaculture Research* **48**, 4707–4717 (2017)a.

69. Clements, J.C., Bourque, D., McLaughlin, J., Stephenson, M., Comeau, L.A. Extreme ocean acidification reduces the susceptibility of eastern oyster shells to a polydorid parasite. *Journal of Fish Diseases* **40**, 1573–1585(2017)b.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

483 70. White, J.M., Buhle, E.R., Ruesink, J.L., Trimble, A.C. Evaluation of Olympia oyster
484 (*Ostrea lurida* Carpenter 1864) status and restoration techniques in Puget Sound,
485 Washington, United States. *Journal of Shellfish Research* **28**, 107–112 (2009).
486 71. Radashevsky, V.I., Pankova, V.V. The morphology of two sibling sympatric *Polydora*
487 species (Polychaeta: Spionidae) from the Sea of Japan. *Journal of the Marine Biological*
488 *Association of the United Kingdom* **86**, 245–252 (2006).
489 72. Rice, S.A., Karl, S., Rice, K.A. The *Polydora cornuta* complex (Annelida: Polychaeta)
490 contains populations that are reproductively isolated and genetically distinct. *Invertebrate*
491 *Biology* **127**, 45–64 (2008).
492 73. Williams, L.G., Karl, S.A., Rice, S., Simon, C.. Molecular identification of polydorid
493 polychaetes (Annelida: Spionidae): Is there a quick way to identify pest and alien species?
494 *African Zoology* **52**, 105–117 (2017).
495 74. Nishitani, G., Nagai, S., Hayakawa, S., Kosaka, Y., Sakurada, K., Kamiyama, T.,
496 Gojobori, T. Multiple plastids collected by the dinoflagellate *Dinophysis mitra* through
497 kleptoplastidy. *Applied and Environmental Microbiology* **78**, 813 (2012).
498

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

499 **Figure Legends**

500

501 **Fig. 1**. *Crassostrea gigas* infested with *Polydora websteri* collected from Oakland Bay, WA.
502 Pathology shown is associated with shell-boring mud worms. (a) Inner surface of an infested
503 valve, (b) opened mud blister, and (c) closed mud blister filled with mud, detritus, and worm
504 feces. In (b) and (c), scale bar indicates 2 mm.

505

506 **Fig. 2**. Map of sampling sites in Southern Puget Sound, Washington state. Oysters were grown in
507 Oakland Bay (n = 72) and Totten Inlet (n = 114).

508

509 **Fig. 3**. *Polydora websteri* from Oakland Bay, Washington extracted from *Crassostrea gigas*. (A)
510 Anterior dorsal view of specimen lacking palps (USNM 000000 H3-61-8). (B) Anterior dorsal
511 view of specimen with basal portion of right palp attached (USNM 000000 H3-61-4). (C)
512 Anterior, right lateral view, same specimen as in A (USNM 000000 H3-61-8). (D) *En face* view
513 of specimen showing anterior end of prostomium, same specimen as in A (USNM 000000 H3-
514 61-8). (E) Lateral view of middle portion of palp, palp removed from specimen shown in B
515 (USNM 000000 H3-61-8). (F) Dorsal view of fifth chaetiger spines (USNM 000000 H3-61-1).
516 (G) Dorsal view of fifth chaetiger spines, same specimen as in B (USNM 000000 H3-61-4). (H)
517 Lateral view of fifth chaetiger spines, close-up, same specimen as in B (USNM 000000 H3-61-
518 4). Scale bars A-C = 250 µm, D = 200 µm, E = 100 µm, F = 50 µm, G, H = 25 µm.

519

520 **Fig. 4.** *Polydora websteri* from Long Island, New York extracted from *Crassostrea virginica*.
521 (A) Anterior dorsal view of specimen lacking palps (USNM 000000 P1-109-2a). (B) Anterior

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

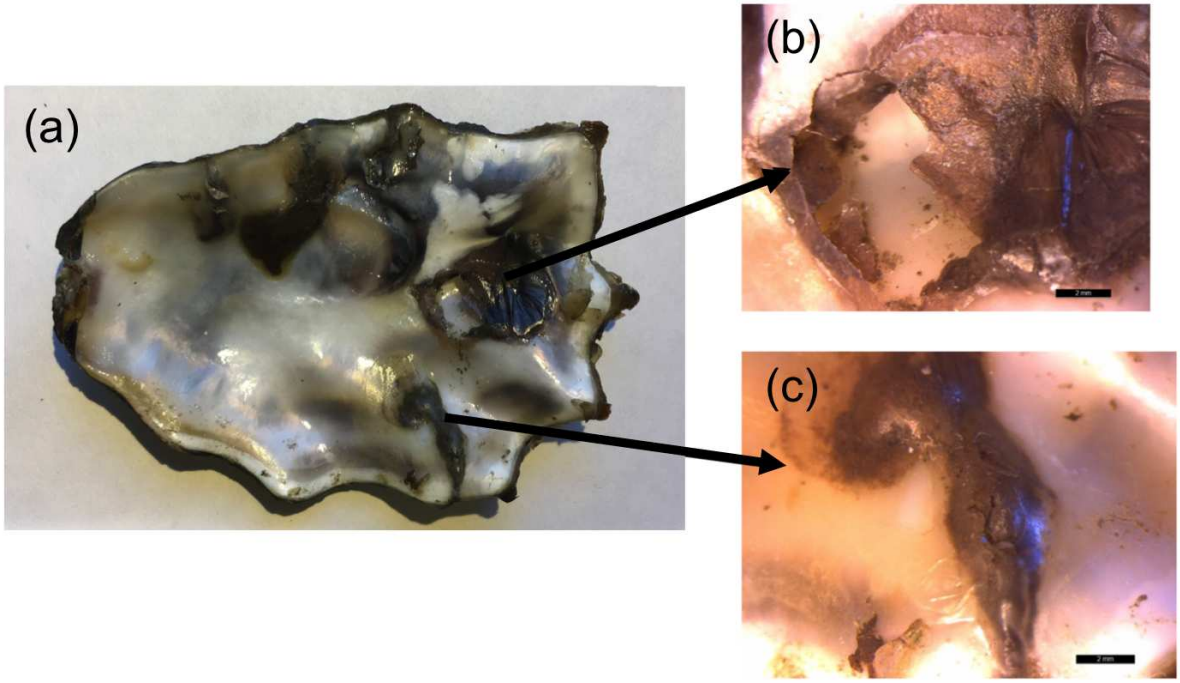
dorsal view of specimen with palps (USNM 000000 P1-109-3a). (C) Anterior, right lateral view, same specimen as in A (USNM 000000 P1-109-2a). (D) *En face* view of specimen showing anterior end of prostomium, same specimen as in B (USNM 000000 P1-109-3a). (E) Lateral view of middle portion of palp, same specimen as in B (USNM 000000 P1-109-3a). (F) Dorsal view of fifth chaetiger spines (USNM 000000 P1-109-4a). (G) Dorsal view of fifth chaetiger spines, same specimen as in B (USNM 000000 P1-109-3a). (H) Lateral view of fifth chaetiger spines, close-up, arrows indicate subdistal "tooth," same specimen as in A (USNM 000000 P1-109-2a). Scale bars A = 200 µm, B = 500 µm, C = 250 µm, D = 100 µm, E = 50 µm, F-H = 25 µm.

Fig. 5. Maximum likelihood phylogeny based on Kimura 2-parameter distances using trimmed 18S1 rRNA sequences (1000 replicates). *Pseudopolydora dayii* (KY677907) was used as an outgroup. Entries accompanied with accession number were acquired from GenBank (Table 3), individuals labeled with OAK and TOT were collected in Oakland Bay and Totten Inlet, respectively.

Fig. 6. Maximum likelihood phylogeny based on Kimura 2-parameter method using trimmed mtCOI sequences (1000 replicates). *Pseudopolydora dayii* (KY677868) was used as an outgroup. Entries accompanied with accession number were acquired from GenBank (Table 3), individuals labeled with OAK and TOT were collected in Oakland Bay and Totten Inlet, respectively.

Fig. 1

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019



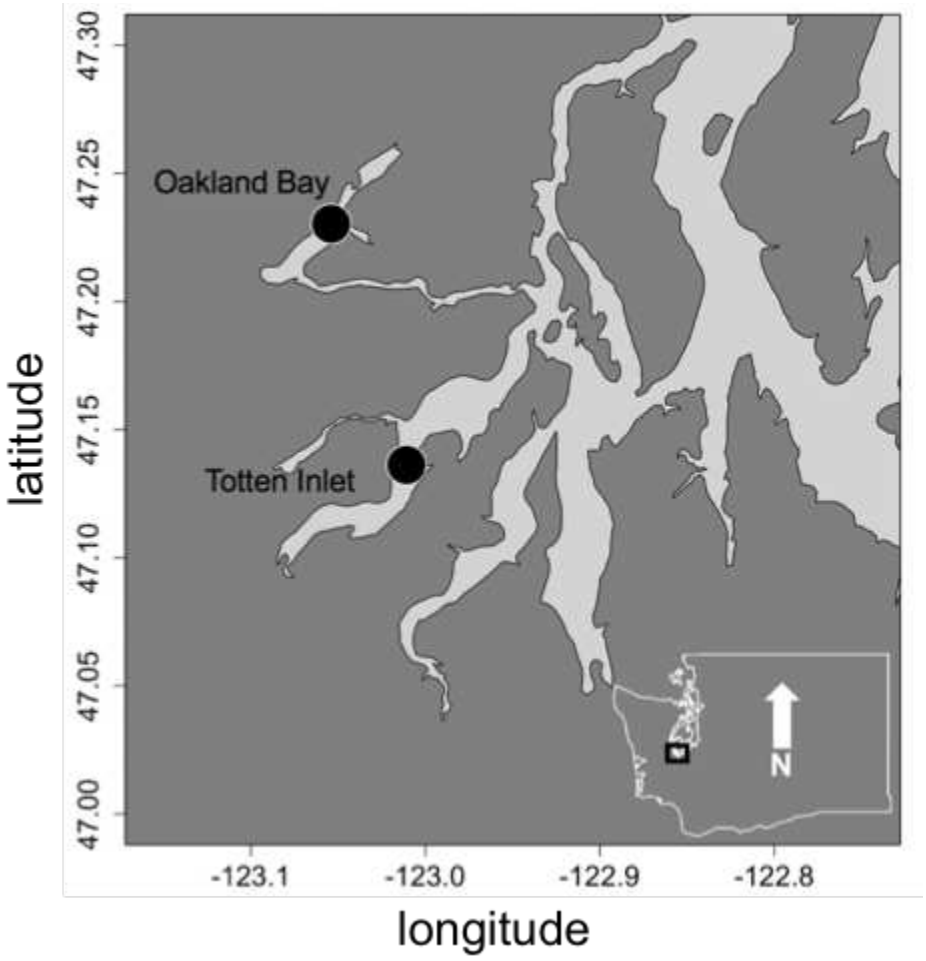
545

546

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

547 **Fig. 2**

548

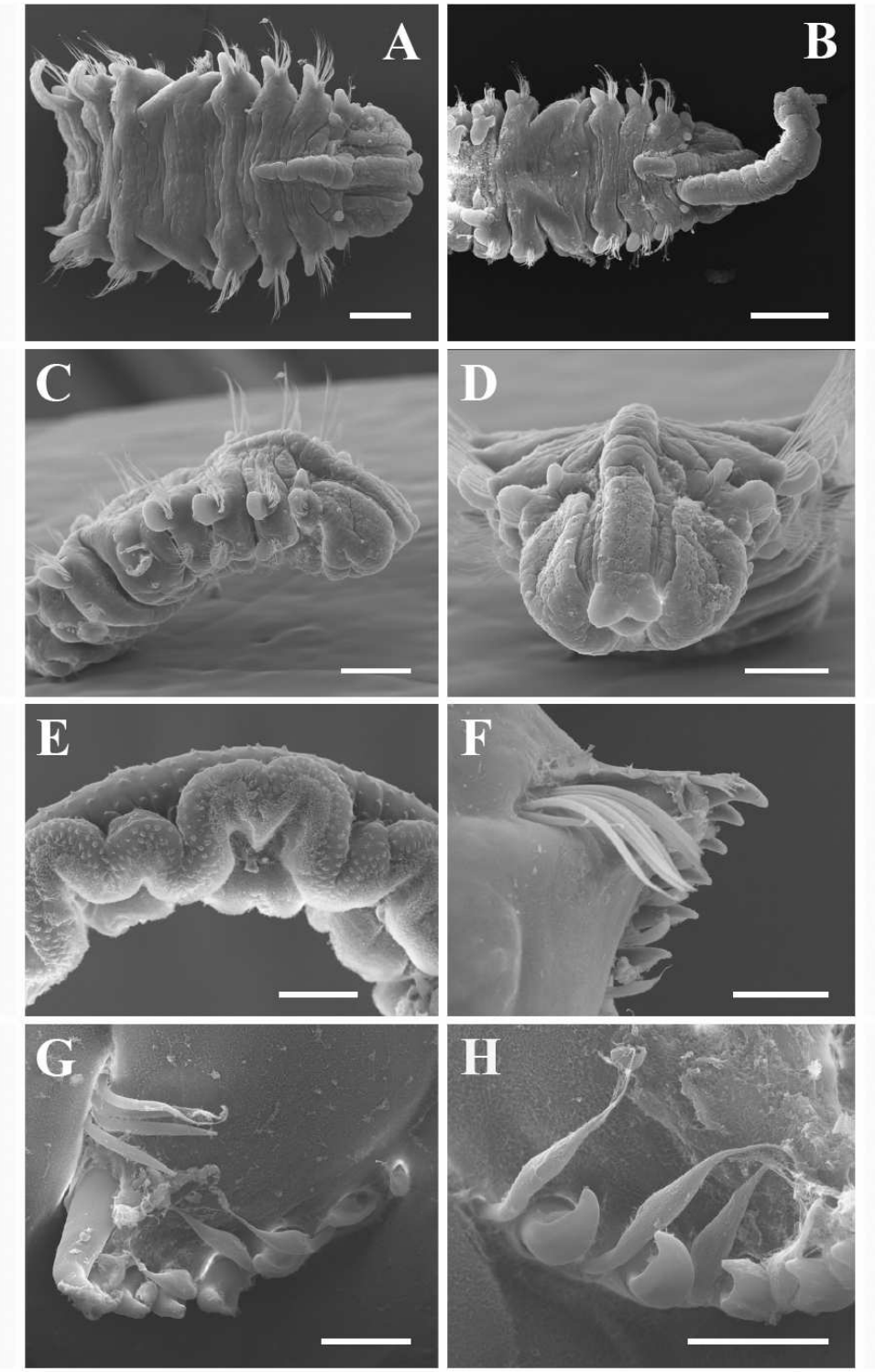


549

550

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

551 **Fig. 3**

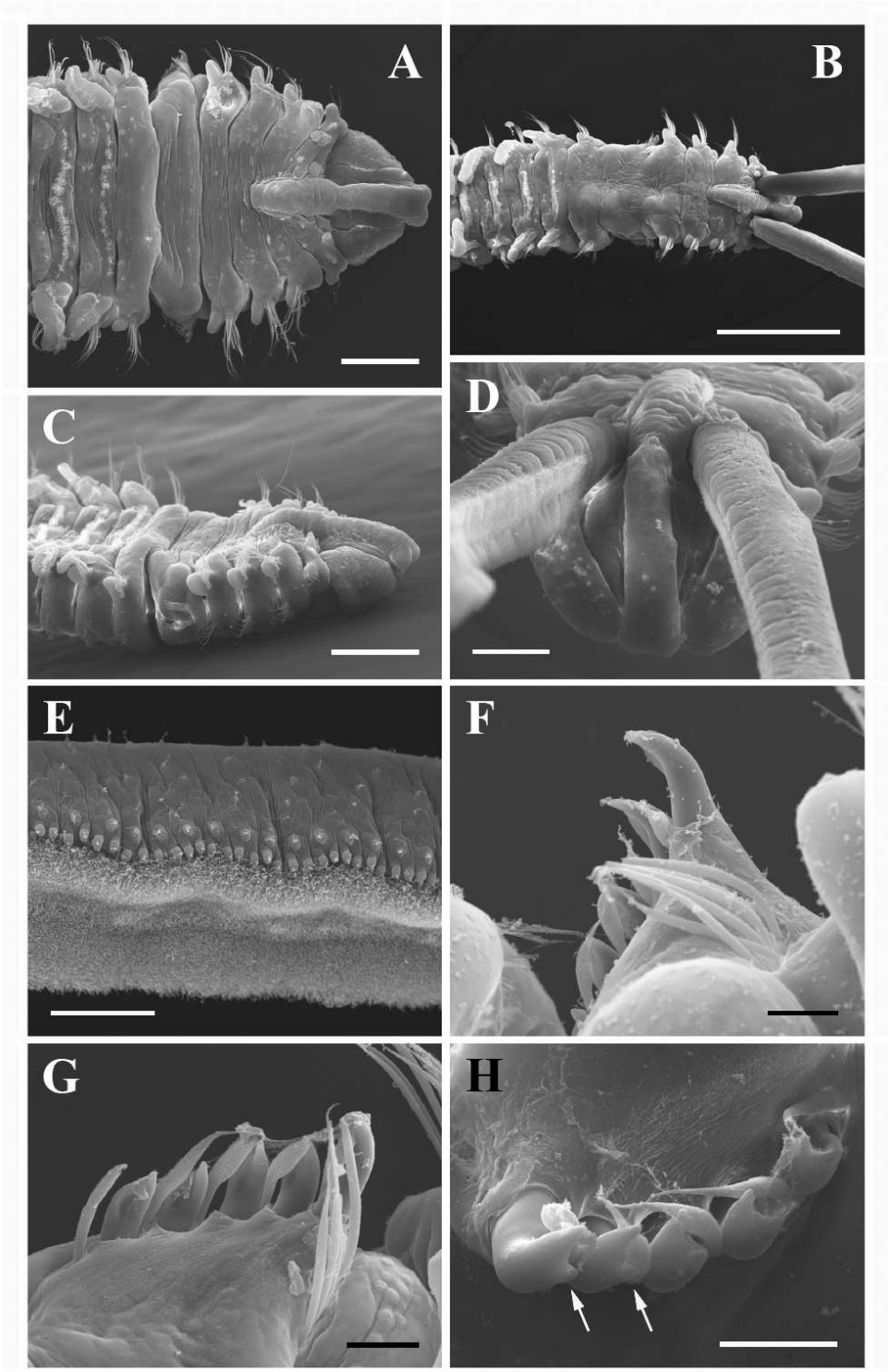


552

553

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

554 **Fig. 4**

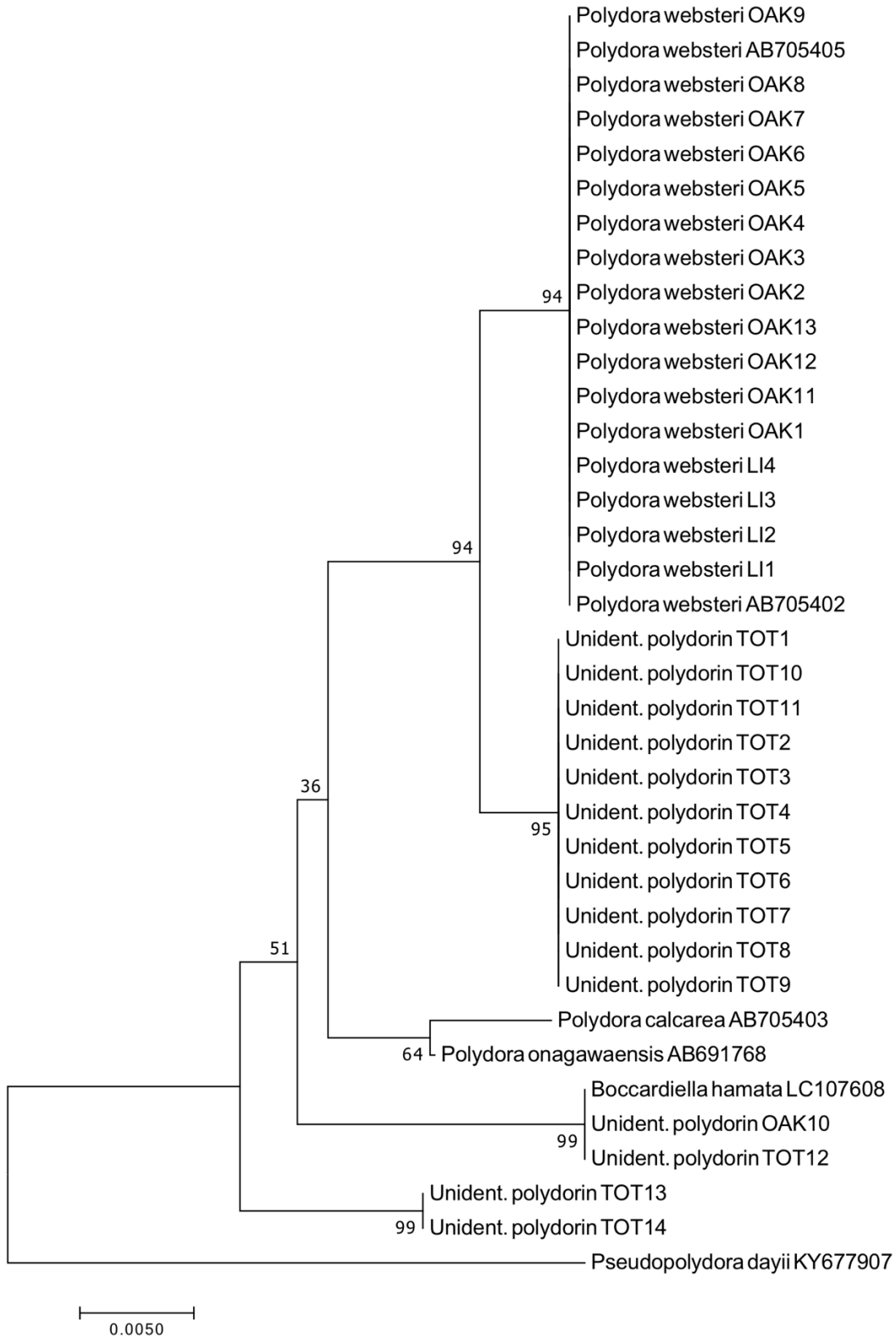


555

556

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

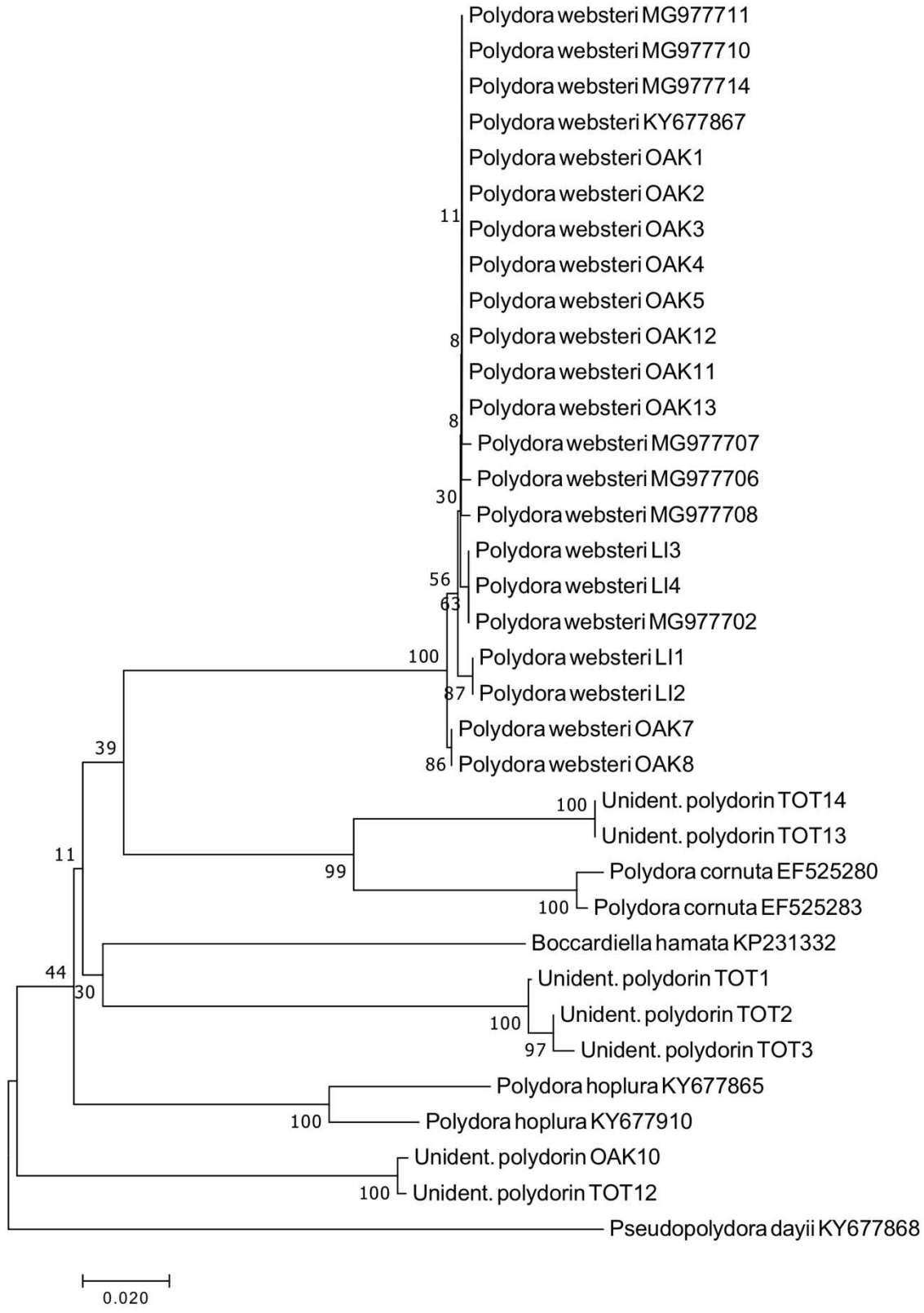
557 **Fig. 5**



558

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

559 **Fig. 6**



560

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

561 **Table 1.** Taxa, sampling location data, museum registration numbers of voucher specimens and
562 GenBank accession numbers of sequences used in the analysis. SEM = specimen prepared for
563 scanning electron micrograph; EtOH = specimen preserved in ethanol. Specimens that were
564 unresolved in the phylogenetic trees are not included in this table.
565

Molecular ID	Morphological ID	Worm ID on trees	Location and host	Coords.	Date	Museum Voucher (SEM or EtOH)	GenBank Accession Numbers	
							18S	*COI*
Polydora websteri	-	OAK1	Oakland Bay, Washington State, USA; from shells of *Crassostrea gigas*	47° 13' 45.93", –123° 3' 19.43"	3 Oct 2017	-	MH891522	MK188730
Polydora websteri	-	OAK2	Same as above	Same as above	3 Oct 2017	-	MH891521	MK188731
Polydora websteri	-	OAK3				-	MH891520	MK188732
Polydora websteri	-	OAK4				-	MH891519	MK188733
Polydora websteri	-	OAK5				-	MH891517	MK188734
Polydora websteri	-	OAK6				-	MH891514	-
Polydora websteri	-	OAK7				-	MH891515	MK188735
Polydora websteri	-	OAK8				-	MH891516	MK188736
Polydora websteri	-	OAK9				-	MH891513	-
Unident. polydorin	-	OAK10				-	MH891518	MK188737
Polydora websteri	*Polydora websteri*	-			15 Aug 2018	H1-61-1(SEM)	MK695999	-
Polydora websteri	*Polydora websteri*	-				H1-61-3(EtOH)	-	-
Polydora websteri	*Polydora websteri*	-				H1-61-4(SEM)	-	-
Polydora websteri	*Polydora websteri*	-				H1-61-5(EtOH)	MK696002	-

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

Polydora websteri	*Polydora websteri*	OAK11				H1-61-6(SEM)	MK696000	MK696586
Polydora websteri	*Polydora websteri*	OAK12				H1-61-7(SEM)	MK696001	MK696587
Polydora websteri	*Polydora websteri*	OAK12				H1-61-8(SEM)	MK696003	MK696588
Polydora websteri	*Polydora websteri*	-				H1-61-9(SEM)	-	-
Unident. polydorin	-	TOT1	Totten Inlet, Washington State, USA; from shells of *Crassostrea gigas*	47° 9' 43.09", –122° 59' 19.62"		-	MH891524	MK188738
Unident. polydorin	-	TOT2	Same as above	Same as above	18 Sep 2017	-	MH891525	MK188739
Unident. polydorin	-	TOT3				-	MH891527	MK188740
Unident. polydorin	-	TOT4				-	MH891530	-
Unident. polydorin	-	TOT5				-	MH891528	-
Unident. polydorin	-	TOT6				-	MH891536	-
Unident. polydorin	-	TOT7				-	MH891534	-
Unident. polydorin	-	TOT8				-	MH891531	-
Unident. polydorin	-	TOT9				-	MH891532	-
Unident. polydorin	-	TOT10				-	MH891523	-
Unident. polydorin	-	TOT11				-	MH891535	-
Unident. polydorin	-	TOT12				-	MH891533	MK188741
Unident. polydorin	-	TOT13				-	MH891529	MK188742
Unident. polydorin	-	TOT14				-	MH891526	MK188743
Polydora websteri	*Polydora websteri*	LI1	North Sea Harbor, Long Island, New York, USA; from shells	40° 56' 24.13"N, 72° 25' 3.97"W	12 Sep 2018	P1-109-2a, b	MK369933	MK696582

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

			of *Crassostrea virginica*					
Polydora websteri	*Polydora websteri*	LI2	Same as above	Same as above		P1-109-3a, b	MK369934	MK696583
Polydora websteri	*Polydora websteri*	LI3	Same as above			P1-109-4a, b	MK369935	MK696584
Polydora websteri	*Polydora websteri*	LI 4	Same as above			P1-109-5a, b	MK369936	MK696585

566

567

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

568 **Table 2.** Details for 18S and COI sequences from GenBank that were used for phylogenetic
569 analyses.
570

Species/Accession number	Marker	Length (bp)	Country	Host	Year
Polydora websteri AB705402	18S	1771	Japan	*Crassostrea gigas*	2013
Polydora websteri AB705405	18S	1771	Australia	*Saccostrea commercialis*	2013
Polydora calcarea AB705403	18S	1771	Japan	*Crassostrea gigas*	2013
Polydora onagawensis AB691768	18S	1771	Japan	*Crassostrea gigas*	2013
Boccardiella hamata LC107608	18S	1772	Japan	*Crassostrea gigas*	2017
Pseudopolydora dayii KY677907	18S	1716	South Africa	N/A	2017
Polydora websteri MG977711	COI	794	United States	*Crassostrea virginica*	2018
Polydora websteri MG977710	COI	794	United States	*Crassostrea virginica*	2018
Polydora websteri MG977714	COI	794	United States	*Crassostrea gigas*	2018
Polydora websteri KY677867	COI	622	South Africa	N/A	2017
Polydora websteri MG977707	COI	794	United States	*Crassostrea virginica*	2018
Polydora websteri MG977706	COI	794	United States	*Crassostrea virginica*	2018
Polydora websteri MG977708	COI	794	United States	*Crassostrea virginica*	2018
Polydora websteri MG977702	COI	794	United States	*Crassostrea virginica*	2018

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

Polydora cornuta EF525280	COI	1020	United States	N/A	2016
Polydora cornuta EF525283	COI	912	United States	N/A	2016
Boccardiella hamata KP231332	COI	918	China	*Crassostrea gigas*	2015
Polydora hoplura KY677865	COI	622	South Africa	N/A	2017
Polydora hoplura KY677910	COI	952	South Africa	N/A	2017
Pseudopolydora dayii KY677868	COI	622	South Africa	N/A	2017

571

572

573

574

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019

575 **Table 3.** Sequences alignment statistics for 18S and COI sequences. Values were calculated
576 using MEGA7.0.26.
577

Variable	**18S**	**COI**
Sample size	27	17
Final length of aligned sequences	614	554
No. variable nucleotides	22/614	164/554
Haplotype diversity	0.009	0.135
Transitions/transversions ratio	1.02	1.31

578
579
580

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27621v2 | CC BY 4.0 Open Access | rec: 21 Nov 2019, publ: 21 Nov 2019